

ORIGINAL ARTICLE

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Analysis of nine short tandem repeat (STR) loci in the Slovenian population

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Abstract A polymerase chain reaction (PCR)-based short tandem repeat (STR) system consisting of nine loci has recently been introduced in Slovenia for use in routine forensic identity testing. Fluorescently labelled PCR products were analysed using an ABI PRISM 310 Genetic Analyzer. The STR loci analysed exhibit between 6 and 14 observed alleles per locus and have a combined matching probability of 2.3×10^{-10} .

Key words PCR · Short tandem repeats · Population genetics · Slovenia

Introduction

STR typing systems are at the forefront of the systems currently used by forensic scientists and are likely to remain so for the immediate future (Bär et al. 1997). Allele and genotype frequencies for nine STR loci were obtained from approximately 240 unrelated, healthy individuals randomly selected from the Slovenian population. A comparison between allele distributions from Slovenian and other European Caucasian population samples is presented and statistical parameters of forensic interest are calculated.

Materials and methods

Genomic DNA was extracted from bloodstains using the chelex extraction procedure. The HUMTH01 (Edwards et al. 1991), HUMVWFA31/A (Kimpton et al. 1992), HUMF13A1 (Polymeropoulos et al. 1991a), HUMFES/FPS (Polymeropoulos et al. 1991b), D21S11 (Sharma and Litt 1992), HUMFIBRA (Mills et al.

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

HUMTH01 (n = 239)		HUMVWFA31/A (n = 237)		HUMFES/FPS (n = 235)	
Allele	Frequency	Allele	Frequency	Allele	Frequency
5	0.002	14	0.110	8	0.017
6	0.272	15	0.120	9	0.002
7	0.119	16	0.181	10	0.317
8	0.115	17	0.281	11	0.413
9	0.207	18	0.224	12	0.194
9.3	0.274	19	0.065	13	0.055
10	0.010	20	0.019	14	0.002

HUMF13A1 (n = 236)		D21S11 (n = 210)		HUMFIBRA (n = 237)	
Allele	Frequency	Allele	Frequency	Allele	Frequency
3.2	0.133	26	0.005	16	0.002
4	0.023	27	0.043	18	0.015
5	0.167	28	0.160	19	0.078
6	0.273	29	0.207	20	0.156
7	0.337	30	0.224	21	0.162
8	0.002	30.2	0.050	21.2	0.006
12	0.002	31	0.098	22	0.177
13	0.006	31.2	0.064	22.2	0.017
14	0.021	32	0.026	23	0.110
15	0.015	32.2	0.093	23.2	0.011
16	0.015	33.2	0.029	24	0.154
17	0.004	34.2	0.002	25	0.084
				26	0.023
				27	0.004

D3S1358 (n = 237)		TPOX (n = 235)		CSF1PO (n = 237)	
Allele	Frequency	Allele	Frequency	Allele	Frequency
13	0.002	8	0.568	8	0.002
14	0.129	9	0.147	9	0.032
15	0.238	10	0.038	10	0.283
16	0.238	11	0.219	11	0.270
17	0.226	12	0.026	12	0.336
18	0.152	13	0.002	13	0.068
19	0.011			14	0.006
20	0.004			15	0.002

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Table 2 Observed and expected heterozygosity and p values of the statistical tests (exact test, χ^2 -test and G test of the HWE hypothesis)

	Observed heterozygosity	Expected heterozygosity ± 1.96 SE	Exact test (p)	χ^2 -test (p)	G test (p)
HUMTH01	0.7741	0.7821 ± 0.0124	0.2660	0.1112	0.1530
HUMVWFA31/A	0.8143	0.8089 ± 0.0134	0.5120	0.4214	0.4490
HUMF13A1	0.7542	0.7661 ± 0.0184	0.8744	0.9646	0.8964
HUMFES/FPS	0.6553	0.6897 ± 0.0203	0.0708	0.1724	0.1298
D21S11	0.8571	0.8554 ± 0.0136	0.8362	0.9346	0.8674
HUMFIBRA	0.8692	0.8695 ± 0.0087	0.4584	0.3708	0.4622
D3S1358	0.7679	0.7973 ± 0.0092	0.7658	0.6148	0.7844
TPOX	0.6298	0.6069 ± 0.0368	0.3590	0.2742	0.6122
CSF1PO	0.7532	0.7297 ± 0.0151	0.5386	0.2156	0.6652

