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SHORT COMMUNICATION

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Validation of the STR system FES/FPS for forensic purposes in an Austrian population sample

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Abstract The short tandem repeat system FES/FPS was amplified by the polymerase chain reaction (PCR) in 211 unrelated Austrians and analysed by horizontal, non-denaturing electrophoresis. The allele distribution was in Hardy-Weinberg equilibrium. No mutations were found in 25 families (50 meioses). The mean exclusion chance was 0.49, the discriminating power 0.86 and the heterozygosity rate 74.4%. Amplification could be achieved with as little as 100 pg of high molecular weight DNA, which could be reduced to 75 pg by using 32 instead of 30 cycles. By reamplifying 1 µl for another 15 cycles, the threshold could be reduced to less than 20 pg. In a degradation experiment DNA extracted from bloodstains stored for up to 24 days in a moist chamber and DNA boiled for up to 18 min could be amplified.

Key words Short tandem repeat system · FES/FPS · Population study · Austria · Sensitivity

Introduction

A large number of short tandem repeat systems are available for forensic stain analysis and paternity testing [1]. These systems need to be further evaluated, not only to prove their suitability for forensic purposes, but also to select the most useful systems for improved inter-laboratory controls [2]. Whereas many studies have been published on some STRs, e.g. TH01 [3] and HumVWA [4], most systems have not yet been sufficiently evaluated. One of these systems is FES/FPS, located in intron V of the human c-fes/fps proto-oncogene on chromosome 15q25–qter [5]. The present study was performed to check the forensic usefulness of FES/FPS in an Austrian population sample.

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Materials and methods

Blood samples from 211 unrelated Austrians and 25 families confirmed by conventional systems and the STRs HumVWA and HumTH01 were extracted using the SuperQuickGene kit (AGTC, Denver, Conn.). For the sensitivity studies cell line DNA (K 562, Promega) was serially diluted to 10 pg/µl. For the DNA quality studies, DNA was degraded by boiling for 6–24 min, and the presence or absence of high-molecular-weight DNA was tested by agar gel electrophoresis followed by ethidium bromide staining. Additionally, bloodstains were kept in a moist chamber at room temperature for 1–40 days and afterwards stored at –80° C until the DNA was extracted using the Chelex 100 method [6].

PCR and non-denaturing electrophoresis were performed according to Möller et al. [7]. A sequenced allelic ladder was kindly donated by Professor B. Brinkmann, Münster.

Statistics

The mean exclusion chance (ME) was calculated according to Krüger et al. [8], and the discriminating power was calculated as 1-Σ (expected phenotype frequencies)^2 [9]. For checking the Hardy-Weinberg expectations, Chi-square tests were performed. Comparisons of the allele frequencies between different populations were done by Chi-square tests of R × C contingency tables, and alleles with less than 10 observations were pooled.

Results

A total of 21 genotypes (Table 1) corresponding to 5 alleles and 2 interalleles (10a,11a) were observed (Table 2). The ME was 0.49, and the discriminating power (DP) was 0.86. No deviation from Hardy-Weinberg expectations were found (Chi-square: 13.48, *df*: 14, P = 0.5–0.6). For comparison of the results of this study with those of other populations [10], the alleles 10/10a, and 11/11a were pooled, as the electrophoretic system used in the other study did not allow differentiation between them [7]. No differences were found from US Caucasians (Chi-square: 0.46, *df*: 3, P = 0.9–0.95), but there were significant differences from Asians (Chi-square: 28.04, *df*: 3, P < 0.0005), US blacks (Chi-square: 31.73, *df*: 4, P < 0.0005), and US Mexican Americans (Chi-square: 13.9, *df*: 3, P = 0.001–0.005).

