TECHNICAL NOTE

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Genetic Variations at Four Tetrameric Tandem Repeat Loci in Korean Population*

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ABSTRACT: Allele and genotype frequencies for four tetrameric short tandem repeat (STR) loci, HumFES/FPS, HumFOLP23, HumGABRB15, and HumCYAR04, have been determined by polymerase chain reaction (PCR) amplification and subsequent polyacrylamide gel electrophoresis from approximately 200 genetically unrelated Koreans. This method allows a single base pair resolution and rapid typing with silver staining. The allele and genotype distributions satisfy Hardy-Weinberg expectation. Also, these STR loci have proven to be useful for forensic analyses and paternity tests in which the variable number of tandem repeat (VNTR) loci have some limitations.

KEYWORDS: forensic science, DNA typing, FES/FPS, FOLP23, GABRB15, CYAR04, short tandem repeat, Hardy-Weinberg equilibrium, population genetics, Korea

The variability of microsatellite and minisatellite regions is so great that a few loci used in combination can be used to distinguish between any two individuals, except identical twins, by revealing a specific DNA fingerprint. This feature makes them valuable for forensic identification (1,2), paternity testing (3), and monitoring the effectiveness of bone marrow transplantation (4). The first hypervariable locus identified in humans was described by Wyman and White (5). Following this observation, similar hypervariable regions were identified flanking several structural loci, such as the human insulin gene (6,7), the ζ-globin pseudogene (8,9), the H-ras gene (10), and the myoglobin gene (11).

To analyze highly polymorphic variable number of tandem repeat (VNTR) regions, the amplified fragment length polymorphism (AmpFLP) technique has been used well in recent years for human identification. The VNTR locus most commonly used by forensic scientists is D1S80 (12–15). This VNTR locus contains repeat units 16 bp in size. In contrast, short tandem repeat (STR) loci consist of tandemly repeated sequences, between 2 and 6 base pair in length core units and which exhibit a high degree of polymorphism in the number of repeat units. The abundance of STR loci in the human genome provide a wide choice of loci (16) for identity testing. Trimeric and tetrameric STRs occur every 300 to 500 Kb on the human X chromosome and appear to be interspersed at this frequency throughout the genome (17). Furthermore, because of their small allele size (generally <300 bp), STR analysis is useful for old or poorly stored specimens that contain only degraded DNA (18,19). Also, the ability to resolve STR alleles differing in size by just 1 base on polyacrylamide gels allows more precise allele designation than for VNTR loci. Tri-, tetra-, and pentameric repeats, which have a wider allele spacing than dinucleotide size appear to be significantly less prone to slippage (i.e., stutter bands) and therefore, are more suitable for forensic identification (17). This paper describes data in the Korean population for four tetrameric STR loci.

Materials and Methods

Preparation of DNA

Whole blood samples were obtained from 200 unrelated Korean donors from the Red Cross National Blood Center. The red blood cells were lysed by washing twice with a red blood cell lysis buffer (10 mM Tris-Cl, pH 8.0, 10 mM NaCl, 5 mM $MgCl_2$). The remaining white blood cell pellets were incubated with lysis buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, 100 mM NaCl, 1% SDS) containing proteinase K (200 μg/mL) for 2 h at 56°C and then extracted twice with phenol/chloroform/isoamylalcohol (25:24:1). The DNA was precipitated by adding 2.5 volumes of absolute ethanol and then spooled out with a glass rod (20). The DNA pellets were air-dried and then dissolved in pure water. All the reagents for DNA preparation were obtained from Sigma Chemical Company, USA. The quantity of DNA was determined spectrophotometrically with Hoechst 33258 dye in a Hoefer DNA Fluorometer TKO 100 (Hoefer Scientific Instruments, San Francisco, CA). Standards of known quantity were used.

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TABLE 1—*Characteristics of STR markers used.**

Locus and STR	Accession No.	Chromosome	Allele No.†	Product Length (bp)†
HumFES/FPS (ATTT)n	X06292	15q25	8	211–235
HumFOLP23 (AAAM)n‡	J00145	6	8	160–184
HumGABRB15 (KATM)n‡	M59216	4p12–13	9	150–178
HumCYAR04 (TTTA)n	M30798	15q21.1	6	153–181

*Adopted from the report (21).
†Our study in Korean population.
‡K signifies G or T and M signifies A or C (21).