1992), D3S1358 (Li et al. 1993), TPOX (Anker et al. 1992) and CSF1PO (Hammond et al. 1994) primer sequences were as described in the literature. The HUMTH01, HUMVWFA31/A, HUMF13A1 and HUMFES/FPS loci were coamplified in the quadruplex reaction according to Kimpton et al. (1993). The HUMFIBRA and D3S1358 loci were coamplified together with HUMVWFA31/A using reagents provided in the AmpFISTR Blue kit (Perkin-Elmer/ABD). The TPOX and CSF1PO loci were coamplified together with HUMTH01 and the X-Y homologous gene amelogenin using reagents provided in the AmpFISTR Green kit (Perkin-Elmer/ABD). Both multiplex reactions were performed according to the manufacturer's instructions. Amplification conditions for D21S11 were as described by Urquhart et al. (1995). 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 was achieved against the AmpFISTR Blue and AmpFISTR Green allelic ladders (Perkin-Elmer/ABD). For HUMF13A1, HUMFES/FPS and D21S11 loci, size windows were determined and final allele designation was performed according to known genotypes of the human cell line DNA 9947A.

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 comparisons between Slovenian and other European Caucasian populations were performed using a computer programme with $R \times C$ contingency tables kindly provided by G. Carmody, Carleton University, Ottawa, Canada.

Table 3 Comparison of allele frequencies for nine STR loci between the Slovenian population and other European Caucasian populations

Compared population	χ^2	p	G-statistic	p
HUMTH01				
SLO $\times$ CRO (Kubat et al. 1995)	4.722	0.603	5.351	0.583
SLO $\times$ I (Trabetti et al. 1995)	8.834	0.173	9.042	0.202
SLO $\times$ GB (Evetts et al. 1997)	13.375	0.054	14.118	0.052
SLO $\times$ PL (Pawlowski et al. 1997)	0.982	0.964	0.983	0.964
SLO $\times$ E (Lorente et al. 1997)	15.168	0.028	16.139	0.032
SLO $\times$ D (Günther and Patzelt 1996)	4.371	0.635	4.725	0.643
SLO $\times$ B (Mertens et al. 1997)	8.448	0.189	8.659	0.217
HUMVWFA31/A				
SLO $\times$ CRO (Kubat et al. 1995)	2.108	0.917	2.137	0.918
SLO $\times$ GB (Evetts et al. 1997)	7.129	0.526	7.619	0.517
SLO $\times$ PL (Pawlowski et al. 1997)	5.660	0.586	6.102	0.583
SLO $\times$ E (Lorente et al. 1997)	7.122	0.310	8.651	0.221
SLO $\times$ D (Günther and Patzelt 1996)	2.393	0.967	2.772	0.968
HUMF13A1				
SLO $\times$ E (Crespillo et al. 1997)	14.845	0.138	16.954	0.157
SLO $\times$ NL (Sjerps et al. 1995)	13.041	0.197	14.836	0.206
SLO $\times$ H (Füredi et al. 1997)	12.784	0.213	13.783	0.247
HUMFES/FPS				
SLO $\times$ I (Piccinini et al. 1996)	0.827	0.933	0.857	0.933
SLO $\times$ A (Klitschar 1995)	2.317	0.687	2.440	0.675
SLO $\times$ NL (Sjerps et al. 1995)	7.358	0.187	8.179	0.182
SLO $\times$ D (Günther and Patzelt 1996)	2.308	0.685	2.312	0.670
D21S11				
SLO $\times$ D (Piccinini et al. 1995)	8.362	0.773	9.376	0.756
SLO $\times$ I (Piccinini et al. 1995)	6.866	0.841	8.810	0.742
SLO $\times$ GB (Evetts et al. 1997)	10.606	0.643	10.987	0.690
SLO $\times$ F (Rousselet et al. 1997)	14.611	0.233	15.903	0.240
SLO $\times$ NL (Ovington et al. 1997)	7.467	0.702	8.009	0.697
HUMFIBRA				
SLO $\times$ I (Betti et al. 1995)	14.212	0.282	17.278	0.210
SLO $\times$ NL (Ovington et al. 1997)	4.733	0.980	5.162	0.980
SLO $\times$ GB (Evetts et al. 1997)	9.621	0.869	10.189	0.899
SLO $\times$ F (Rousselet et al. 1997)	5.978	0.966	6.476	0.968
SLO $\times$ P (Santos et al. 1996)	8.391	0.861	10.128	0.826
D3S1358				
SLO $\times$ P (Santos et al. 1996)	4.961	0.554	6.392	0.454
TPOX				
SLO $\times$ P (Santos et al. 1996)	3.251	0.519	3.244	0.526
SLO $\times$ E (Martin et al. 1995)	7.346	0.181	7.642	0.189
CSF1PO				
SLO $\times$ P (Santos et al. 1996)	4.663	0.630	5.318	0.638
SLO $\times$ E (Martin et al. 1995)	5.600	0.475	5.814	0.553
SLO $\times$ B (Mertens et al. 1997)	6.960	0.321	8.463	0.249

