TECHNICAL NOTE

Toshimichi Yamamoto,[1] Ph.D.; Rieko Uchihi,[1] M.S.; Hideki Nozawa,[1] M.D.; Xiu-Lin Huang,[1] M.D.; Yim-Kheng Leong,[1] M.S.; Miwa Tanaka,[1] B.D.S.; Masaki Mizutani,[1] M.D.; Keiji Tamaki,[1] M.D.; and Yoshinao Katsumata,[1] M.D.

Allele Distribution at Nine STR Loci—D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820—in the Japanese Population by Multiplex PCR and Capillary Electrophoresis*

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ABSTRACT: Nine tetranucleotide short tandem repeat (STR) loci, D3S1358, vWA, FGA TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820, were analyzed in the Japanese population with a newly released kit for personal identification using multiplex PCR with fluorescent-labeled primers following capillary electrophoresis. The observed heterozygosities were 0.67, 0.77, 0.82, 0.61, 0.62, 0.73, 0.78, 0.81 and 0.74, respectively, and the combined discrimination power of the nineplex was 0.9999999991. None of the nine loci deviated from Hardy-Weinberg equilibrium expectations using the chi-square test, homozygosity test, likelihood ratio test and exact test after the grouping of the alleles. The nine STR loci allele frequencies were significantly different from those of other ethnic populations.

KEYWORDS: forensic science, DNA typing, population genetics, multiplex PCR, short tandem repeats, D3S1358, vWA, FGA TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820, capillary electrophoresis, Japanese

The importance of short tandem repeat (STR) analysis is steadily increasing in forensics (1–5). STRs are usually genotyped by PCR using fluorescent or isotopic labeled primers and gel electrophoresis following detection by laser or autoradiography. Genotypes may be also detected simply by silver staining after gel electrophoresis of the PCR product. Nominal alleles are identified by direct visualization by side-by-side comparison with the allelic ladder markers (1,2,6). However, as an alternative to gel electrophoresis, a capillary electrophoresis system with multi-fluorescent dyes has been developed (7,8). This system makes it possible to determine the size of STR alleles so accurately that the error of sizing is within 0.5 base (9). Multiplex PCR has also been applied to STRs and it is more effective because of the potential simultaneous typing of several loci. Recently, a STR typing kit using multiplex PCR for nine STR loci, the AmpFℓSTR Profiler PCR Amplification Kit, has been available commercially. We present population data for the STR systems at D3S1358 (10), vWA (11), FGA (12), TH01 (13), TPOX (14), CSF1PO (15), D5S818 (16), D13S317 (16) and D7S820 (17) in the Japanese population using this kit. We also compare our data with those of other ethnic populations.

Materials and Methods

Sample Preparation—Whole blood samples were collected from 206 unrelated Japanese individuals under informed consent, and DNA was extracted as previously described (18).

STR Typing—PCR amplification was performed according to the protocol supplied with the AmpFℓSTR Profiler PCR Amplification Kit (PE Applied Biosystems, USA) including each triplex STR amplification system, the Blue, Green I and Yellow kit, and the PCR product was analyzed by capillary electrophoresis with the Genetic Analyzer 310 (PE Applied Biosystems, USA). The Gene Scan 2.0.2 and the Genotyper 2.0 (PE Applied Biosystems, USA) software were used for sizing and typing, respectively.

Statistical Analysis—Tests for Hardy-Weinberg equilibrium (HWE) were carried out using the chi-square test, homozygosity test (19), likelihood ratio test (20) and exact test (21). To apply the chi-square test, alleles were pooled with adjacent alleles so that each event of the allele could be under the ''rule of five'' (22), which implies that the expected values of all genotypes under HWE are not less than one and that the proportion of the alleles of which the expected values are less than five is less than 20%. As for the other three tests, alleles containing less than five events

[1] Associate Professor, Assistant Professor, Postgraduate Students, Associate Professor and Professor, respectively, Department of Legal Medicine, Nagoya University School of Medicine, Nagoya 466-8550, Japan.

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were also pooled with the adjacent alleles so that no allele would have less than five events (23). The probabilities of the likelihood ratio test and the exact test were estimated based on 10 000 shuffling experiments. Each allele frequency calculated in this study was compared with that of African-American ($n = 195$) and U.S. Caucasian ($n = 200$) mentioned in the protocol.

Results and Discussion

Allele frequencies at nine STR loci in 206 Japanese are shown in Table 1. No significant deviations from HWE were observed using the chi-square test, homozygosity test, likelihood ratio test and exact test after the grouping of the alleles except using the exact test at vWA and CSF1PO (Table 2). However, because these p-values are close to 0.05 ($p = 0.0461$ and 0.0218), the departures from HWE are not highly significant as has been observed for other population studies (24,25). When rare classes of alleles were pooled with adjacent alleles, the p-values exceeded 0.05. The distributions of allele frequencies significantly deviated from those of other ethnic populations, African-American ($n = 195$) and U.S. Caucasian ($n = 200$) (shown in the protocol) (all $p < 0.01$). Alleles 24.2 and 25.2 at FGA locus, allele 14 at TPOX, and allele 15 at CSF1PO, all of which were not found in both populations were observed in this study.

