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# Population data and forensic efficiency values for the STR systems HumVWA, HumMBP and HumFABP

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**Summary.** Population studies were carried out on Caucasians from north-west Germany using the short tandem repeat (STR) systems HumVWA (locus: 12p12-12pter), HumMBP (locus: 18q23-pter) and HumFABP (locus: 4q28-q31). After electrophoresis 9 alleles could be identified for HumVWA in a sample size of 321 unrelated individuals and 4 alleles were found for HumFABP in 106 individuals. For HumMBP-A 10 alleles and for HumMBP-B 7 alleles and 1 intermediate allele were determined in a sample size of 143 individuals. No deviations from Hardy-Weinberg equilibrium could be observed. In a small family study (HumVWA – $n = 129$; HumMBP – $n = 59$; HumFABP – $n = 48$) no new mutations could be found for HumMBP-A and HumFABP whereas 2 mutations were found in HumMBP-B and one mutation in HumVWA. Positive results could be obtained from 1 ng–20 pg (HumVWA, HumFABP) and 1 ng–100 pg (HumMBP) template DNA.

**Key words:** Short tandem repeats – HumVWA – HumMBP – HumFABP – Forensic validation – Population studies

**Zusammenfassung.** Populationsstichproben nordwestdeutscher Kaukasier wurden mit den 3 Short tandem repeat (STR)-Systemen HumVWA (Locus: 12p12-12pter), HumMBP (Locus: 18q23-pter) und HumFABP (Locus: 4q28-q31) untersucht. Nach gelelektrophoretischer Auftrennung konnten 9 Allele für HumVWA in einer Bevölkerungsstichprobe von 321 nicht verwandten Personen und 4 Allele für HumFABP in einer Stichprobe von 106 Individuen differenziert werden. 10 Allele für HumMBP-A und 7 Allele sowie ein Zwischenallel für HumMBP-B wurden in einer Bevölkerungsstichprobe von 143 nicht verwandten Personen gefunden. Eine Abweichung vom Hardy-Weinberg-Gleichgewicht wurde nicht festgestellt. Erste Familienstudien zeigten für HumMBP-A (Meiosen = 118) und HumFABP (Meiosen = 97) keine Neumutationen, während bei HumMBP-B (Meiosen = 118) 2 Mutationen und bei HumVWA (Meiosen = 258) 1 Mutation auftraten. Die Nachweisgrenze der STR's lag im Bereich von 1 ng–20 pg (HumVWA, HumFABP) bzw. 1 ng–100 pg (HumMBP) template-DNA.

**Schlüsselwörter:** Short tandem repeats – HumVWA – HumMBP – HumFABP – Forensische Validierung – Populationsstudien

## Introduction

During the last 2 years STRs have become increasingly important in forensic case work. At present they represent the most sensitive VNTR polymorphisms with a very low susceptibility to degradation (Brinkmann 1992; Wiegand et al. 1993).

The aim of the present study was to validate 3 further STR polymorphisms including optimization of PCR parameters, analysis of population and family samples, experimental investigations in stains together with some applications (DNA recommendations 1992).

### *Brief description of systems*

*HumVWA.* Von Willebrand factor, intron A. This is the first intron out of 3 in the whole gene. Chromosomal localization: 12p12-12pter. Repeat: TATA (Mercier et al. 1991; Kimpton et al. 1992).

*HumMBP.* Myelin basic protein. Using one pair of primers 2 intron polymorphisms are amplified. Chromosomal localization: 18q23-pter. Repeat: TGGA – locus A and B (Boylan et al. 1990; Polymeropoulos et al. 1992).

*HumFABP.* (= HUMFABP: Human intestinal fatty acid binding protein, intron B, Edwards et al. 1992). Chromosomal localization: 4q28-q31. Repeat: ATT.

## Materials and methods

DNA was extracted from blood samples as previously described (Brinkmann et al. 1991). Bloodstains were extracted with 150 µl

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chelex (Walsh et al. 1991) containing 50 µl proteinase K (2 mg/ml). Extraction of vaginal swabs was performed as described by Wiegand et al. (1992). The DNA concentration was determined by fluorimetry (Bontemps et al. 1975) and additionally in a 1% agarose gel by comparison with a known lambda standard (DRIgest III, Pharmacia) or known aliquots of the cell line K562.

### *PCR protocol*

*HumMBP:* 1 ng template DNA, 0.8 U Taq polymerase (ITC Biotechnology, Heidelberg, Germany), 1 µM each primer, 100 µM each nucleotide, 5 µl 10×PCR buffer (ITC, Heidelberg, Germany) diluted to a final concentration of 50 µl in 0.2 ml tubes (Perkin-Elmer).