TABLE 2—*Primer sequences of STR markers.**

Locus	Primer sequence (5'->3')	Reference
HumFES/FPS	U:5'-GGGATTTCCCTATGGATTGG-3'	Polymeropoulos et al. (34)
	L:5'-GCGAAAGAATGAGACTACAT-3'	GenBank X06292
HumFOLP23	U:5'-ATTGTAAGACTTTTGGAGCCATTT-3'	Polymeropoulos et al. (35)
	L:5'-TTCAGGGAGAATGAGATGGGC-3'	GenBank J00145
HumGABRB15	U:5'-CTAGAAAGCTAGCAAGGTGGAT-3'	Dean et al.(36)
	L:5'-GCTCATTAAACACTGTGTTCCT-3'	GenBank M59216
HumCYAR04	U:5'-CTCTGGAAAACAACTCGACCCTTC-3'	Polymeropoulos et al. (37)
	L:5'-TGGGTGATAGAGTCAGAGCCTGTC-3'	GenBank M30798

*Adopted from the report (21).

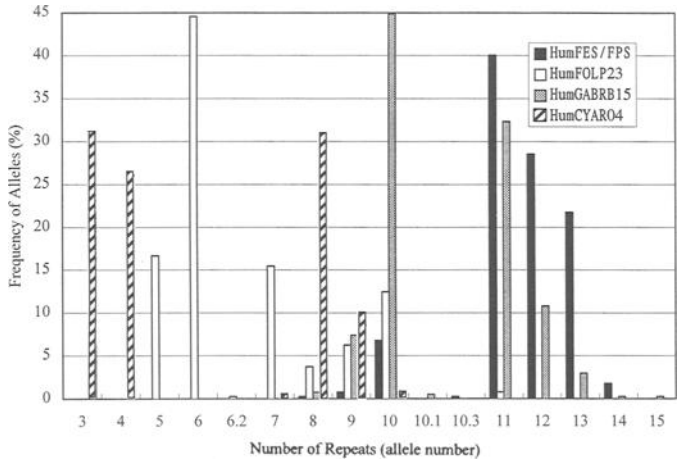

FIG. 1—*Relative allele frequency histograms of 4 STR loci studied. The data were generated from a minimum of 200 individuals from Korean population. The distributions plotted from the data presented in Table 3. Allele designation number is represented on the X axis. Allele frequency (%) is shown on the Y axis.*

TABLE 3—*Allele frequencies (%) in four STR loci in Korean population.*

	Locus			
Allele*	HumFES/FPS (n = 200)	HumFOLP23 (n = 201)	HumGABRB15 (n = 204)	HumCYAR04 (n = 202)
3	0.00	0.00	0.00	31.19
4	0.00	0.00	0.00	26.49
5	0.00	16.67	0.00	0.00
6	0.00	44.53	0.00	0.00
6.2†	0.00	0.25	0.00	0.00
7	0.00	15.42	0.00	0.50
8	0.25	3.73	0.74	30.94
9	0.75	6.22	7.35	10.15
10	6.75	12.44	44.85	0.74
10.1†	0.00	0.00	0.49	0.00
10.3†	0.25	0.00	0.00	0.00
11	40.00	0.75	32.35	0.00
12	28.50	0.00	10.78	0.00
13	21.75	0.00	2.94	0.00
14	1.75	0.00	0.25	0.00
15	0.00	0.00	0.25	0.00

*Alleic designation refers to the number of repeats of the core sequence motif indicated in the locus column.
†This is a nonconcensus allele that does not differ in size by the expected 4 bp from the adjacent alleles. n refers to the number of individuals sampled.

PCR Amplification for STR Typing

The STR loci in this study were HumFES/FPS, HumFOLP23, HumGABRB15, and HumCYAR04 (21) (Table 1). All oligonucleotide primers used were synthesized commercially by Korea Biotech and the nucleotide sequences are displayed in Table 2. Ten ng of each DNA sample were amplified in 0.2 mL thin-walled tubes (Perkin Elmer, Foster City, CA) in a total reaction volume of 20 μL consisting of 1X PCR buffer (50 mM KCl, 10 mM Tris-Cl, pH 8.3, 1.5 mM $MgCl_2$, 0.1% Triton X-100), 1 unit of Taq polymerase (Perkin Elmer, Foster City, CA), 200 μM of each dNTP, and 0.5 μM of each primer. Amplification was carried out with a Perkin Elmer GeneAmp PCR System 2400 Thermal Cycler (Perkin Elmer, Foster City, CA) under following conditions: (HumFES/FPS) 30 cycles at 95°C for 45 s, 54°C for 30 s, and 72°C for 30 s; (HumFOLP23, HumCYAR04) 30 cycles at 94°C for 45 s, 60°C for 30 s, and 72°C for 30 s; and (HumGABRB15) 28 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The allelic ladders were made by the method of Hochmeister et al. (22) by reamplification of an aliquot of a 10^5 or 10^6 dilution of the PCR reaction mixture containing the already amplified allelic DNA sample in this study.

