ANTHROPOLOGIE • XXXV/1 • pp. 91–96 • 1997

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IDENTIFICATION OF INDIVIDUALS BY POLYMERASE CHAIN REACTION AND COMPARISON OF THEIR BLOOD SAMPLES WITH BLOOD TRACES FOUND AT THE SCENE OF THE CRIME

ABSTRACT: The polymerase chain reaction (PCR) was used to compare blood traces found at the place where a dead woman had been discovered with the peripheral blood samples of 17 suspects. The comparisons, which took almost two years to complete, were based on three loci of the human genome: MCT 118 (chromosome 1), Apo B (chromosome 2) and YNZ 22 (chromosome 17). The results of the molecular biology tests indicate the accuracy of determinations using this set of highly polymorphic loci of human genomic DNA.

KEY WORDS: Polymerase chain reaction – Tandem repeats – Blood Traces

INTRODUCTION

In developed countries, the polymerase chain reaction has been used in forensic practices for almost ten years. In the Czech Republic, molecular biology methods started to be used to determine paternity or to identify persons after 1990. The human genome, which contains many regions which vary not only interindividually but also from population to population, is organized into genes and regions of non-coding DNA sequences. Eucaryotic cells contain about 100 times more DNA sequences which are non-coding for protein structures than DNA sequences which are coding for proteins. The non-coding regions contain the so-called Alu-repetitive sequences, representing 5 % of the total DNA. Hypervariable regions (VNTR) were also compared in the samples investigated in this study. The loci MCT 118, ApoB and YNZ 22 were studied on chromosomes 1, 2 and 17, respectively. VNTR loci show high variability, and when three VNTR-DNA loci are used in combination for making an identification, the results obtained are of a high informational value.

MATERIALS AND METHODS

DNA isolation

A. DNA samples were isolated from blood traces (minimum size 2×2 mm) found at the scene of the crime – V1, V2, V3, V4, V5 – and from the possessions of one suspect person – V6, V7.

Samples:
V1 – blood stain, size 1.0×0.4 cm (white cotton cloth)
V2 – blood stain, size 0.5×0.5 cm (white cotton cloth)
V3 – blood stain, size 0.5×1.0 cm (white cotton cloth)
V4 – blood stain, size 2.0×1.5 cm (white cotton cloth)
V5 – blood stain, size 5.0×5.5 cm (white cotton cloth)
V6 – blood stain, size 4.5×2.0 cm (colored cotton cloth)
V7 – blood stain, size 2.0×1.0 cm (colored cotton cloth)
V8 – dried blood from victim

In our extractions, we performed the DNA isolation methods on dry blood stains (Kobayashi *et al.* 1988, Kanter *et al.* 1986). DNA isolation was carried out using sterile

solutions and sterile material. PCR contamination was examined by positive and negative controls and an Amplitype system (Hoffmann-La Roche, USA).

B. Nucleic acid from 17 fresh blood samples K1–K17 were prepared using common methods (Maniatis *et al.* 1982).

Polymerase chain reaction (PCR)

The polymerase chain reaction was performed according to the Perkin Elmer Cetus (USA) protocol.
Chemicals (reaction volume 50 ml)
67 mM TRIS - HCl, pH = 8.8
2 mM MgCl2
250 mM dATP, dCTP, dGTP, dTTP
2 mg/ml bovine serum albumine
0.5 U Taq DNA polymerase

Primers, temperature regimen and number of cycles

Chromosome 2 (Apo B - locus)

primer sequences
5´ – ATggA AACgg AgAAA TTATg – 3´
5´ – CCTTC TCACT TggCA AATAC – 3´
PCR temperatures 94°C, 1 min
59°C, 1 min
72°C, 4 min
cycles 30

Chromosome 1 (MCT - 118 locus)

primer sequences
5´ - gAAAC TggCC TCCAA ACACT gCCCg CCg - 3´
5´ - gTCTT gTTgg AgATg CACgT gCCCC TTgC - 3´
PCR temperatures 94°C, 1 min
65°C, 1 min
72°C, 8 min
cycles 28

Chromosome 17 (p YNZ 22 locus)

primer sequences
5´-AAACT gCAgA gAgAA AggTC gAAgA gTgAA gTg -3´
5´- AAAgg ATCCC CCACA TCCgC TCCCC AAgT - 3´
PCR temperatures 94°C, 1 min
61°C, 1 min
72°C, 5 min
cycles 30

Electrophoresis

PCR products were electrophoretically separated after amplification on a 6% polyacrylamide gel (Budowle *et al.* 1991, Allen *et al.* 1989). Electrophoresis was performed for 2 hours at 100V.