*HumVWA:* 1 ng template DNA, 1 U Taq polymerase (Promega, USA), 1 µM each primer, 200 µM each nucleotide, 2 µl 10×PCR buffer (Promega, USA) diluted to a final concentration of 25 µl. The reaction assay was overlayed with 30 µl oil.

*HumFABP:* 1 ng template DNA, 1 U Taq polymerase (Promega, USA), 1 µM each primer, 100 µM each nucleotide, 2 µl 10×PCR buffer (Promega, USA) diluted to a final volume of 25 µl and overlayed with 30 µl oil.

PCR amplification was performed using negative (without DNA) and positive (cell line K562, laboratory control) control samples.

### *Primer sequences*

HumMBP (Polymeropoulos et al. 1992)

5'GAA CCT CGT GAA TTA CAA TC 3'
5'ATT TAC CTA CCT GTT CAT CC 3'

HumVWA (Kimpton et al. 1992)

5'CCC TAG TGG ATA AGA ATA ATC 3'
5'GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG 3'

HumFABP (Edwards et al. 1992)

5'GTA GTA TCA GTT TCA TAG GGT CAC C 3'
5'CAG TTC GTT TCC ATT GTC TGT CCG 3'

### *Amplification conditions*

HumMBP: 94°C – 10 s, 64°C – 10 s, 72°C – 1 s; 31 cycles (Perkin-Elmer 9600)

HumVWA: 94°C – 1 min, 50°C – 1 min, 72°C – 1.5 min; 30 cycles (Thermocycler: Biometra Triothermoblock, Germany)

HumFABP: 93°C – 1 min, 60°C – 1 min, 72°C – 1.5 min; 28 cycles (Thermocycler: Biometra Triothermoblock, Germany)

### *Gels (according to Allen et al. 1989)*

*HumMBP:* polyacrylamide (9% T, 3% C, 750 µm), piperazine diacrylamide as crosslinker, 84 mM formate, 18 cm separation distance, 2% agarose plugs in 2×tris/borate (0.5 M tris, 0.28 M borate pH9).

*HumVWA:* polyacrylamide (9% T, 3% C, 750 µm), piperazine diacrylamide as crosslinker, 126 mM formate, 10 M urea, 50 mM CHES (Cyclohexylaminoethane sulfonic acid). The gel solution was sterile filtered (pore sizes 0.45 µm) before the addition of ammonium peroxodisulfate (10%) and TEMED (N'N'N'N'-Tetramethylethylendiamine). Separation distance 20 cm, 2% agarose plugs in 2×tris/borate.

*HumFABP:* polyacrylamide (7.5% T, 3% C, 750 µm) piperazine diacrylamide as crosslinker, 140 mM formate, 10 M urea, 40 mM CHES. The gel solution was sterile filtered before the addition of ammonium peroxodisulfate (10%) and TEMED. Separation distance 20 cm, 2% agarose plugs in 2% tris/borate.

### *Electrophoresis*

*HumMBP:* Initial 1000 V, 40 mA and 5 W with ramping every 90 min up to 15 W which was continued until the bromophenol blue marker front had reached the anode. Bands were visualized by silver staining (Budowle et al. 1991).

*HumVWA and HumFABP:* Initial 1000 V, 40 mA and 5 W with ramping every 60 min up to 30 W which was continued until the bromophenol blue marker front had reached the anode. The gel was rinsed in 10% (v/v) ethanol/10% (v/v) acetic acid for 15 min to remove the urea from the gel. The silver staining was carried out as described.

### *Allelic ladders*

Initially preliminary allelic ladders were used composed of human alleles from each polymorphism. The designation is according to the number of repeats. The different ranges of the allelic ladders from the 3 STR systems are shown in Fig. 1.

The HumMBP ladder A has 8 alleles starting with allele 3, ladder B has 6 alleles starting with 7, HumVWA has 7 alleles starting with 8 and HumFABP contains 3 alleles starting with 10.

### *Statistics*

A total of 115 triplet families and 109 unrelated individuals were used for the population genetic analysis regarding the loci HumVWA, HumMBP-A, HumMBP-B and HumFABP.

Using the Family Analysis Package FAP (Seuchter et al. 1991) allele frequencies were the straightforward result of gene counting. In addition, on the basis of the available family data, it was possible to test the assumption of allelic independence regarding different loci. For the 4 loci, FAP assigns each individual to 4 locus haplotypes (the 4 alleles occurring in the same parental gamete) compatible with segregation and observed phenotypes. In the case

