Int J Legal Med (1999) 113 : 60–62

SHORT COMMUNICATION

F. Neuhuber · M. Radacher · N. Meisner
E. Tutsch-Bauer

Nine STR markers plus amelogenin (AmpFℓSTR Profiler Plus): a forensic study in an Austrian population

Received: 28 September 1998 / Received in revised form: 2 March 1999

Abstract Genetic efficiency data of nine short tandem repeat (STR) loci were determined by multiplex PCR using fluorescently labeled primers and subsequent analysis by capillary electrophoresis (ABI 310). For each locus 7–14 alleles were detected. The combined matching probability is about 1×10^{-11}. No deviations from Hardy-Weinberg equilibrium were observed.

Key words PCR · Multiplex · Short tandem repeats · Population study · Austria

Introduction

STR typing is the most powerful technique for individualization of biological stains and in addition is very well suited for the investigation of paternity cases. The technique of multiplexing is well established (Sparkes et al. 1996a,b; Evett et al. 1997), leads to faster results and less material is needed. Allele and genotype frequencies of nine STR loci (AmpFℓSTR Profiler Plus, Perkin Elmer) were determined in an Austrian population sample consisting of 194 unrelated Caucasian individuals. A comparison between populations was performed and forensically relevant parameters were calculated.

Materials and methods

Blood samples were taken from 194 unrelated individuals living in the Salzburg region of Austria. Genomic DNA was isolated from the samples using the Quiagen blood kit. The loci D3S1358 (Li et al. 1993), vWA (Kimpton et al. 1992), FGA (Mills et al. 1992), D8S1179 (Oldroyd et al. 1995), D21S11 (Sharma and Litt 1992), D18S51 (Urquhart et al. 1995), D5S818, D13S317 (Hudson et al. 1995), D7S820 (Green et al. 1991) and amelogenin (Sullivan et al.

F. Neuhuber (✉) · M. Radacher · N. Meisner · E. Tutsch-Bauer
Institute of Legal Medicine, Ignaz-Harrer-Str. 79,
A-5020 Salzburg, Austria
e-mail: franz.neuhuber@sbg.ac.at
Tel. +43-662-8044-3823; Fax +43-662-8044-3829

Table 1 Allele frequencies of the nine investigated STR markers in the Austrian population (n = 194 individuals)

D3S1358		VWA		FGA	
Allele	Frequency	Allele	Frequency	Allele	Frequency
11	0.003	11	0.005	18	0.005
13	0.003	14	0.113	19	0.072
14	0.131	15	0.080	20	0.148
15	0.201	16	0.242	21	0.181
16	0.247	17	0.253	21.2	0.008
17	0.211	18	0.222	22	0.184
18	0.180	19	0.072	22.2	0.013
19	0.021	20	0.010	23	0.158
20	0.003	21	0.003	23.2	0.003
				24	0.122
				25	0.059
				26	0.023
				27	0.021
				28	0.003
D8S1179		D21S11		D118S51	
8	0.026	26	0.008	10	0.005
9	0.021	27	0.010	11	0.013
10	0.085	28	0.168	12	0.147
11	0.075	29	0.191	13	0.157
12	0.142	30	0.226	14	0.170
13	0.301	30.2	0.049	15	0.174
14	0.234	31	0.072	16	0.101
15	0.090	31.2	0.106	17	0.090
16	0.023	32	0.018	18	0.064
17	0.003	32.2	0.108	19	0.041
		33.2	0.036	20	0.015
		34.2	0.008	21	0.010
				22	0.013
D5S818		D13S317		D7S820	
7	0.003	8	0.157	7	0.018
9	0.049	9	0.090	8	0.152
10	0.070	10	0.070	9	0.206
11	0.304	11	0.253	10	0.268
12	0.412	12	0.302	11	0.188
13	0.152	13	0.077	12	0.144
14	0.010	14	0.046	13	0,021
		15	0.005	14	0.003