RESULTS

As is evident from *Figure 1*, the spots found on the colored cotton tissues labeled V6 and V7 do not agree with the genotype found on a dried blood sample of the victim. On the other hand, *Figures 2, 3, 4* and *5* show that the blood traces from the white cotton tissues labeled V2 and V3 agree in their genotypes with the two samples of peripheral blood labeled K8 and K12. Sample V5 is identical in genotype with V7, but is not identical with any of the remaining samples tested in locus ApoB. When compared with the samples of peripheral blood from the suspects, sample V5 is identical with genotypes K1, K7, K13 and K16. Because of their small size, spots V1 and V4 were used only in further testing.

DNA amplification in VNTR - locus, Apo B (*Figures 1–5*, *Table 1*).

TABLE 1. Genotype differences of tested samples in Apo B - locus.

V 2	30–36		
V 3	30–36		
V 5	34–36		
V 6	36–36		
V 7	34–36		
V 8	34–34		
K 1	34–36	K 10	32–34
K 2	34–48	K 11	34–36
K 3	36–40	K 12	30–36
K 4	34–46	K 13	34–36
K 5	34–50	K 14	34–46
K 6	36–48	K 15	36–48
K 7	34–36	K 16	34–36
K 8	30–36	K 17	36–36
K 9	30–34		

DNA amplification in VNTR - locus, MCT 118 (*Figure 6, Table 2*)

TABLE 2. Genotype differences of tested samples in MCT 118 - locus.

V 1	24–26		
V 3	24–26		
V 4	24–26		
V 5	26–32		
K 8	24–26	K 13	24 -25
K 9	18–32	K 14	22–24
K 10	24–26	K 15	18–20
K 11	23–24	K 16	18–25
K 12	18–18	K 17	18–25

DNA amplification in VNTR - locus, p YNZ 22 (*Figure 7*, *Table 3*)

TABLE 3. Genotype differences of tested samples in p YNZ 22 - locus.

V 1	1–3
V 3	1–3
V 5	3–3
V 6	3–3
V 8	2–2
K 8	1–3

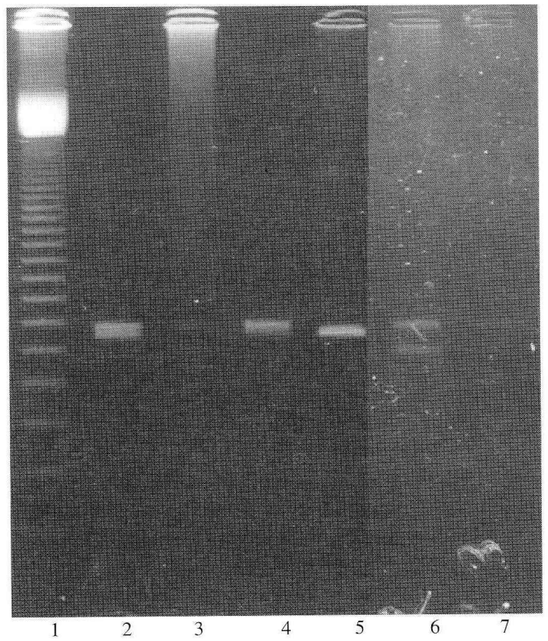


FIGURE 1. Apo B polymorphism.

1 – DNA marker 100 bp, 2 – sample V5, 3 – sample V6, 4 – sample V7, 5 – sample V8, 6 – sample V2, 7 – sample V3.

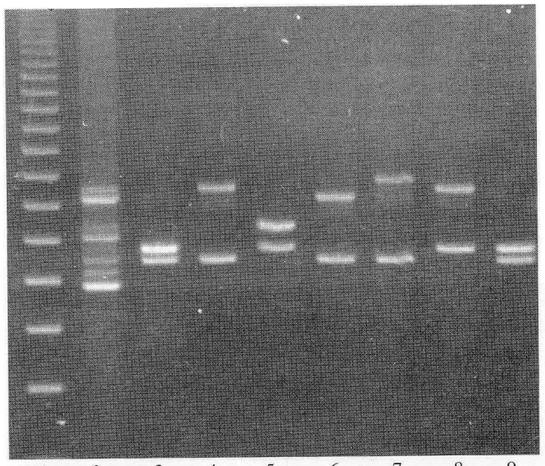


FIGURE 2. Apo B polymorphism.

1 – DNA marker 100 bp, 2 – Apo B alelles, 3–9 – DNA samples from fresh peripheral blood no. 1–7.