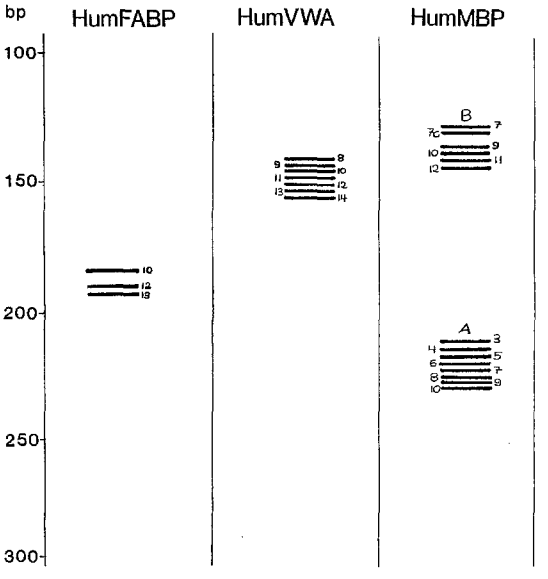


**Fig. 1.** Schematic representation of the previous allelic ladders used for the systems HumMBP, HumVWA and HumFABP

**Table 1.** Frequency values for HumVWA, HumFABP and HumMBP (literature see Fig. 2)

| HumVWA | | | HumFABP | | |
|---|---|---|---|---|---|
| Allele | Germany | UK | Allele | Germany | USA |
| 7 | 0.0047 | – | | | |
| 8 | 0.110 | 0.11 | 10 | 0.471 | 0.497 |
| 9 | 0.098 | 0.08 | 11 | 0.184 | 0.174 |
| 10 | 0.210 | 0.23 | 12 | – | 0.034 |
| 11 | 0.270 | 0.28 | 13 | 0.38 | 0.248 |
| 12 | 0.210 | 0.21 | 14 | – | 0.044 |
| 13 | 0.077 | 0.08 | 15 | 0.009 | – |
| 14 | 0.017 | 0.01 | | | |
| 15 | 0.0016 | – | | | |
| HumMBP-A | | | HumMBP-B | | |
| Allele | Germany | UK | Allele | Germany | USA |
| 2 | 0.003 | – | 7 | 0.374 | 0.26 |
| 3 | 0.066 | – | 7c | 0.003 | |
| 4 | 0.049 | 0.07 | 8 | | 0.01 |
| 5 | 0.28 | 0.32 | 9 | 0.038 | 0.13 |
| 6 | 0.01 | 0.19 | 10 | 0.073 | 0.14 |
| 7 | 0.035 | 0.08 | 11 | 0.339 | 0.25 |
| 8 | 0.472 | 0.20 | 12 | 0.164 | 0.21 |
| 9 | 0.052 | 0.04 | 13 | 0.007 | – |
| 10 | 0.028 | 0.10 | | | |
| 11 | 0.003 | – | | | |

of non-unique solutions all possibilities are taken into account in an iterative process leading to the maximum likelihood estimation of the four locus gametic frequencies. Estimated frequencies are then compared to the expectation under the hypothesis of independence, i.e. the product of the involved allele frequencies.

## Results

### *Allele frequencies*

Testing for population homogeneity revealed that only the population data (Table 1 and Fig. 2) of HumVWA showed no deviation ($P$=0.83) compared to other investigations. More striking differences can be seen for HumMBP and HumFABP possibly due to the small size of the US population. We also observed 3 more fragments in HumMBP-A (allele 1, 2, 11 – see Fig. 2b), one intermediate (allele 7c – see Fig. 2c) and one regular additional allele in HumMBP-B (allele 13 – see Fig. 2c).

In order to reach the necessary sample size for Hardy-Weinberg calculation, alleles associated with small numbers of observations were grouped together for HumMBP and HumVWA (Rand et al. 1992). No significant deviation from Hardy-Weinberg equilibrium was observed (Table 2).

### *Haplotype frequencies and linkage disequilibrium*

Table 3 shows the results of the two-locus haplotype frequencies for the loci HumMBP-A and HumMBP-B to-

