Int J Legal Med (1997) 110 : 101–103

CASE REPORT

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STR analysis on perforating FMJ bullets and a new VWA variant allele

Received: 7 November 1996 / Received in revised form: 5 December 1996

Abstract In three separate shooting incidents involving multiple gunshots, two FMJ bullets and one bullet fragment found at the scene (one from each case) were investigated for the presence of biological material from the victim after perforation. The surface of the missiles, which did not show obvious tissue traces when examined under a macroscope, was swabbed. PCR typing of up to five STR loci was performed on the small amounts of DNA extracted, which were even below the detection limit of the slot blot quantification in one case. Nevertheless, individualisation of cellular material from the perforating projectiles was successful in each of the three cases presented. Consequently, identification of the victim wounded by a perforating bullet can reliably be achieved if contamination or removal of evidentiary material by improper handling is prevented. This technique is especially useful in cases where more than one person has fired a gun because the bullet carrying DNA can be linked to the firearm by investigation with a comparison microscope. As a by-product of this investigation, a variant allele 14 (14+4) at the VWA locus was detected.

Key words Perforating bullets · Microsatellites · New VWA variant 14 (+4)

Introduction

Individualisation of biological traces on a perforating bullet can identify the person through which the bullet has passed. This is especially important if more than one person is injured or several gunshots have been fired. In a previous publication (Karger et al. 1996), experimental DNA typing of biological material on perforating bullets was performed using calves. Due to the lack of suitable polymorphic markers in bovines, only a species identification was carried out, but it was suggested that the application of a set of short tandem repeat (STR) systems would also be successful in cases of human individualisation. This approach will be illustrated in three human gunshot cases.

In three separate multiple shooting incidents the victims were hit by some of the missiles fired. In the first two cases the discrimination between the bullets which had perforated and those which had missed the bodies was crucial. In the third case the question arose whether the bullet had perforated a particular person.

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Case reports

Case 1

A man (victim I) was found dead inside his car with two gunshot wounds to the head including one exit wound. Four empty cartridge cases were discovered inside and outside the car and the car windows showed two perforations. Among others, a fired and non-deformed 7.65 mm full metal jacketed (FMJ) bullet (bullet I) was recovered outside the car at a distance of 3 m.

Case 2

A man (victim II) was found dead lying on the ground with four intracranial gunshot wounds, two of them perforating, and with one penetrating gunshot wound of the thorax. A fired, non-deformed 6.35 mm FMJ bullet (bullet II) was recovered 1 m from the body.

Case 3

A major gunfight in a railway station resulted in two fatalities with multiple hits including perforating and penetrating bullets. Among others, a 0.4×1.2 cm fragment of a bullet jacket was found at the scene at a distance of several metres from the two victims. Since none of the victims (victims A and B) showed fragment wounds, the bullet had most likely fragmented on concrete after perforation of the body.

Materials and methods

The FMJ bullets and the bullet fragment were viewed under a macroscope (30 × magnification) for adhering biological material. In case 1 no material was visible except on the base of the bullet, where some black/brownish spots – presumably powder residues – could be seen. Consequently, a presumptive test for blood (Kastle-Meyer) on these stains was negative. A single minute piece of red/brown material attached to the base of bullet II (case 2) showed a positive reaction when tested for the presence of blood. On the bullet fragment (case3), some brownish spots could be seen under the macroscope which gave a positive reaction for the presence of blood. As no or only minute amounts of material were recovered by visual inspection, the whole surface of the bullets was swabbed in each case with approx. 5 moistened fibreglass applicators (3 × 5 mm; Pharmacia, Sweden). The detailed procedure of removing cellular material from bullets is given elsewhere (Karger et al. 1996). DNA was extracted using Chelex 100 and Proteinase K (Wiegand et al. 1993) and quantified by the slot blot technique as previously described (Waye et al. 1989). PCR typing of the STR loci was performed on 2-20 µl aliquots of DNA extract followed by high resolution non-denaturing gel electrophoresis (Allen et al. 1989) and visualisation by silver staining (Budowle et al. 1991). The PCR primers and amplification conditions used were as described by the following authors:

ACTBP2 (Polymeropoulos et al. 1992; Möller and Brinkmann 1994)

TH01 (Edwards et al. 1992; Meyer et al. 1995)

VWA (Kimpton et al. 1992; Möller et al. 1994)

FES (Polymeropoulos et al. 1991; Alper et al. 1995)

FGA (Barber et al. 1996)

Taq-cycle-sequencing and sequence analysis of the VWA allele 14 (+4) was performed as described by Möller and Brinkmann (1994).