Table 2 shows that spots V1, V3 and V4 are identical in their genotype, and this is why they were used in the mixture as one sample (*Figure 6*) in order to provide more illustrative documentation. *Figure 6* and *Table 2* evidence that, as in the locus ApoB, the blood spots on the white cotton tissues labeled V1, V3 and V4 are identical in genotype with the sample of peripheral blood numbered K8, whereas, according to the findings related to locus p YNZ 22, the blood sample numbered K12 is to be excluded. Sample V5 is not identical to any of the peripheral blood samples tested in relation to this locus.

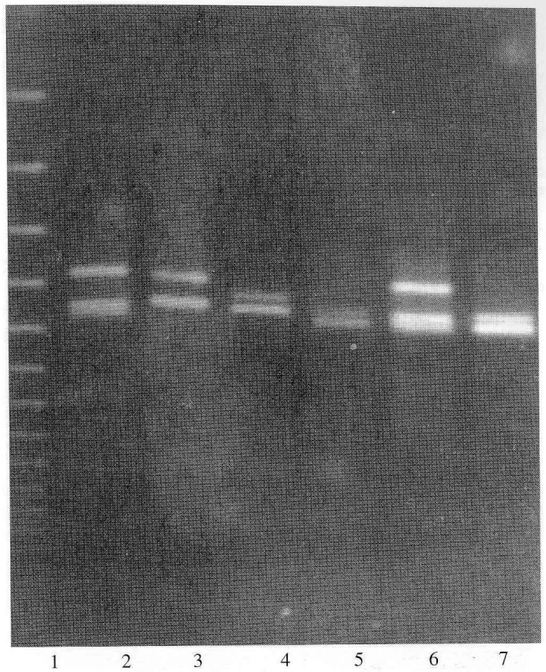


FIGURE 3. Apo B polymorphism.

1 – DNA marker 100 bp, 2–7 – DNA samples from fresh peripheral blood no. 8–13.

As can be seen from *Figure 7* and *Table 3*, blood traces V1 and V3 are identical in genotype to the peripheral blood sample of a suspect numbered K8. Samples V5 and V6 are differ in genotype with regard to either dried blood sample V8 or the peripheral blood sample of a suspect numbered K8. The latter is identical in genotype on locus pYNZ 22 to blood traces V1 and V3.

DISCUSSION

Even though methods of molecular genetics are being used in criminal investigations with increasing frequency, this case is of interest both for the high number of suspects and the variety of the isolation procedures used. Modifications of DNA separation from dried human peripheral blood (victim) and dried blood spots on textiles (traces from the scene of the crime and the subsequently-supplied clothes of the suspects) extensively saturated with either sweat or organic solvents (Kobayashi et al. 1988, Kanter *et al.* 1986, Maniatis *et al.* 1982, Prinz *et al.* 1990, Feddersen, VanNess 1989, Williams *et al.* 1987) were used.

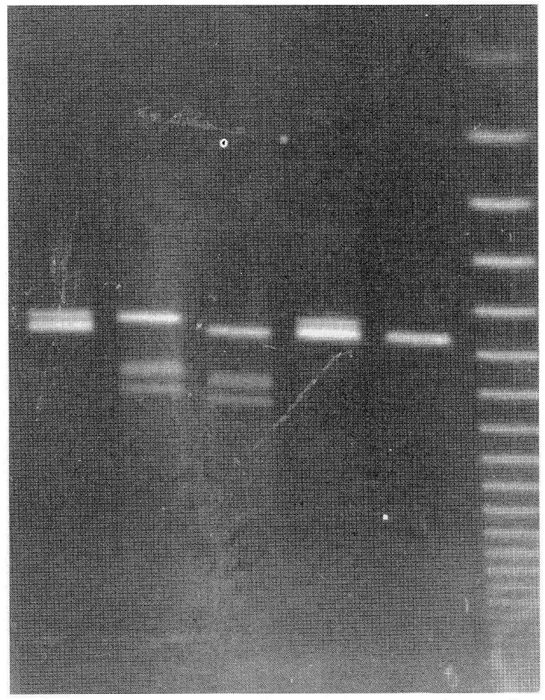


FIGURE 4. Apo B polymorphism.

