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A Korean population study of the nine STR loci FGA, VWA, D3S1358, D18S51, D21S11, D8S1179, D7S820, D13S317 and D5S818

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Abstract DNA typing was performed on 379 randomly selected unrelated Koreans using the nine short tandem repeat loci FGA, VWA, D3S1358, D18S51, D21S11, D8S1179, D7S820, D13S317 and D5S818 present in the AmpF*l*STR Profiler Plus PCR amplification kit. Allele frequencies, heterozygosity, power of discrimination, mean exclusion chance, and polymorphism information content of each locus were calculated by statistical analysis. All nine loci were in Hardy-Weinberg equilibrium. The combined discrimination index and the combined mean exclusion chance in Koreans was 2.31×10^{-12} and 0.99983, respectively. By evaluation of 297 children from 128 families, 2 mutations were found at the FGA locus and 1 each at the D18S51 and D13S317 loci. This study demonstrates that this multiplex system is a useful and convenient tool for forensic identification and parentage testing in Korea.

Key words STR · Allele frequency · Korea

Introduction

Analysis of short tandem repeat by the polymerase chain reaction (PCR) has had a remarkable impact in determination of biological relatedness between individuals (Alford et al. 1994) and in human identity test (Gill et al. 1994, 1995; Hammond et al. 1994). Currently, DNA typing after PCR amplification of short tandem repeat (STR) or variable numbers of tandem repeat (VNTR) loci is becoming the method of choice for forensic identification and paternity testing. Particularly STR loci are widely used due to the ease of amplification by PCR even in cases with degraded DNA. According to the vast progress in techniques, various multiplex PCR systems detectable with laser fluorescence have been developed and recently, a multiplex amplification kit for 12 loci became commercially available (Lazaruk et al. 1998). We used the AmpF*l*STR Profiler Plus PCR amplification Kit (PE Applied Biosystems, Foster City, Calif.) to amplify nine STR loci. In a single amplification tube this kit amplifies FGA, VWA, and D3S1358, all labelled with 5-FAM, D18S51, D21S11 and D8S1179, labelled with JOE, D7S820, D13S317 and D5S818, labelled with NED and amelogenin labelled with JOE. To introduce a new STR system, a population database for the relevant population must be established for statistical analysis of forensic cases. In this study, we studied the nine short tandem repeat (STR) loci present in the AmpFISTR Profiler Plus PCR amplification kit for allele frequency distributions and characteristics in a population of Koreans. In addition, we studied Korean families to investigate the genetic stability of the systems.

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Materials and methods

Sample preparation and PCR amplification

Buccal swab samples were obtained from 636 legally proven Korean family members and from these 379 unrelated individuals have been used to calculate the allele frequencies of the nine STR loci. DNA was extracted using standard proteinase-K digestion, phenol/chloroform extraction and ethanol precipitation. Each DNA was quantified with a spectrophotometer (DU-650, Beckmann, Westbury, NY). For PCR 2 ng of genomic DNA was amplified in a GeneAmp PCR system 9600 (PE Applied Biosystems) for 11 min preincubated at 95 °C, followed by 28 cycles of 1 min at 94 °C, 1 min at 59 °C, 1 min at 72 °C and a final extension for 45 min at 60 °C.

Electrophoresis and data analysis

Aliquots of 1 µl of PCR product were mixed with 0.5 µl of GeneScan-500 ROX (PE Applied Biosystems) size standard and 2.5 µl of deionized formamide. The samples were then denatured at 95 °C for 2–3 min and snap-cooled in an ice-water bath. Electrophoresis was carried out on a 4% polyacrylamide sequencing gel

Table 1 Allele frequencies for the nine STR loci in 379 unrelated Koreans (*MEC* mean exclusion chance, *PD* power of discrimination, *PIC* polymorphism information content)

Allele	FGA	vWA	D3S1358	D18S51	D21S11	D8S1179	D7S820	D13S317	D5S818
7				0.001			0.007	0.001	0.015
8						0.004	0.133	0.269	0.005
9				0.001		0.002	0.059	0.140	0.084
10						0.116	0.186	0.132	0.199
11				0.008		0.087	0.313	0.235	0.312
12			0.004	0.046		0.150	0.261	0.177	0.222
13			0.001	0.248		0.251	0.033	0.038	0.152
14		0.212	0.044	0.194		0.166	0.004	0.007	0.011
15		0.028	0.389	0.186		0.152	0.004	0.001	
16		0.169	0.274	0.079		0.062			
17	0.001	0.307	0.207	0.065		0.010			
18	0.032	0.186	0.078	0.057					
19	0.063	0.087	0.003	0.061					
20	0.050	0.011		0.026					
21	0.108			0.016					
21.2	0.001								
22	0.182			0.008					
22.2	0.004								
23	0.211			0.003					
23.2	0.007								
24	0.174								
24.2	0.005								
25	0.095			0.001					
25.2	0.003								
26	0.051								
26.2	0.001								
27	0.011				0.002				
28	0.001				0.042				
28.2					0.012				
29					0.215				
30					0.371				
30.2					0.009				
30.3					0.003				
31					0.117				
31.2					0.067				
32					0.018				
32.2					0.092				
33					0.003				
33.2					0.042				
34					0.003				
34.2					0.004				
Exact test for HWE	$p = 0.936$	$p = 0.575$	$p = 0.541$	$p = 0.543$	$p = 0.954$	$p = 0.579$	$p = 0.820$	$p = 0.659$	$p = 0.445$
Observed heterozygosity	0.871	0.787	0.728	0.813	0.805	0.807	0.752	0.815	0.768
Expected heterozygosity	0.861	0.789	0.722	0.846	0.785	0.839	0.777	0.803	0.783
MEC	0.723	0.586	0.482	0.697	0.603	0.678	0.568	0.609	0.576
PD	0.996	0.924	0.876	0.959	0.929	0.954	0.916	0.932	0.920
PIC	0.846	0.758	0.676	0.829	0.761	0.816	0.743	0.774	0.750

on an ABI 377 Genetic Analyzer (PE Applied Biosystems) for 2 h at a constant 3000 V with a fixed temperature of 51 °C. Fragment sizes were determined automatically using Genescan software ver 2.1 (PE Applied Biosystems), and typed by comparison with the allelic ladder using Genotyper software ver 2.1.