SLO-Slovenia, CRO-Croatia, I-Italy, GB-Great Britain, PL-Poland, E-Spain, D-Germany, B-Belgium, NL-Holland, H-Hungary, A-Austria, F-France, P-Portugal

Table 4 Statistical parameters of forensic interest for nine STR loci in the Slovenian population

	MEC	MEP	PIC	pM	D
HUMTH01	0.5684	0.5662	0.7456	0.0860	0.9140
HUMVWA31/A	0.6200	0.6157	0.7802	0.0670	0.9330
HUMF13A1	0.5513	0.5378	0.7286	0.0915	0.9085
HUMFES/FPS	0.4291	0.4126	0.6317	0.1534	0.8466
D21S11	0.7090	0.7056	0.8371	0.0398	0.9602
HUMFIBRA	0.7325	0.7336	0.8532	0.0352	0.9648
D3S1358	0.5920	0.5940	0.7637	0.0735	0.9265
TPOX	0.3650	0.2992	0.5569	0.2130	0.7870
CSF1PO	0.4816	0.4758	0.6789	0.1307	0.8693

Results and discussion

The distributions of observed allele frequencies for the nine STR loci are shown in Table 1. According to the statistical tests (Table 2), no deviations from the HWE hypothesis were detected. Quantitative comparisons of allele frequencies between this study and other population studies are shown in Table 3. The Slovenian population does not differ significantly from other European populations. Deviation was observed only for the HUMTH01 locus when comparing our data with that of the Spanish population (Lorente et al. 1997). The data support the concept that most European populations have similar allele distributions for these STR loci. The forensic efficiency values shown in Table 4 suggest that the STR loci investigated are very discriminating in the Slovenian population, with a combined discrimination power of 0.99999999977.