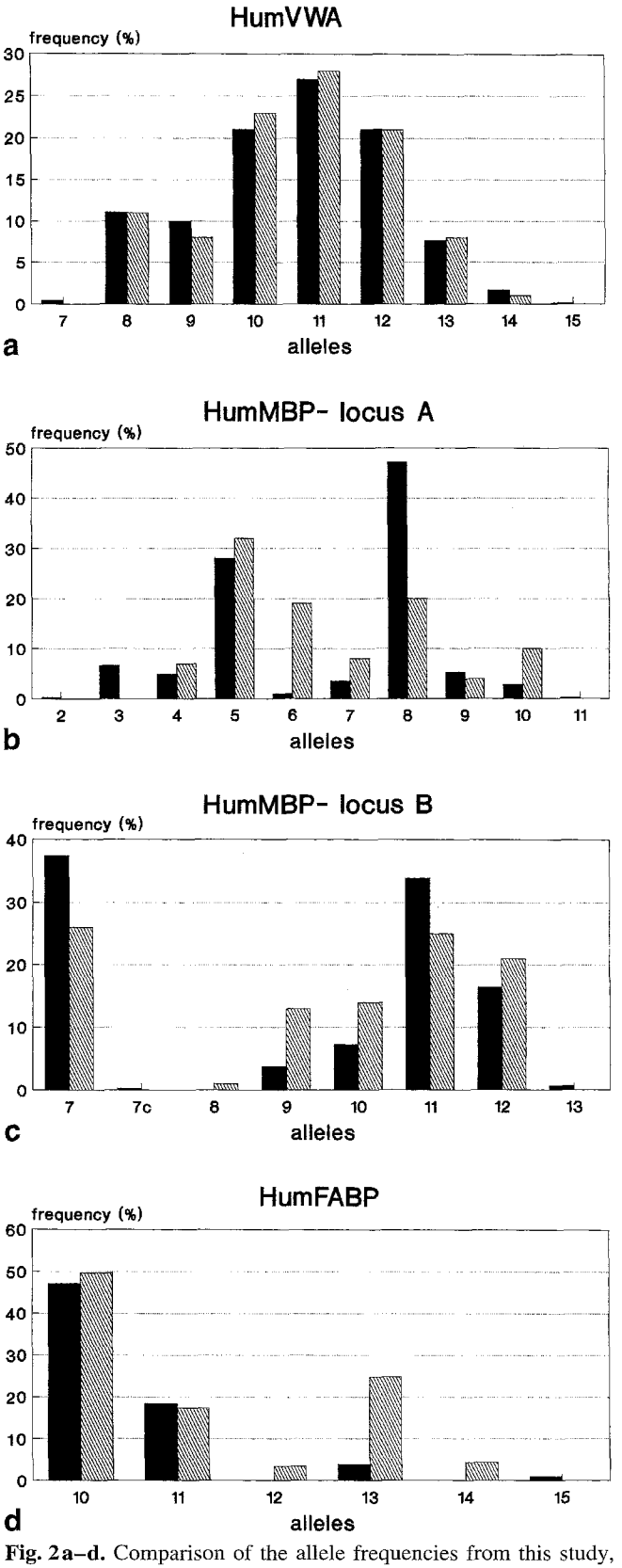


**Fig. 2a–d.** Comparison of the allele frequencies from this study, UK (HumVWA, Kimpton et al. 1992) and USA (Hum MBP-A, HumMBP-B, Polymeropoulos et al. 1992; HumFABP, Edwards et al. 1992) for HumVWA (**a**), HumMBP-A (**b**), HumMBP-B (**c**) and HumFABP (**d**). **a** ■ Germany ($n$= 321 ind.), ▨ UK ($n$=200 ind.); **b** ■ Germany ($n$=143 ind.), ▨ USA ($n$=36 ind.); **c** ■ Germany ($n$=143 ind.), ▨ USA ($n$=36 ind.); **d** ■ Germany ($n$= 106 ind.), ▨ USA ($n$=149 ind.)

**Table 2.** Chi²-test for deviations from Hardy-Weinberg equilibrium for HumVWA, HumMBP and HumFABP (according to Rand et al. 1992)

| Alleles (groups) | HumVWA | HumMBP-A | HumMBP-B | HumFABP |
|---|---|---|---|---|
| I | Allele 7,8,9 | Allele 2,3,4 | Allele 7 | Allele 10 |
| II | Allele 10 | Allele 5 | Allele 7c,8,9,10 | Allele 11 |
| III | Allele 11 | Allele 6,7 | Allele 11 | Allele 12 |
| IV | Allele 12 | Allele 8 | Allele 12,13 | Allele 13 |
| V | Allele 13,14,15 | Allele 9,10,11 | | |
| *df* | 14 | 14 | 9 | 9 |
| Chi | 7.17 | 11.58 | 1.19 | 10.06 |
| *P* | 0.9–0.95 | 0.6–0.7 | 0.995–0.999 | 0.3–0.4 |