1–5 – DNA samples from fresh peripheral blood no. 13–17
6 – DNA marker 100 bp

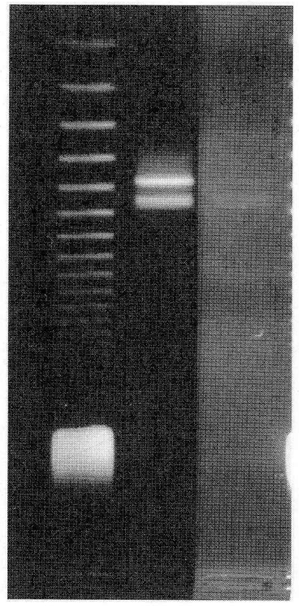
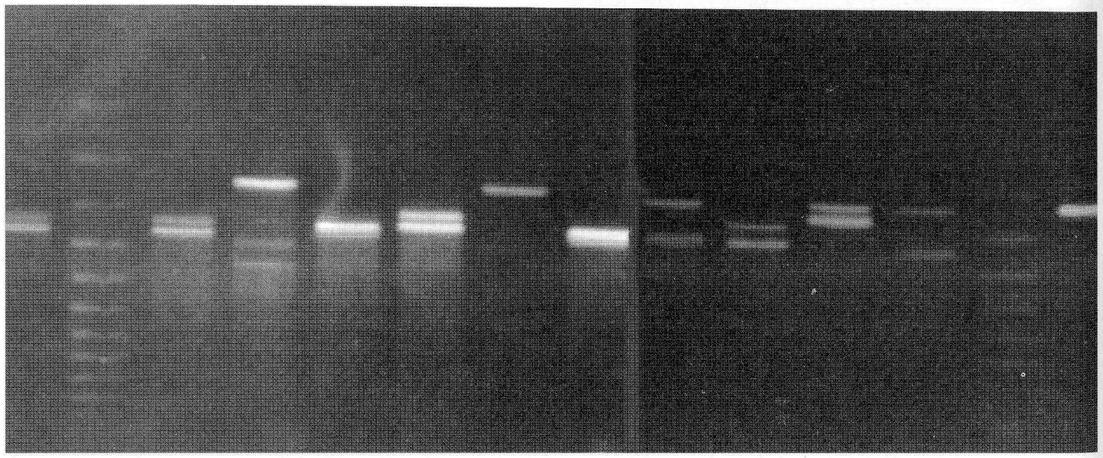


FIGURE 5. MCT 118 polymorphism.

1 – DNA marker 100 bp
2 – DNA sampleprepared from DNA samples (V2, 3)
3 – DNA sample from fresh peripheral blood no. 8

FIGURE 6. MCT 118 polymorphism.

1 – DNA sample prepared from DNA samples (V2, 3), 2 – DNA marker 100 bp, 3–12 – DNA samples from fresh peripheral blood no. 8–17, 13 – DNA marker 100 bp, 14 – DNA samples from fresh peripheral blood no. 12



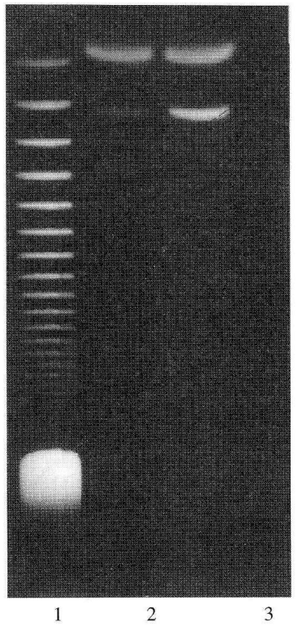


FIGURE 7. YNZ-22 polymorphism.

1 – DNA marker 100 bp
2 – DNA sample from fresh peripheral blood no. 8
3 – DNA sample prepared from DNA samples (V1, V3 and V4)

In addition, the blood spots studied were of very small size. It is also interesting to notice how the suspects' blood samples were gradually eliminated in the different steps of the investigation, i.e., those concerning the different VNTR loci. The VNTR schedule used, described as a frequently and successfully used combination in the investigation of serious crimes (Ludwig *et al.* 1989, Kasai *et al.* 1990, Higuchi *et al.* 1989, Jeffreys *et al.* 1988, Horn *et al.* 1989) also contributed in elucidating the facts of this case, which would likely have been impossible to solve using conventional serological procedures.

CONCLUSION

On the basis of the numerous data resulting from PCR analyses in VNTR regions ApoB, MCT 118 and YNZ 22, it is possible to state that one of the peripheral blood samples of the suspects was found identical in all VNTR loci studied on the dried blood samples on the white cotton tissue belonging to the victim. Since the probability of finding an individual among the population with the same genotype configuration on the same loci is estimated to be 3.3×10–6, i.e., 1 individual per more than 3 million people, it could be concluded that, given the current state of knowledge, the presence of the mentioned suspect at the scene of the crime may be considered as virtually proved.

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