TABLE 4—*Genotype frequency of the HumFES/FPS locus in Korean population (n = 200).*

Genotype	Observed (%)	Expected (%)
8–14	1 (0.50)	1.75 (0.00)
9–10	1 (0.50)	0.20 (0.10)
9–11	1 (0.50)	1.21 (0.60)
9–12	1 (0.50)	0.86 (0.43)
10–10	2 (1.00)	0.91 (0.46)
10–11	10 (5.00)	10.86 (5.43)
10.3–11	1 (0.50)	0.40 (0.20)
10–12	5 (2.50)	7.70 (3.85)
10–13	7 (3.50)	5.87 (2.94)
11–11	32 (16.00)	32.40 (16.20)
11–12	43 (21.50)	45.89 (22.94)
11–13	39 (19.50)	35.20 (17.60)
11–14	2 (1.00)	2.40 (1.20)
12–12	22 (11.00)	16.24 (8.12)
12–13	20 (10.00)	24.80 (12.40)
12–14	1 (0.50)	2.00 (1.00)
13–13	9 (4.50)	9.46 (4.73)
13–14	3 (1.50)	1.52 (0.76)

TABLE 5—*Genotype frequency of the HumFOLP23 in Korean population (n = 201).*

Genotype	Observed (%)	Expected (%)
5–5	7 (3.48)	5.58 (2.78)
5–6	31 (15.42)	29.84 (14.84)
5–7	10 (4.98)	10.33 (5.14)
5–8	3 (1.49)	2.50 (1.24)
5–9	4 (1.99)	4.17 (2.07)
5–10	4 (1.99)	8.33 (4.15)
5–11	1 (0.50)	0.50 (0.25)
6–6	38 (18.91)	39.85 (19.83)
6–7	28 (13.93)	27.76 (13.81)
6–8	7 (3.48)	6.71 (3.34)
6–9	10 (4.98)	11.19 (5.57)
6–10	25 (12.44)	22.26 (11.08)
6–11	1 (0.50)	1.34 (0.67)
6.2–7	1 (0.50)	0.15 (0.08)
7–7	2 (1.00)	4.78 (2.38)
7–8	2 (1.00)	2.31 (1.15)
7–9	7 (3.48)	3.86 (1.91)
7–10	10 (4.98)	7.71 (3.83)
8–9	2 (1.00)	0.93 (0.46)
8–10	1 (0.50)	1.87 (0.93)
9–10	3 (1.49)	3.11 (1.55)
10–10	3 (1.49)	3.11 (1.55)
10–11	1 (0.50)	0.37 (0.19)

TABLE 6—*Genotype frequency of the HumGABRB15 locus in Korean population (n = 204).*

Genotype	Observed (%)	Expected (%)
8–10	3 (1.47)	1.35 (0.66)
9–9	1 (0.49)	1.10 (0.54)
9–10	17 (8.33)	13.67 (6.70)
9–11	7 (3.43)	9.70 (4.76)
9–12	4 (1.96)	3.24 (1.59)
10–10	35 (17.15)	41.04 (20.12)
10–11	67 (31.37)	59.21 (29.02)
10.1–11	2 (0.98)	0.65 (0.32)
10–12	19 (9.31)	19.74 (9.67)
10–13	6 (2.94)	5.38 (2.64)
10–15	1 (0.49)	0.45 (0.22)
11–11	19 (9.31)	21.35 (10.47)
11–12	14 (6.86)	14.24 (6.98)
11–13	3 (1.47)	3.88 (1.90)
11–14	1 (0.49)	0.32 (0.16)
12–12	2 (0.98)	2.37 (1.16)
12–13	3 (1.47)	1.29 (0.63)

TABLE 7—*Genotype frequency of the HumCYAR04 in Korean population (n = 202).*

Genotype	Observed (%)	Expected (%)
3–3	20 (9.90)	19.65 (9.73)
3–4	33 (16.34)	33.37 (16.52)
3–7	1 (0.50)	0.62 (0.31)
3–8	40 (19.80)	38.99 (19.30)
3–9	12 (5.94)	12.79 (6.33)
4–4	16 (7.92)	14.17 (7.01)
4–8	32 (15.84)	33.11 (16.39)
4–9	9 (4.50)	10.86 (5.38)
4–10	1 (0.50)	0.79 (0.39)
7–9	1 (0.50)	0.20 (0.10)
8–8	21 (10.40)	19.34 (9.57)
8–9	10 (4.95)	12.69 (6.28)
8–10	1 (0.50)	0.93 (0.46)
9–9	4 (1.98)	2.08 (1.03)
9–10	1 (0.50)	0.30 (0.15)

Gel Electrophoresis and Detection

Amplified DNA fragments were separated in 7%T 4%C (HumFES/FPS) and 7%T 5%C (HumFOLP23, HumGABRB15, and HumCYAR04) polyacrylamide gels (42 by 32 by 0.1 cm). Electrophoresis was carried out in 0.28 M Tris-borate (pH 9.0) buffer for 5 to 6 h, with the voltage progressively being increased from 500 V to 1000 V. DNA fragments were visualized by the silver staining method described by Budowle et al. (13). All the reagents for electrophoresis were obtained from Sigma Chemical Company, USA.

