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SHORT COMMUNICATION

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Fluorescence based co-amplification and automated detection of the STR loci HUMFIBRA and HUMD21S11 in a Hungarian Caucasian population sample

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Abstract Population data were generated for the STR systems HUMFIBRA and HUMD21S11 for a Hungarian Caucasian population sample residing in Baranya County, Hungary (127 unrelated individuals). The loci were co-amplified using a fluorescence based PCR method and were typed automatically. For both loci 12 different alleles could be found including some variants. No deviations from Hardy-Weinberg expectations were observed. Both loci proved to be highly discriminating and valuable polymorphisms for forensic analyses.

Key words Short tandem repeats · HUMFIBRA · HUMD21S11 · Hungary

Introduction

Numerous Hungarian Caucasian databases have already been generated for conventional and DNA polymorphisms [1–3], but there are no data for the STR loci HUMFIBRA and HUMD21S11 at present. The aim of this work was to set up databases for these polymorphisms in a Hungarian Caucasian population sample residing in Baranya County, in the south-west part of Hungary.

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Despite the high level of polymorphisms of both loci which may candidate the systems for inclusion into routine forensic applications only a few studies have been published in detail [4–7].

Materials and methods

Blood samples were obtained from 127 unrelated Hungarians. DNA was extracted using a phenol-chloroform method [8]. Briefly, bloodstains were incubated in 100 µl TNE buffer (10 mM Tris-HCl pH 7.6, 100 mM NaCl, 1 mM EDTA) at 70° C for 3 h. An equal volume of TE-saturated phenol (containing 0.1% 8-hydroxyquinoline) was added and the samples were mixed and centifuged. These steps were repeated three more times with the aqueous phase followed by a standard phenol-chloroform extraction and ethanol precipitation. DNA concentration was determined spectrophotometrically.

The HUMFIBRA [9] and HUMD21S11 [10] STR loci were coamplified using a modification of a previously described method [11].

The amplification cocktail consisted of 10 ng of DNA, 10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 1 U of Taq DNA polymerase (Promega Corporation, Madison, USA), 0.2 µM of each HUMFIBRA primer, 0.3 µM of each HUMD21S11 primer (the forward primers for both loci were labeled at the 5′-end with Texas Red) and 200 µM of each dNTP, in a total volume of 25 µl. The amplification conditions were (Mini-Cycler, MJ Research, Watertown, Mass.): 95° C for 3 min, followed by 28 cycles of 95° C for 45 s, 60° C for 30 s, 72° C for 30 s with a final extension step of 72° C for 6 min.

For electrophoresis 2 µl of PCR product was mixed with 2 µl of loading dye (83% formamide, 8.3 mM EDTA pH 8.0, 0.0025% methyl violet 6B). The samples were heat-denatured at 95° C for 3 min and 2 µl was loaded on a 6% Long Ranger gel (27 cm long and 0.3 mm thick) containing 6.1 M urea and 1.2 × TBE. The gels were run at 1000 V for 3 h on the SQ-5500-S DNA sequencer (Hitachi Electronics Engineering). Alleles were determined automatically by comparison with the allellic ladders generated previously using the Fluorescent Image Analysis Software FRAGLYS Version 2 (Hitachi Electronics Engineering). In order to create an allelic ladder, different alleles were sequenced on the SQ-5500-S DNA sequencer and reamplified. Allele designations of the HUMFIBRA [12] and HUMD21S11 [13] loci were based on the ISFH recommendations [14].

The Hardy-Weinberg equilibrium was tested using the exact test [15]. The forensic efficiency data were determined as published previously [16, 17]. For population comparisons we used a χ^2 method [18].

Table 1 Observed allele frequencies and forensic efficiency data for the two STR loci in 127 unrelated Hungarian Caucasian individuals (Hobs = observed hererozygosity; Pd = power of discrimination; PIC = polymorphic information content)

Allele		HUMFIBRA	Allele	HUMD21S11
18		0.004	27	0.035
19		0.13	28	0.126
20		0.098	29	0.224
21		0.169	30	0.236
22		0.205	30.2	0.407
22.2		0.008	31	0.087
23		0.138	31.2	0.106
23.2		0.004	32	0.012
24		0.146	32.3	0.083
25		0.075	33.2	0.016
26		0.016	34	0.024
27		0.008	34.2	0.004
H obs.	=	0.905		0.819
PD	=	0.96		0.96
PIC	=	0.84		0.831
Exact test	=	0.335		0.949

Results and discussion

Incomplete tetranucleotide repeat arrays were found in both systems. No significant deviations from Hardy-Weinberg expectation could be detected (Table 1).

The HUMD21S11 allele frequency distribution is similar to those of some other published European databases [4–6, 19]. No statistically significant differences could be found between Hungarians, two German populations [5, 19] (Hungarian-German [Münster]: $\chi^2 = 8.687$; df = 10; $0.6 < P < 0.5$; Hungarian-German [Frankfurt]: $\chi^2 = 5.364$, df = 9; $0.9 < P < 0.8$) and Italians [5] ($\chi^2 = 9.482$; df = 10; $0.5 < P < 0.4$). However, for the HUMFIBRA locus allele frequency data revealed significant differences between Hungarian and Portuguese [20] ($\chi^2 = 33.051$ df = 10; $P < 0.0005$) and between Hungarians and Japanese [21] ($\chi^2 = 94.66$; df = 8; $P < 0.0005$).

The allele frequency distributions for these STR systems can be used to estimate DNA profiles in Hungarian Caucasians.

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