Table 2 Observed (H. obs.) and expected (H. exp.) heterozygosities and *P* values of the exact test (HWE hypothesis) and genetic efficiency data of the nine STR loci

	H. obs	H. exp	*P*-value (exact test)	MEC	PIC	D
D3S1358	0.79	0.80 ± 0.056	0.84	0.606	0.773	0.931
VWA	0.75	0.81 ± 0.056	0.15	0.612	0.775	0.928
FGA	0.83	0.87 ± 0.048	0.83	0.718	0.848	0.964
D8S1179	0.86	0.81 ± 0.055	0.85	0.636	0.788	0.938
D21S11	0.80	0.85 ± 0.050	0.96	0.705	0.835	0.961
D18S51	0.85	0.87 ± 0.047	0.41	0.738	0.856	0.966
D5S818	0.76	0.71 ± 0.064	0.78	0.467	0.659	0.863
D13S317	0.83	0.80 ± 0.056	0.68	0.612	0.772	0.929
D7S820	0.83	0.81 ± 0.056	0.82	0.613	0.777	0.927

1993) were amplified in a single PCR reaction. Analysis of the fluorescently labeled amplified fragments was performed on an ABI 310 capillary electrophoresis instrument using GeneScan Analysis and Genotyper DNA fragment analysis software (Perkin Elmer). Allele designation was performed according to the AmpFℓSTR Profiler Plus user's manual (Perkin Elmer) and is in accordance with the DNA recommendations (Bär et al. 1997).

The mean exclusion chance (MEC) (Krüger et al. 1968), polymorphism information content (PIC) (Botstein et al. 1980) and the discrimination power (D) (Jones 1972) were determined using the computer programme HWE-Analysis, Version 3.1 (Christoph Puers, Institute of Legal Medicine, University of Münster). To test if the genotype distribution is in accordance with Hardy-Weinberg equilibrium (HWE) expectations, the same software was used performing the exact test (Guo and Thompson 1992).

A pairwise population comparison test (R × C contingency test; G. Carmody, Ottawa, Canada) was used to test for significant differences between the Austrian and other populations. The calculation of parentage indices was performed using the "Popstats 5.1" software kindly provided by the FBI.

Table 3 Pairwise population comparison test (R × C contingency table). Data from the Austrian population were compared to U.S. Caucasians and U.S. Blacks (AmpFℓSTR Profiler Plus user's manual)

		P	G statistic	*P*
A-US Cau				
D3S1358	14.22	0.075	15.29	0.096
VWA	9.97	0.233	10.78	0.264
FGA	23.82	0.041	27.43	0.029
D8S1179	8.85	0.452	8.90	0.494
D21S11	22.58	0.054	26.14	0.037
D18S51	8.73	0.828	9.38	0.818
D5S818	14.80	0.047	16.06	0.053
D13S317	8.04	0.422	8.45	0.454
D7S820	13.74	0.111	14.61	0.134
		P	G statistic	*P*
A-US black				
D3S1358	52.68	0.0	58.27	0.0
VWA	52.74	0.0	57.03	0.0
FGA	53.45	0.0	57.22	0.0
D8S1179	71.91	0.0	77.53	0.0
D21S11	63.90	0.0	76.78	0.0
D18S51	104.59	0.0	111.15	0.0
D5S818	41.60	0.0	49.97	0.0
D13S317	75.15	0.0	79.89	0.0
D7S820	29.31	0.0	32.96	0.0

Table 4 Parentage indices (PI) and number of exclusion constellations (# Excl.) in 57 paternity cases (*n* = number of paternity cases)

PI	*n*	%
100– 1000	0	0
1000– 10 000	11	31
10 000–100 000	13	36
> 100 000	12	33
#Excl.	***n***	**%**
< 4	0	0
4	3	14
5	9	43
6	4	19
7	2	10
8	3	14

Results and discussion

Allele frequencies of the nine STR loci investigated are shown in Table 1. No system showed a significant deviation from the HWE hypothesis (Table 2) and the statistical parameters of the loci are summarized in Table 2. A comparison of the Austrian population with a US Caucasian population and a US Black population showed only minor differences between Austrians and US Caucasians, whereas highly significant differences were found between Austrians and US blacks. The χ^2 and G statistic values with the corresponding *P* values are listed in Table 3.

