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Parentage testing with 14 STR loci and population data for 5 STRs in the Slovenian population

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Abstract In order to apply a set of 14 short tandem repeat (STR) loci in parentage testing, we performed a population genetic study on a sample of 260 unrelated people from the Slovenian population. Genotypes for the 14 STRs were determined using three multiplex polymerase chain reactions (PCR) and automated fluorescent detection. The allele frequencies of the STR loci D5S818, D13S317, D7S820, D8S1179 and D18S51 showed no deviation from the Hardy-Weinberg equilibrium and agreed well with other Caucasian populations. We resolved a series of 181 parentage disputes of which 29 were exclusions. In all cases, evidence for exclusion was obtained by at least 4 informative STRs out of the 14 loci analysed. The 14 loci combined comprise a highly discriminating test suitable for paternity and identity testing in the Slovenian population, with an average estimated mutation rate of 1.2×10^{-3}, a combined calculated power of exclusion of 99.99974% and paternity index (PI) value of $>10^6$ in 72% of the inclusion cases and $>10^5$ in 91% of the inclusion cases.

Key words PCR · Short tandem repeats · STRs · Paternity testing · Population genetics · Slovenia

Introduction

The introduction of STR typing into routine paternity analysis is desirable as it will allow faster turnaround times, the use of smaller and more convenient sample types, and is amenable to automation. There are several published validations of STR loci for application in parentage testing (Alford et al. 1994; Mertens et al. 1997; Thomson et al. 1999). Here we present validation of a STR system consisting of the 14 STR loci D3S1358, HUMVWFA31/A, HUMFIBRA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, HUMTH01, HUMTPOX, HUMCSF1PO, HUMFES/FPS and HUMF13A1 for use in paternity investigations. With the number of alleles ranging from 6 to 15, these STR loci are known to be highly informative. Allele frequencies for five STR loci are presented and statistical parameters of forensic interest are calculated. The characteristics of the nine other STRs are as previously reported (Zupanič et al. 1998).

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Materials and methods

Genomic DNA was extracted from bloodstains and oral swabs using the chelex extraction procedure. The D5S818, D13S317, D7S820, D8S1179 and D18S51 loci were co-amplified with D3S1358, HUMVWFA31/A, HUMFIBRA and D21S11 using reagents provided in the AmpF*l*STR Profiler Plus kit (Perkin-Elmer/ABD). The HUMTH01, HUMTPOX and HUMCSF1PO loci were co-amplified with the X-Y homologous gene amelogenin using reagents provided in the AmpF*l*STR Green I kit (Perkin-Elmer/ABD). Both multiplex reactions were performed according to the manufacturer's instructions except that the PCR volume was 10 μl using 1–2 ng of template DNA. The HUMFES/FPS and HUMF13A1 loci were co-amplified in the duplex reaction according to Kimpton et al. (1993). The genotype data were determined by fluorescence-based automated detection on an ABI PRISM 310 Genetic Analyzer using the ABI PRISM GeneScan Analysis software, version 2.0.2 and Genotyper DNA fragment analysis software, version 2.0 (Perkin-Elmer/ABD). The allele designation for the loci D5S818, D13S317, D7S820, D8S1179 and D18S51 was achieved against the AmpF*l*STR Profiler Plus allelic ladders (Perkin-Elmer/ABD).

In order to assess the accordance of STR genotype configuration for a particular locus with the Hardy-Weinberg equilibrium (HWE) hypothesis, exact, χ^2 and G tests were performed using the HWE-Analysis software, version 3.2 (C. Puers, Institute for Legal Medicine, University of Münster, Germany). The determination of the mean exclusion chance (MEC), the mean exclusion probability (MEP), the polymorphism information content (PIC), the probability of match (pM), the discrimination power (D), and the heterozygosity were performed for each locus as indicators of their discrimination potential in human identification and paternity analysis applications using the same software. The frequency profile