**Table 3.** Estimated linkage disequilibria between markers HumMBP-A and HumMBP-B

| MBPB | MBPA | | | | | | | | | | SUM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | |
| 7 | 34 | 285 | 268 | 2703 | 101 | 17 | 218 | 34 | 0 | 0 | 3657 |
| | 9 | 27 | 133 | 1684 | 64 | –118 | –1512 | –164 | –110 | –12 | |
| | 0.21 | 0.06 | 0.57 | 0.95 | 1.00 | –0.88 | –0.87 | –0.83 | –1.00 | –1.00 | |
| | 0.1 | 0.1 | 6.4* | 181.4*** | 5.2* | 5.0* | 118.0*** | 6.7** | 5.3* | ... | |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 34 | 34 |
| | –0 | –2 | –1 | –9 | –0 | –1 | –16 | –2 | –1 | 33 | |
| | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | |
| | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | |
| 9 | 34 | 352 | 0 | 0 | 0 | 0 | 34 | 17 | 0 | 0 | 436 |
| | 31 | 321 | –16 | –121 | –4 | –16 | –172 | –7 | –13 | –1 | |
| | 0.48 | 0.79 | –1.00 | –1.00 | –1.00 | –1.00 | –0.84 | –0.29 | –1.00 | –1.00 | |
| | ... | 112.9*** | ... | 5.2* | ... | ... | 8.5** | 0.0 | ... | ... | |
| 10 | 0 | 50 | 101 | 0 | 0 | 318 | 244 | 38 | 0 | 0 | 751 |
| | –5 | –3 | 73 | –209 | –8 | 291 | –111 | –2 | –23 | –3 | |
| | –1.00 | –0.05 | 0.21 | –1.00 | –1.00 | 0.85 | –0.31 | –0.06 | –1.00 | –1.00 | |
| | ... | 0.0 | 6.4* | 9.3** | ... | 102.1*** | 2.1 | 0.0 | 0.7 | ... | |
| 11 | 0 | 17 | 0 | 67 | 0 | 0 | 2765 | 318 | 302 | 0 | 3468 |
| | –23 | –227 | –128 | –899 | –35 | –128 | 1124 | 131 | 197 | –12 | |
| | –1.00 | –0.93 | –1.00 | –0.93 | –1.00 | –1.00 | 0.62 | 0.37 | 1.00 | –1.00 | |
| | 1.00 | 10.4** | 6.0* | 53.0*** | 1.6 | 6.0* | 66.8*** | 4.4* | 17.4*** | ... | |
| 12 | 0 | 0 | 0 | 17 | 0 | 34 | 1470 | 67 | 0 | 0 | 1587 |
| | –11 | –112 | –58 | –425 | –16 | –25 | 719 | –19 | –48 | –5 | |
| | –1.00 | –1.00 | –1.00 | –0.96 | –1.00 | –0.43 | 0.86 | –0.22 | –1.00 | –1.00 | |
| | ... | 4.2* | 2.1 | 20.1*** | ... | 0.3 | 46.3*** | 0.1 | 1.7 | ... | |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 67 | 0 | 0 | 67 |
| | –0 | –5 | –2 | –19 | –1 | –2 | –32 | 63 | –2 | –0 | |
| | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | –1.00 | 1.00 | –1.00 | –1.00 | |
| | ... | ... | ... | 0.7 | ... | ... | 1.8 | ... | ... | ... | |
| SUM | 67 | 704 | 369 | 2786 | 101 | 369 | 4730 | 541 | 302 | 34 | 10000 |

1: haplotype frequencies/10000
2: delta/10000
3: relative delta
4: chi-square

* $P$=0.05; ** $P$=0.01; *** $P$=0.001
Sample size: 298

gether with the estimated linkage disequilibrium and a corresponding statistical test for its deviation from zero. Obviously extreme associations are present between the alleles of these two loci – for example A5 (allele 5, HumMBP-A) is almost exclusively present in B7 (allele 7, HumMBP-B) carrying gametes, stroy positive associations for A3 and B9, A7 and B10, A8 and B11, A8 and B12 and consequently numerous negative associations

**Table 4.** Forensic efficiency values for the STR systems HumVWA, HumMBP and HumFABP (discrimination index was calculated according to Sensabaugh 1982, mean exclusion chance according to Krüger et al. 1968)

| System | Heterozy. | Disc. ind. | Mean excl. ch. |
|---|---|---|---|
| HumVWA | 0.81 | 0.92 | 0.63 |
| HumMBP-A | 0.72 | 0.85 | 0.46 |
| HumMBP-B | 0.70 | 0.86 | 0.46 |
| HumFABP | 0.75 | 0.80 | 0.36 |

**Table 5.** Family studies and mutation rates for HumVWA, HumMBP-A, HumMBP-B and HumFABP

| System | HumVWA | HumMBP-A | HumMBP-B | HumFABP |
|---|---|---|---|---|
| Families | 129 | 59 | 59 | 48 |
| Meiosis | 258 | 118 | 118 | 97 |
| Mutation rates (%) | 0.3 | – | 1.8 | – |