A total of 57 paternity cases (36 inclusions, 21 exclusions) previously tested with 5 single locus probes (MS1, MS31, MS43a, g3 and YNH24) were retested using the Profiler Plus and none gave a parentage index (Pi) below 1400 with the highest value being 4.3×10^7. All exclusions were confirmed in at least four loci (Table 4). No new mutations were found for any locus.

In addition the Profiler Plus has been used for stain analysis in our laboratory over an 8-month period. It proved to be very robust and very sensitive and 90% of the stains that could be typed by singleplexing STRs gave a full profile at the first attempt. About half of the residual 10% could be typed successfully at a second or third attempt by increasing the amount of DNA for PCR. The rest gave partial profiles which showed a clear and simple correlation between the length of the fragments and PCR fail-

ure in the multiplex system. The Profiler Plus is now routinely used for stain cases.

The reported data suggest that the Profiler Plus is a very efficient STR multiplex system with a combined discrimination power of 99.99999999898% in the Austrian population.

References

Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA recommendations: further report of the DNA Comission of the ISFH regarding the use of short tandem repeat systems. Int J Legal Med 110:175–176

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Evett 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

Green ED, Mohr RM, Idol JR, Jones M, Buckingham JM, Deaven LL, Moyzis RK, Olson MV (1991) Systematic generation of sequence-tagged sites for physical mapping of human chromosomes: application to the mapping of human chromosome 7 using yeast artificial chromosomes. Genomics 11:548–564

Guo SW, Thompson EA (1992) Performing the exact test of Hardy Weinberg proportion for multiple alleles. Biometrics 48:361–372

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, Baptista R, Kruglyak L, Xu SH (1995) An STS based map of the human genome. Science 270:1945–1954

Jones DA (1972) Blood samples: probabilities an discriminations. J Forensic Sci Soc 12:355–359

Kimpton CP, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the vWF gene. Hum Mol Genet 1:287

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

Li H, Schmidt L, Wei M-H, Hustad T, Lerman MI, Zbar B, Tory K (1993) Three tetranucleotide polymorphisms for loci: D3S1352; D3S1358; D3S1359. Hum Mol Genet 2:1327

Mills KA, Even D, Murray JC (1992) Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum Mol Genet 1:779

Oldroyd NJ, Urquhart AJ, Kimpton CP, Millican ES, Watson SK, Downes T, Gill PD (1995) A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification. Electrophoresis 16:334–337

Sharma V, Litt M (1992) Tetranucleotide repeat polymorphism at the D21S11 locus. Hum Mol Genet 1:67

Sparkes R, Kimpton C, Watson S, Oldroyd N, Clayton T, Barnett L, Arnold J, Thompson C, Hale R, Chapman J, Urquhart A, Gill P (1996a) The validation of a 7-locus multiplex STR test for use in forensic casework: (I) mixtures, ageing, degradation and species studies. Int J Legal Med 109:186–194

Sparkes R, Kimpton C, Gilbard S, Carne P, Andersen J, Oldroyd N, Thomas D, Urquhart A, Gill P (1996b) The validation of a 7-locus multiplex STR test for use in forensic casework: (II) artefacts, casework studies and success rates. Int J Legal Med 109:195–204

Sullivan KM, Mannucci A, Kimpton CP, Gill P (1993) A rapid and quantitative sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechniques 15:636–641

Urquhart A, Oldroyd NJ, Kimpton CP, Gill P (1995) Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. Biotechniques 18:116–121