Table 1 Distribution of the observed allele frequencies for five STR loci in the Slovenian population (*n* number of individuals)

Allele	D5S818 (*n* = 278)	D13S317 (*n* = 276)	D7S820 (*n* = 260)	D8S1179 (*n* = 263)	D18S51 (*n* = 259)
7	–	–	0.012	–	–
8	0.004	0.152	0.152	0.008	–
9	0.032	0.087	0.204	0.002	–
10	0.088	0.062	0.269	0.068	0.008
11	0.347	0.348	0.183	0.059	0.017
12	0.358	0.232	0.140	0.144	0.122
13	0.162	0.072	0.038	0.344	0.116
14	0.007	0.042	0.002	0.257	0.139
15	0.002	0.005	–	0.089	0.169
16	–	–	–	0.029	0.162
16.2	–	–	–	–	0.002
17	–	–	–	–	0.114
18	–	–	–	–	0.071
19	–	–	–	–	0.027
20	–	–	–	–	0.033
21	–	–	–	–	0.008
22	–	–	–	–	0.010
23	–	–	–	–	0.002
Minimum Frequency	0.005	0.005	0.006	0.006	0.006

comparisons between Slovenian and other European Caucasian populations were performed using a computer programme with R×C contingency tables kindly provided by G. Carmody, Carleton University, Ottawa, Canada. A *p*-value of less than 0.05 was considered significant. The power of exclusion for individual loci and for combined loci, paternity index and posterior probability of paternity were determined according to Weir (1996).

Results and discussion

The distributions of observed allele frequencies for five STR loci are shown in Table 1. The genotype frequency distributions showed no deviations from the HWE hypothesis based on the exact test, χ^2-test and G-test (data not shown). Quantitative comparisons of allele frequencies between this study and other population studies were performed (data not shown). The Slovenian population data for the five STR loci were compared with Italian Caucasians (Garofano et al. 1998), Swiss Caucasians (Gehrig et al. 1999), Spanish Caucasians (Entrala et al. 1998, 1999; Martin et al. 1998, 1999; Gene et al. 1998) and British Caucasians (Evett et al. 1997; Thomson et al. 1999). The allele frequencies for the STR loci D5S818, D13S317 and D7S820 were also compared with German population data (Hantschel et al. 1999) and the STR loci D8S1179 and D18S51 with French population data (Rousselet et al. 1997). The Slovenian population does not differ significantly from other European Caucasian populations. Deviations were observed only for the STR loci D7S820 and D8S1179 when comparing our data with that of the Italian population (Garofano et al. 1998) (D7S820: $\chi^2 = 24.62$, $p = 0.00$, G = 24.10, $p = 0.00$; D8S1179: $\chi^2 = 18.68$, $p = 0.02$, G = 20.66, $p = 0.01$). For D18S51 we found an intermediate allele 16.2, not previously found in Caucasians. The forensic efficiency values shown in Table 2 suggest that the five STR loci investigated in this study are very discriminating in the Slovenian population which, together with the nine other STR loci investigated previously (Zupanič et al. 1998) have a combined matching probability of 4.5×10^{-16}, a mean observed heterozygosity of 0.773 and a mean expected heterozygosity of 0.777.

Table 2 Statistical parameters of forensic interest for the five STR loci in the Slovenian population (H_{obs} observed heterozygosity, H_{exp} expected heterozygosity, *MEC* mean exclusion chance, *PIC* polymorphism information content, *D* discrimination power)

Locus	H_{obs}	H_{exp}±1.96 SE	MEC	PIC	D
D5S818	0.730	0.718±0.018	0.473	0.668	0.864
D13S317	0.808	0.785±0.019	0.591	0.755	0.920
D7S820	0.804	0.810±0.011	0.618	0.780	0.930
D8S1179	0.761	0.779±0.019	0.580	0.747	0.917
D18S51	0.846	0.878±0.008	0.750	0.864	0.970