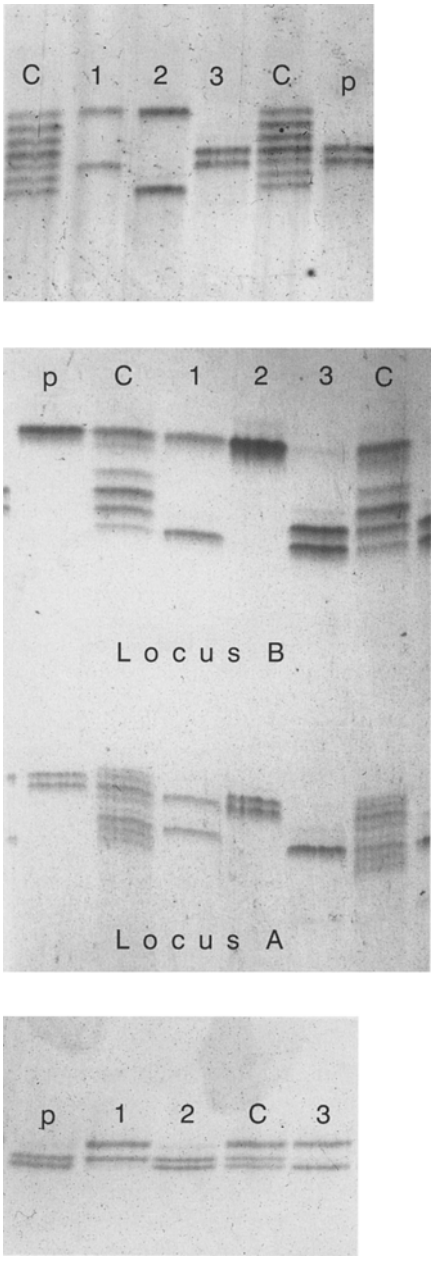


**Fig. 3a–c.** Amplification patterns from paternity cases for HumVWA (**a**), HumMBP (**b**) and HumFABP (**c**). For every STR system 1 ng template-DNA was amplified according to the described standard protocol. Positive (cell line K562) and negative (without DNA) control samples were included in each analysis. C: allelic ladder (preliminary); 1: child; 2: mother; 3: putative father; p: positive control

compensating for the positive deviations from independence.

In contrast to the results regarding HumMBP-A and HumMBP-B all other pairwise combinations of the 4 investigated loci showed no significant gametic associations. Estimated "haplotype frequencies" were in good accordance with expected frequencies resulting from the product of the involved gene frequencies.

As a consequence it is clear that loci HumVWA, HumFABP and one of HumMBP-A or HumMBP-B can be used independently for forensic analyses by multiplication of the involved allele frequencies. The simultaneous use of both HumMBP-A and HumMBP-B by way of multiplication is not permissible. If both loci are included the two locus haplotype frequencies in Table 3 would have to be used, but the gain in information would be minimal due to the extreme associations of the alleles of the 2 closely linked loci.

*Forensic efficiency*

Due to differences in the number of alleles and frequency of alleles the efficiency data show some variation (Table 4) but the single discrimination indices are always greater than 80% and the combined mean probability of exclusion reaches 93%.

*Family analyses*

Families which had been investigated by RFLP analyses were also tested using the 3 STR systems (Fig. 3a–c). The best results for successful amplification of human DNA were obtained with 1 ng template DNA. Regular segregations were observed in HumFABP (97 meioses) and HumMBP-A (118 meioses). In contrast, two mutations were observed in HumMBP-B whereas one mutation was found in HumVWA (Table 5). In order to verify this rate of new mutation the sample size for HumMBP and HumFABP should be enlarged.

*Forensic data*

The sensitivity of the 3 STR systems was studied in serial dilutions of the cell line K 562 as template and the following minimum amounts were established:

HumVWA, HumFABP: 20 pg
HumMBP: (31 PCR-cycles, hot start): 100 pg

Reproducible results could be obtained with less than 50 pg template-DNA dependent on the quality and quantity of the stain material.

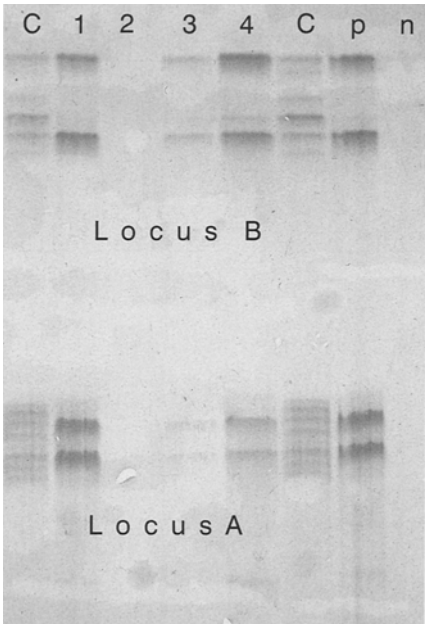


**Fig. 4.** STR system HumMBP. Amplification pattern from 5 hair shafts plucked from the back of the head (without roots) were incubated overnight at 56°C with ProK (0.230 mg/ml) and DTT (Dithiothreitol, 0.0188 M) following phenol/chloroform extraction and centricon 100 purification, 10 µl (25 pg) centricon eluate was used for amplification. Re-amplification was carried out with 1 µl of the PCR assay using 10 and 15 cycles. p: positive control; n: negative control; C: allelic ladder (preliminary); 1: positive sample (DNA was extracted from blood); 2: amplification pattern of DNA extracted from 5 hair shafts (standard protocol); 3: amplification pattern of DNA extracted from 5 hair shafts after reamplification (10 cycles); 4: amplification pattern of DNA extracted from 5 hair shafts after reamplification (15 cycles)