Table 1 Allelic frequencies (%) found in this study (Austrians) and in that of Hammond et al. [10] for FES/FPS. The brackets indicate that the electrophoretic system used by Hammond's group could not distinguish between 10 and 10a or 11 and 11a

	Austrians $n = 422$	US Caucasians $n = 364$	US Blacks $n = 328$	US Mexican Americans $n = 318$	US Asians $n = 134$
7			0.3		
8	0.7	1.4	7.3	0.6	0.7
9			4.6	1.5	1.5
10a	23.5				
10	5.2	30.7	24.0	16.6	9.7
11a	2.2				
11	40.0	39.0	35.7	43.4	47.0
12	22.5	22.0	23.2	27.7	21.6
13	5.4	6.3	4.8	7.8	18.7
14		0.5		1.6	

Table 2 Genotype frequencies found in this study for the STR system FES/FPS

Genotype	No. observed
8, 10a	2
8, 12	1
10a, 10a	13
10a, 10	3
10a, 11a	2
10a, 11	43
10a, 12	17
10a, 13	3
10, 10	1
10, 11a	1
10, 11	8
10, 12	9
10, 13	1
11a, 11	5
11a, 13	1
11, 11	30
11, 12	44
11, 13	7
12, 12	9
12, 13	10
13, 13	1
Total	211

Family studies

FES/FPS was also tested in 25families (50 meioses) for whom results obtained with conventional systems and the STRs HumVWA and HumTH01 were also available. The segregations followed Mendelian inheritance and no mutations were found.

Sensitivity studies

K562 cell line DNA was diluted stepwise and amplified. Standard PCR as described yielded results down to approximately 100 pg template DNA. When 32, rather than 30, cycles were used the threshold could be lowered to 75 pg. When 1 ml of the PCR product was reamplified for 15 cycles, the detection limit was less than 20 pg.

In another experiment, previously typed DNA extracted from 10 experimental stains relevant for forensic purposes by the Chelex method (6 bloodstains, 1 semen stain, 1 cigarette end, 2 hair roots) were amplified at 50% and 25%, the DNA quantity that would have been necessary for successful typing without reamplification. On reamplification of 1 ml of the product from the first PCR for another 15 cycles, 9 of the 10 samples with 50% of the DNA amplified and could be typed correctly, but none with 25% of the DNA could be amplified. No allelic drop-out or ladder bands were observed. A 4-min hot start before the second PCR did not improve the results.

DNA quality studies

Typing of blood stains on white cotton was successful in the case of those kept for up to 26 days in a moist chamber at room temperature. For the boiled DNA, amplification could be achieved after up to 18 min of boiling.

Forensic case work

The STR system FES/FPS has been used in our laboratory in six criminal cases. A total of 42 trace stains (22 bloodstains, 7 hair roots, 1 handkerchief with nasal secretion, 2 semen stains, and debris from 10fingernails) that had been typed successfully with HumTH01 and HumVWA were amplified. Amplification and correct typing was possible in all cases.

Discussion

Non-denaturing electrophoresis made it possible to distinguish 2 sub-alleles of 10 and 11 repeat lengths; each variant (10 a and 11a) showed one single base substitution only [7]. This discrimination is not possible in denaturing gels. Therefore the ME and DP for our population are 0.49 and 0.86, respectively, while pooling of the subtypes (which should give the same results as for denaturing electrophoresis systems) resulted in slighly lower values (0.42 and 0.82). Despite this obvious advantage it must be noted that clear differentiation of the sub-alleles requires optimal running conditions. Minimal impairments (e.g. poor buffers, heat inhomogeneities, etc.) might lead to misinterpretations.

The STR system tested in this study proved to be sufficiently sensitive and resistant to degradation of DNA. The sensitivity was in the same range as that of STRs such as HumTH01 and ACTBP2 [3], while lower than VWA [4].

Reamplification could lower the threshold for amplification considerably. Although no allelic drop-out or extra bands were observed, these possible hazards need further investigation.

In conclusion, the STR system FES/FPS seems to be helpful in the analysis of biological traces for forensic

purposes. Nevertheless, as the ME and DP are distinctly lower for this system than those for other STRs, such as HumVWA and HumTH01 [11], and some AmpFLP systems [12], this system seems to be less suitable for paternity testing unless it is used as part of a multiplex system, as proposed for example by Lygo et al. [13]

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