Genotyping

Allele and genotype assignments were accomplished by side-by-side comparison with an allelic ladder, composed of amplified samples of known genotype. Phenotypes including apparent homozygotes were assumed to be the same as the genotype. Allele designations, based on the number of repeat units, were determined by comparison of PCR fragments with those of an allelic ladder. All designations were made according to the recommendations of the DNA Commission of the International Society of Forensic Haemogenetics.

Statistical Analysis

The frequency of each allele for each locus was calculated from the numbers of each genotype in the sample set. Unbiased estimates of expected heterozygosity were computed as described by Edwards et al. (23). Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (24–26), the likelihood ratio test (23,27,28), and the exact test (29). The expected number of distinct homozygous and heterozygous genotype classes and their standard error (SE) was calculated according to the method described by Chakraborty et al. (27). An interclass correlation criterion (30) was used for detecting disequilibrium between loci. The power of discrimination (PD)

TABLE 8—*Forensic value of the STRs studied using various statistical parameters in Korean population.*

Locus	Observed Heterozygote %	Homozygosity Test *p* value	Likelihood Ratio Test *p* value	Exact Test *p* value	Unbiased Estimate	PD	PIC
HumFES/FPS	67.50	0.300	0.283	0.158	0.708	0.8611	0.3423
HumFOLP23	74.63	0.630	0.778	0.731	0.731	0.8939	0.5446
HumGABRB15	71.08	0.296	0.688	0.835	0.677	0.8410	0.5239
HumCYAR04	69.80	0.334	0.766	0.580	0.728	0.8740	0.4913

PD, power of discrimination: PIC, polymorphism information content: *p*, probability.

TABLE 9—*Observed and expected heterozygous and homozygous classes for HumFES/FPS HumFOLP23, HumGABRB15, and HumCYAR04 in unrelated Koreans.*

	HumFES/FPS	HumFOLP23	HumGABRB15	HumCYAR04
Heterozygotes Observed	14	19	13	11
Heterozygotes Expected ± SE*	12 ± 1.7	17.2 ± 1.6	14.3 ± 1.9	9.6 ± 1.4
Homozygotes Observed	4	4	4	4
Homozygotes Expected ± SE	3.7 ± 0.6	4.7 ± 0.7	3.8 ± 0.7	3.9 ± 0.4

*SE equals standard error.

was calculated using Fisher's equation (31). The polymorphism information content (PIC) was calculated using Botstein's method (32). Expected gene diversity, or heterozygosity, was calculated from observed number of alleles (24).

Results and Discussion

The allele and genotype frequencies for loci, HumFES/FPS, HumFOLP23, HumGABRB15, and HumCYAR04 were determined in a Korean population sample. The major considerations for selection of loci were discriminating power, consistence with Hardy-Weinberg expectations, low levels of stutter bands (33), and resolution of alleles. The repeat unit at each locus was defined as the first in frame repeat unit on the strand listed in the GenBank database (21). The allele and genotype frequencies for the four STR loci are summarized in Tables 3 through 7. The most frequent allele type for each locus is HumFES/FPS: 11, HumFOLP23: 6, HumGABRB15: 10, and HumCYAR04: 3. The most common allele frequency ranged from 0.31 to 0.45.

There was no deviation from expected values for the four STR loci. Furthermore, the four STR loci do not deviate from HWE based on the homozygosity test, likelihood ratio test, and the exact test (Table 8). A test for independence of the alleles within a locus based on the number of distinct heterozygote and homozygote genotype classes was performed (Table 9). An inter-class correlation test analysis demonstrated that there is no detectable evidence for correlation between the alleles at any of the pair-wise comparisons of the four loci. The PD values for the 4 STR markers studied here ranged from 0.841 to 0.894.

The PIC values were calculated by the method of Botstein et al. (33). Under this formulation, two loci (HumFOLP23, HumGABRB15) were highly informative (PIC > 0.5), and the other two loci (HumFES/FPS, HumCYAR04) were reasonably informative ($0.25 < PIC < 0.5$).

In conclusion, four STR loci described here are well suited for routine identification of individuals. It is clear that after appropriate validation work, the selection of these highly polymorphic STR loci will provide forensic scientists with a useful tool for discriminating amongst individuals.

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