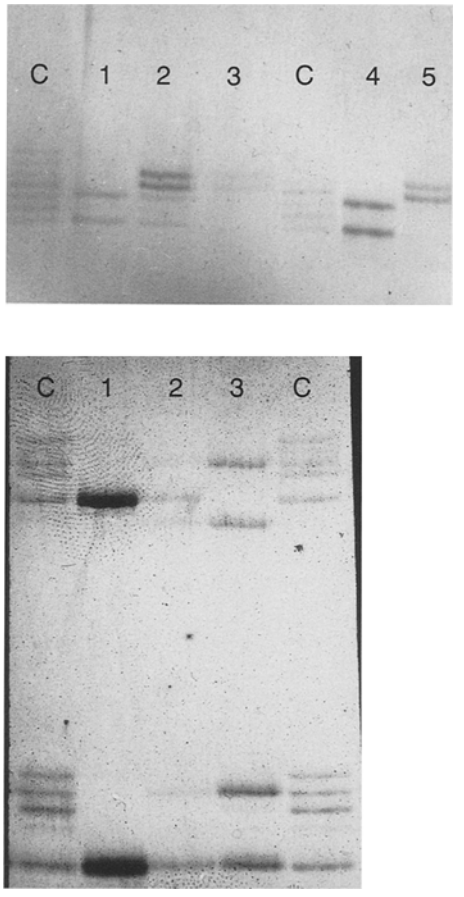


**Fig. 5a.** STR system HumVWA. Mixed stain analysis in a rape case (mild lysis extraction; Wiegand et al. 1992). The suspect was also injured by the victims fingernails. A microbloodstain (1×1 mm) from the suspect could be found on the brassiere. Each PCR was performed with 1 ng template DNA. Positive (K562) and negative controls reacted accordingly. C: allelic ladder (preliminary); 1: bloodstain on the brassiere; 2: mixed stain (vaginal swab) containing DNA from spermatozoa and vaginal cells; 3: supernatant from the mixed stain (lysed vaginal cells); 4: suspects pattern; 5: victims pattern. **b** STR system HumMBP. Rape case in which only highly degraded DNA could be extracted from the mixed stain (mild lysis extraction). Each PCR was performed with 1 ng template DNA. Positive (K562) and negative controls reacted accordingly. C: allelic ladder (preliminary); 1: suspects pattern; 2: mixed stain (spermatozoa/vaginal cells); 3: victims pattern (DNA was extracted from bone)

Experimental investigations were carried out to validate the forensic significance of the STR systems. DNA was extracted from different amounts of hair shafts without roots (1 hair = 5 cm, 5 hairs = 25 cm and 15 hairs = 75 cm) which had been plucked from the back of the head. A total of 5 out of 13 different DNA samples each extracted from 15 hair shafts could be successfully amplified with HumVWA. In contrast only one DNA sample extracted from 1 hair showed positive results. Clear amplification patterns were obtained with 5 hair shafts for HumMBP after reamplification (10 cycles) using 1 µl of the first PCR assay (Fig. 4).

In two rape cases we have successfully applied the systems HumVWA and HumMBP in addition to other PCR-VNTRs. The patterns allowed a differentiation between victim and perpetrator and the assignment of relevant bands to the perpetrator. In both cases the suspect' pattern could be found in the mixed stain pattern (Fig. 5a,b).

## Discussion

The populations investigated so far show a high degree of similarity with regards to HumVWA. This degree of correlation is only slightly less for HumFABP and is so because two rare alleles observed in the US survey were not detected in our population while another allele (No. 15) was additionally observed in our study. Discrepancies are much more pronounced in both HumMBP loci but these should only be discussed after enlargement of the sample size.

Additional alleles found in our study (HumMBP-A: 1, 2 and 11; HumMBP-B: 7c and 13) will be sequenced before finally designated.

The optimization of PCR parameters was especially effective for HumMBP which had previously shown ladder bands due to incorrect polymerisation (slippage effect). It could be demonstrated that "hot start" before the PCR reaction and reamplification (10 cycles) is very efficient even with small amounts of DNA (<100 pg). After optimization of the PCR conditions we no longer observed artefacts such as additional bands, ladder bands or allelic drop out.