References

- Anker R, Steinbrueck T, Donis-Keller H (1992) Tetranucleotide repeat polymorphism at the human thyroid peroxidase (hTPO) locus. *Hum Mol Genet* 1:137
- Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA recommendations: further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. *Int J Legal Med* 110:175–176
- Betti F, Giacomazzo B, Ghio F, Piccinini A (1997) North Italian population genetic data on the STR system HumFGA. *Int J Legal Med* 110:110–111
- Crespillo M, Luque JA, Fernandez R, Ramirez E, Garcia P, Valverde JL (1997) Allele frequency distributions of 13 PCR-based systems in a population from North-East Spain. *Int J Legal Med* 110:223–225
- Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am J Hum Genet* 49:746–756
- Eveit IW, Gill PD, Lambert JA, Oldroyd N, Frazier R, Watson S, Panchal S, Connolly A, Kimpton C (1997) Statistical analysis of data for three British ethnic groups from a new STR multiplex. *Int J Legal Med* 110:5–9
- Füredi S, Woller J, Padar Z (1997) A population study of the STR loci HUMLPL, HUMF13B, and HUMF13A01 in Hungary. *Int J Legal Med* 110:107–108
- Günther S, Patzelt D (1996) Population data for the STR systems Hum TH01, HumVWA and FES/FPS in a population sample from Lower Franconia. *Int J Legal Med* 109:102–103
- Hammond HA, Jin L, Zhong Y, Caskey CT, Chakraborty R (1994) Evaluation of thirteen STR loci for use in personal identification applications. *Am J Hum Genet* 55:175–189
- Kimpton C, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the vWF gene. *Hum Mol Genet* 1:287
- Kimpton CP, Gill P, Walton A, Urquhart A, Millican ES, Adams M (1993) Automated DNA profiling employing multiplex amplification of short tandem repeat loci. *PCR Methods Appl* 3:13–22
- Klitsch M (1995) Validation of the STR system FES/FPS for forensic purposes in an Austrian population sample. *Int J Legal Med* 108:162–164
- Kubat M, Wiegand P, Brinkmann B (1995) Population genetic study from the Zagreb area using 3 STR systems. *Int J Legal Med* 107:219–221
- Li H, Schmidt L, Wei M, Hustad T, Lerman MI, Zbar B, Tory K (1993) Three tetranucleotide polymorphisms for loci: D3S1352; D3S1358; D3S1359. *Hum Mol Genet* 2:1327
- Lorente M, Lorente JA, Wilson MR, Budowle B, Villanueva E (1997) Spanish population data on seven loci: D1S80, D17S5, HUMTH01, HUMVWA, ACTBP2, D21S11 and HLA-DQA1. *Forensic Sci Int* 86:163–171
- Martin P, Alonso A, Budowle B, Albarran C, Garcia O, Sancho M (1995) Spanish population data on 7 tetrameric short tandem repeat loci. *Int J Legal Med* 108:145–149
- Mertens G, Mommers N, Heylen H, Gielis M, Muylle L, Vandenberghe A (1997) Allele frequencies of nine STR systems in the Flemish population and application in parentage testing. *Int J Legal Med* 110:177–180
- Mills KA, Even D, Murray SC (1992) Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). *Hum Mol Genet* 1:779
- Ovington A, Daselaar P, Sjerps M, Kloosterman A (1997) A Dutch population study of the STR loci D21S11 and HUMFIBRA. *Int J Legal Med* 110:14–17
- Pawlowski R, Maciejewska A, Paszkowska R, Wetz A (1997) Frequencies for five short tandem repeat (STR) systems in a population from North Poland. *Int J Legal Med* 110:10–13
- Piccinini A, Jüriling A, Junge A (1995) Short tandem repeat (STR) system HumD21S11: population genetic study on an Italian population. *Int J Legal Med* 108:165–166
- Piccinini A, Möller K, Wiegand P (1996) HumFES/FPS and HumF13B: population genetic data from North Italy. *Int J Legal Med* 108:283–284
- Polymeropoulos MH, Rath DS, Xiao H, Merrill CR (1991a) Tetranucleotide repeat polymorphism at the human coagulation factor XIII subunit gene (F13A1). *Nucleic Acids Res* 19:4306
- Polymeropoulos MH, Rath DS, Xiao H, Merrill CR (1991b) Tetranucleotide repeat polymorphism at the human c-fes/fps proto-oncogene (FES). *Nucleic Acids Res* 19:4018
- Rousselet F, Pfützinger H, Mangin P (1997) A pentaplex automated fluorescent typing system for forensic identification and French Caucasian population data. *J Forensic Sci* 42(3):500–503
- Santos SMM, Budowle B, Smerick JB, Keys KM, Moretti TR (1996) Portuguese population data on the six short tandem repeat loci – CSF1PO, TPOX, TH01, D3S1358, VWA and FGA. *Forensic Sci Int* 83:229–235
- Sharma V, Litt M (1992) Tetranucleotide repeat polymorphism at the D21S11 locus. *Hum Mol Genet* 1:67
- Sjerps M, van der Geest N, Pieron C, Gajadhar M, Kloosterman A (1995) A Dutch population study of the STR loci HUMTH01, HUMFES/FPS, HUMVWA31/1 and HUMF13A1, conducted for forensic purposes. *Int J Legal Med* 108:127–134
- Trabetti E, Galavotti R, Casartelli A, Magalini G, De Leo D, Pignatti PF (1995) Allele and genotype frequencies of eight DNA polymorphisms in the Italian population. *Mol Cell Probes* 9:183–188
- Urquhart A, Oldroyd NJ, Kimpton CP, Gill P (1995) Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. *Biotechniques* 18:116–121