Statistical analysis

The frequency of each allele for each locus was calculated from the observed number of each genotype. To evaluate Hardy-Weinberg equilibrium (HWE), the exact test (Guo and Thompson 1992) was performed using the DNA-View program (Charles Brenner). The observed heterozygosity (obs-H) and the expected heterozygosity (exp-H) (Nei and Roychoudhury 1974), the polymorphism informa-

Table 2 Comparison of the discrimination indexes for the nine STR loci

	Discrimination index			Mean exclusion chance		
	African-American[a]	US Caucasian[a]	Korean	African-American[a]	US Caucasian[a]	Korean
FGA	0.035	0.036	0.004	0.720	0.717	0.723
VWA	0.058	0.065	0.076	0.639	0.617	0.586
D3S1358	0.102	0.078	0.124	0.526	0.580	0.482
D18S51	0.028	0.030	0.041	0.752	0.741	0.697
D21S11	0.033	0.045	0.071	0.728	0.684	0.603
D8S1179	0.075	0.067	0.046	0.593	0.613	0.678
D7S820	0.081	0.061	0.084	0.574	0.631	0.568
D13S317	0.131	0.074	0.068	0.473	0.595	0.609
D5S818	0.097	0.140	0.080	0.538	0.455	0.576
Combined	1.48×10^{-11}	1.04×10^{-11}	2.31×10^{-12}	0.99986	0.99988	0.99983

[a] AmpF*l*STR Profiler Plus PCR amplification kit user's manual

Table 3 The age distribution of the parents at the birth of the child

Father		Mother	
Age	No. of meioses	Age	No. of meioses
<19	0	<19	4
19–24	18	19–24	84
25–30	196	25–30	304
30–34	246	30–34	124
35–39	76	35–39	24
≥ 40	8	≥ 40	4
Unknown	50	Unknown	50
Total	594	Total	594

tion content (PIC) (Botstein et al. 1980), the mean exclusion chance (MEC) (Kruger et al. 1968), the power of discrimination (PD) and the discrimination index (DI) (Jones 1972) were also calculated.

Among the 636 individuals, 297 children from 128 families and their parents were analysed for possible mutations at each locus.

Results and discussion

Population data

The allele frequencies of the nine loci are shown in Table 1 and a total of 56 different genotypes and 18 alleles were found for FGA, 25 different genotypes and 7 alleles for VWA, 17 different genotypes and 8 alleles for D3S1358, 62 different genotypes and 16 alleles for D18S51, 47 different genotypes and 15 alleles for D21S11 and 35 different genotypes and 10 alleles for D8S1179. At the D21S11 locus, two 30.3 alleles were observed that have not yet been reported. A total of 27 different genotypes and 9 alleles for D7S820, a total of 26 genotypes and 9 alleles for D13S317, and a total of 26 different genotypes and 8 alleles for D5S818 were observed.

The heterozygosity and other parameters of forensic importance of each locus were calculated from allele frequencies (Table 1). The FGA, D18S51 and D8S1179 loci exhibited a higher mean exclusion chance and power of discrimination than the other six loci. From the exact test, all nine loci were found to be in Hardy-Weinberg equilibrium. We also calculated the combined discrimination index (DI) and combined mean exclusion chance (MEC) and compared these with data from African-American and American-Caucasian populations (PE Applied Biosystems) (Table 2). A smaller combined DI and a larger combined MEC in Koreans than in African-Americans and American-Caucasians means that this multiplex system has a higher forensic efficiency for the forensic identification and paternity testing for Koreans than other races. By comparison of the allele frequenies for FGA, D21S11 and VWA with other Korean population studies, no significant differences were found (data not shown).

Table 4 Mutations observed at the FGA, D18S51, D13S317 loci from 594 meiosis

Locus	FGA				D18S51		D13S317	
	Case 1		Case 2		Case 1		Case 1	
	Genotype	Age	Genotype	Age	Genotype	Age	Genotype	Age
Father	23–25	33	19–24	53	14–21	50	13–13	42
Child	24–24	4	25–25	20	13–15	44	10–13	39
Mother	19–24	30	22–25	50	13–20	20	12–13	16
Mutation	Paternal				Paternal		Paternal or maternal	
Mutation rate	0.00338				0.00168		0.00168	

Mutation rate

Samples from 297 children and their parents from 128 families were analysed and the age distribution of the parents at the birth of the children is shown in Table 3. The youngest father and mother were aged 28 and 27 years, respectively and the oldest father and mother were both 69 years of age. From 297 children, 2 mutations were found at the FGA locus and 1 mutation was found at each of the loci D18S51 and D13S317 (Table 4) and each mutation was found in a different family. We confirmed these mutations by sequencing both strands of the mutated and non-mutated alleles and the paternity by testing eight additional STRs, THO1, TPOX, CSF1PO, ACTBP2, F13A1, FES/FPS, D12S391, GABARB1 and two VNTRs D1S80, D17S5.

In conclusion, this multiplex system is a useful and convenient tool for forensic identification and paternity testing in the Korean population.

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