Results and discussion

In an experimental approach (Karger et al. 1996) it was found that far less DNA was recovered from the smooth surfaces of FMJ bullets compared to hollow point bullets. The two FMJ bullets investigated in this report also showed only one minute particle (bullet II) or no attaching material (bullet I) under the macroscope. This resulted in extremely small amounts of extracted DNA which were sometimes below the detection limit of the slot blot quantification. But this small amount of DNA extract was sufficient for successful PCR typing in several STR systems, even in the case of intact FMJ bullets, which represent the most difficult challenge (Table 1). Consequently, individualisation of biological material from perforating bullets and thus identification of the victim can, in our experience, be reliably achieved because of the high sensitivity of STR analysis. A slot blot quantification will consume precious sample, therefore, it is not recommended in the cases of minute DNA extracts because the STRs applied are known to be human specific. The brownish small particles predominantly located at the base of a bullet can either be dried blood stains and/or gun powder residues. A presumptive test for blood (Kastle-Meyer) allows a good appraisal of the kind of the staining and the expected DNA yield.

In the third case the bullet fragment could be attributed beyond a reasonable doubt to have perforated one (victim A) of the two persons under question. This was achieved by the use of only two STR systems because person B could be excluded in both systems (Table 1). In general, the number and selection of the STR systems applied has to depend on the actual case and on the questions to be answered. In cases involving several possible victims a few systems will usually be sufficient for an exclusion. In the case of only one victim, the verification of DNA is already a strong indicator that this bullet perforated the victim, so that two or three matching STR systems are more than sufficient, even if the combined frequency is low compared to identification purposes. It is also worth mentioning that sufficient cells for STR analysis were present on the bullet fragment, although the violent impact and fragmentation most likely took place after perforation of the body, thus increasing the chances of cells becoming lost.

In the two intact bullets, the only particle visible under the macroscope was located at the base of bullet II. This again corroborates the assumption that cells are likely to collect at the base of FMJ bullets for aerodynamic reasons (Karger et al. 1996). So for recovery of DNA, the whole bullet and especially the base should be swabbed thoroughly. It should also be stressed that this sampling should be done early to prevent contamination or loss of biological material by improper handling or investigative procedures such as comparison of firing marks. This will not cause a delay in further investigations because the recovery can be done with a minimum of equipment outside the

Table 1 Typing results of 2 perforating bullets and 1 bullet fragment (KM: Kastle-Meyer test; Neg: negative; Pos: positive; –: not tested)

	KM	Slot blot (ng/µl DNA)	ACTBP2	TH01	VWA	FES	FGA	Combined frequency
Bullet I	Neg.	Neg.	No result	8, 9.3	18, 18	10A, 11	21, 22	1 in 26.500
Victim I	–	–	N24, N30	8, 9.3	18, 18	10A, 11	21, 22	
Bullet II	Pos.	0.5	N20, N28m	6, 9	14 (+4), 16	11, 12	25, 25	1 in 300 Mio
Victim II	–	–	N20, N28m	6, 9	14 (+4), 16	11, 12	25, 25	
Fragment	Pos.	0.3	7, 20	6, 9	–	–	–	1 in 1200
Victim A		–	7, 20	6, 9	–	–	–	
Victim B		–	5, 13	8, 9	–	–	–	Exclusion

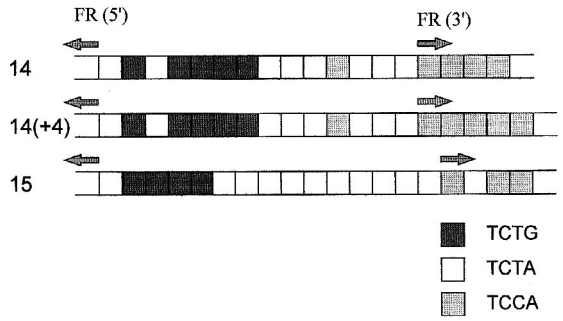

Fig. 1 Schematic representation of the tandem arrays of VWA alleles 14, 14 (+4) and 15. FR = flanking region, beginning marked by arrows. According to the nomenclature introduced by Urquhart et al. (1994) the TCCA repeats located in the 3′-direction of the tandem arrays belong to the flanking region and are not included in the allele nomenclature

laboratory within a short time. Since very minute quantities of DNA template are to be expected in the cases of FMJ bullets, extreme care must be taken while handling the evidence in order to avoid PCR artefacts such as differential amplification or additional bands. DNA typing of material on perforating bullets can be regarded as an additional method which can supplement conventional crime scene reconstruction in cases when it is necessary to know which victim was injured by which perforating bullet.

An interesting by-product of this investigation was the detection of a variant allele 14 at the VWA locus in one of the victims (victim II; a Turkish Caucasian individual). This allele migrated slightly faster in the native gel system used than consensus allele 15. Nucleotide sequencing of this allele revealed a different arrangement of TCTG- and TCTA repeats within the tandem array relative to the consensus allele 15, but a molecular structure nearly identical to VWA allele 14 (Möller et al. 1994). The only difference that could be observed between alleles 14 and the variant allele 14 (+4) was an additional TCCA repeat in the 3′-flanking region of the 14 (+4) allele (Fig. 1).

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