**Table 6.** Combined discrimination index (DI) for the STR systems HumACTBP2 (SE33, Wiegand et al. 1993), HumTH01 (TC11, Wiegand et al. 1993), HumVWA, HumMBP and HumFABP

| | HumACTBP2 | HumTH01 | HumVWA | HumMBP-B | HumFABP | Comb. |
|---|---|---|---|---|---|---|
| DI | 0.99 | 0.91 | 0.91 | 0.86 | 0.80 | $2 \times 10^{-6}$ |

Denaturing PAA gel electrophoresis using 10M urea was effective for HumVWA because shadow bands were eliminated.

Experimental stains from blood, semen, hairs and urine as well as from mixed body fluids were used to forensically validate the STR systems. Studies with hair shafts showed that increasing the amount of hair shafts is paralleled with an increase of the success rate for PCR amplification. For each STR system and stain type 10–20 experimental stains were examined. Of special concern was the susceptibility to degradation. This criterion was closely associated to the fragment sizes under investigation.

The enlargement of our package of STR systems would lead to a higher discrimination index (Table 6). Due to the close association of HumMBP-A and HumMBP-B and the greater sensitivity, the calculation of the combined disrimination index (DI) was only performed with HumMBP-B.

Comparative studies show that the new generation of STRs are much more sensitive than AmpFLPs (Brinkmann 1993; Rand et al. 1992). Using STRs even highly degraded DNA extracted from old and little stain material could be successfully amplified whereas AmpFLPs gave no results. The evaluation of more than 100 cases shows a success rate of over 90% while the previous generation of AmpFLPs was associated with a rate of only 50–60% (Du Chesne et al. 1993; Brinkmann 1993).

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## References

Allen RC, Graves G, Budowle B (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. Biotechniques 7:736–744

Bontemps J, Houssier C, Fredericq E (1975) Physico-chemical study of the complexes of 33258 Hoechst with DNA and nucleohistone. Nucleic Acids Res 2:971–984

Boylan KB, Ayres M, Popko B, Takahashi N, Hood LE, Prusiner SB (1990) Repetitive DNA (TGGA) 5' to the human myelin basic protein gene: a new form of oligonucleotide repetititve sequence showing length polymorphism. Genomics 6:16–22

Brinkmann B (1993) Experiences using PCR analysis in forensic casework in Germany. Crime Lab Dig (in press)

Brinkmann B (1992) The use of STRs in stain analysis. In: Proceedings from the Third International Symposium on Human Identification. Promega Corporation, Madison, USA, pp 357–373

Brinkmann B, Rand S, Wiegand P (1991) Population and family data of RFLP's using selected single- and multi-locus systems. Int J Leg Med 104:81–86

Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC (1991) Analysis of the variable number of tandem repeats locus D1S80 by the polymerase chain reaction followed by high resolution polyacrylamide gel electrophoresis. Am J Hum Genet 48:137–144

DNA recommendations – 1992 report concerning recommendations of the DNA commission of the International Society of Forensic Haemogenetics relating to the use of PCR-based polymorphisms. Int J Leg Med 104:63–64

Du Chesne A, Rand S, Brinkmann B (1993) Spurenuntersuchungen mit DNA-Technologie – eine Retrospektivanalyse. Arch Kriminol (in press)

Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12: 241–253

Kimpton CP, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the vWF gene. Hum Mol Gen 1:28

Krüger J, Fuhrmann W, Lichte KH, Steffens C (1968) Zur Verwendung des Polymorphismus der sauren Erythrocytenphosphatase bei der Vaterschaftsbegutachtung. Dtsch Z Gerichtl Med 64:127–146

Mercier B, Gaucher C, Mazurier C (1991) Characterisation of 98 alleles in 105 unrelated individuals in the F8vWF gene. Nucleic Acids Res 19:4800

Polymeropoulos MH, Xiao H, Merril CR (1992) Tetranucleotide repeat polymorphism at the human myelin basic protein gene (MBP). Hum Mol Genet 1:658

Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B (192) Population genetics and forensic efficiency data of 4 AMPFLPs. Int J Leg Med 104:329–333

Sensabaugh GF (1982) Biochemical markers of individuality. In: Saferstein R (ed) Forensic science handbook. Prentice-Hall, Englewood Cliffs, pp 340–415

Seuchter SA, Neugebauer M, Baur MP (1991) User manual for FAP, version 2.0, Institute for Medical Statistics, University of Bonn, Germany

Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513

Wiegand P, Schürenkamp M, Schütte U (1992) DNA extraction from mixtures of body fluids using mild preferential lysis. Int J Leg Med 104:358–360

Wiegand P, Budowle B, Rand S, Brinkmann B (1993) Forensic validation of the STR systems SE33 and TC11. Int J Leg Med 105:315–320