Among the statistical parameters important for parentage testing the observed heterozygosity for the 14 STRs range from 60.98% for the HUMTPOX to 86.92% for the HUMFIBRA. The calculated power of exclusion ranged from 0.40 (HUMTPOX) to 0.76 (D18S51) and the experimentally observed power of exclusion from 0.41 (D5S818) to 0.79 (HUMFIBRA). The combined calculated power of exclusion for the 14 loci was estimated to be 99.99974%. We resolved 181 paternity disputes of which 29 turned out to be exclusions. Figure 1 shows the frequency distribution of the informative STRs in the 29 cases of excluded paternity, where the number of excluding STR loci varied from 4 to 12 and the experimentally observed mean number of excluding loci was 7.93. In 5 out of 152 cases of inclusion we observed apparent germ line mutation at 1 STR locus, and the 13 other STR loci did not exclude paternity and gave a paternity index (PI) of 4.2×10^7, 2.6×10^7,

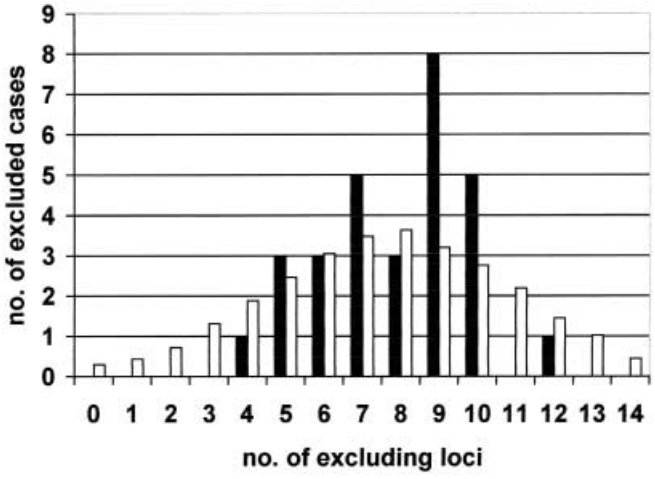

Fig. 1 Number of excluding STR loci for the 29 exclusion cases reported. The expected number of excluding loci was calculated by the method of Chakraborty and Schull (1976), mean 8.19, σ 3.24, ■ observed number of cases of exclusion, □ expected number of cases of exclusion

5.7×10^5, 3.9×10^5 and 1.3×10^4, respectively. In spite of high paternity index values, these five inclusions were subjected to further investigation and confirmed with five additional polymorphic markers (D3S1359, D12S391, SE33, D1S80 and ApoB) which did not exclude paternity in any of the five cases. In three cases we observed a mutation at locus HUMCSF1PO, in one case at locus HUMFES/FPS and in one case at locus HUMVWFA31/A. We have examined 304 meioses and mutation rates based on observed mutations can be estimated for HUMFES/FPS and HUMVWFA31/A as 3.3×10^{-3}, for HUMCSF1PO 9.8×10^{-3} and for all other loci $< 3.3\times10^{-3}$ (no mutations observed). Mutation rates between 0 and 7×10^{-3} per locus per gamete per generation have been reported for other STR loci (Brinkmann et al. 1998). We estimated an average mutation rate of 1.2×10^{-3} for all 14 loci analysed. Further investigation and data are required before reliable estimates of mutation rates for all these STR loci can be made.

The data from resolved parentage disputes were analysed to give information on the paternity index and posterior probability of paternity for the STR multiplex system consisting of 14 STR loci. Our results show that in the experimental data set the paternity index (PI) calculated for the alleged father of each matching trio was $> 10^6$ in 72% and $> 10^5$ in 91% of the inclusion cases. The probability of paternity (W) was > 99.9999% in 72% and > 99.999% in 91% of the inclusion cases. When only STR loci are used for paternity testing, typing using the 14 loci included in our assay seems to be sufficient to conclude that paternity is proven beyond reasonable doubt.

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