The observed heterozygosity, expected heterozygosity (unbiased), power of discrimination (PD) and polymorphism information content (PIC) at the nine STR loci are shown in Table 3. The combined PD with the nineplex was calculated at 0.9999999991. This value was not substantially lower than those of African-American (0.9999999999) and U.S. Caucasian (0.9999999997) mentioned in the protocol supplied with the kit.

We also calculated the probability of paternity exclusion (PE) value at each locus and the combined PE value of nine loci (Table 4). In general, the PE values of Japanese tend to be higher in the

TABLE 1—*Allele frequencies at nine STR loci in the AmpFℓSTR Profiler Kit in the Japanese population.*

Allele	D3S1358	vWA	FGA	THO1	TPOX	CSF1PO	D5S818	D13S317	D7S820
6	. . .	. . .	. . .	0.189	. . .	. . .	. . .	. . .	. . .
7	. . .	. . .	. . .	0.238	. . .	0.005	0.005	. . .	0.002
8	. . .	. . .	. . .	0.046	0.490	. . .	0.007	0.226	0.090
9	. . .	. . .	. . .	0.485	0.070	0.039	0.083	0.114	0.051
9.3	. . .	. . .	. . .	0.034	. . .	. . .	. . .	. . .	. . .
10	. . .	. . .	. . .	0.007	0.041	0.204	0.214	0.146	0.216
11	. . .	. . .	. . .	. . .	0.354	0.211	0.252	0.238	0.350
12	. . .	. . .	. . .	. . .	0.036	0.427	0.272	0.206	0.243
13	. . .	. . .	. . .	. . .	0.005	0.107	0.146	0.061	0.049
14	0.034	0.189	. . .	. . .	0.002	0.005	0.019	0.010	. . .
15	0.415	0.019	. . .	. . .	. . .	0.002	0.002	. . .	. . .
16	0.291	0.175	. . .	. . .	. . .	. . .	. . .	. . .	. . .
17	0.199	0.262	0.005	. . .	. . .	. . .	. . .	. . .	. . .
18	0.056	0.240	0.022	. . .	. . .	. . .	. . .	. . .	. . .
19	0.005	0.102	0.073	. . .	. . .	. . .	. . .	. . .	. . .
20	. . .	0.012	0.121	. . .	. . .	. . .	. . .	. . .	. . .
21	. . .	. . .	0.138	. . .	. . .	. . .	. . .	. . .	. . .
21.2	. . .	. . .	0.002	. . .	. . .	. . .	. . .	. . .	. . .
22	. . .	. . .	0.216	. . .	. . .	. . .	. . .	. . .	. . .
22.2	. . .	. . .	0.002	. . .	. . .	. . .	. . .	. . .	. . .
23	. . .	. . .	0.163	. . .	. . .	. . .	. . .	. . .	. . .
24	. . .	. . .	0.143	. . .	. . .	. . .	. . .	. . .	. . .
24.2	. . .	. . .	0.002	. . .	. . .	. . .	. . .	. . .	. . .
25	. . .	. . .	0.068	. . .	. . .	. . .	. . .	. . .	. . .
25.2	. . .	. . .	0.005	. . .	. . .	. . .	. . .	. . .	. . .
26	. . .	. . .	0.029	. . .	. . .	. . .	. . .	. . .	. . .
27	. . .	. . .	0.010	. . .	. . .	. . .	. . .	. . .	. . .

TABLE 2—*Tests for Hardy-Weinberg equilibrium.*

Locus	Chi-square Test*	Homozygosity Test† (19)	Likelihood Ratio Test† (20)	Exact Test† (21)
D3S1358	0.9120 [3]	0.2002 [5]	0.8102 [5]	0.6341 [5]
vWA	0.0685 [4]	0.2864 [7]	0.1272 [7]	0.0461 [7]
FGA	0.6620 [6]	0.1213 [9]	0.9666 [9]	0.8868 [9]
THO1	0.0887 [3]	0.0521 [5]	0.2027 [5]	0.2868 [5]
TPOX	0.4281 [3]	0.7673 [5]	0.1352 [5]	0.0880 [5]
CSF1PO	0.2561 [3]	0.6432 [5]	0.0500 [5]	0.0218 [5]
D5S818	0.2283 [5]	0.8125 [7]	0.5388 [7]	0.2727 [7]
D13S317	0.5567 [5]	0.8548 [6]	0.2779 [6]	0.2664 [6]
D7S820	0.5057 [4]	0.5677 [6]	0.7388 [6]	0.7753 [6]

* Alleles were pooled with the adjacent alleles so that each event of the allele would be under the ''rule of five'' (22).
† Alleles containing less than five entries were also pooled with adjacent alleles so that no alleles would have less than five events (23).
Numbers in brackets are the number of ''alleles'' for testing.

TABLE 3—*Statistical properties of nine loci in Japanese by the AmpFℓSTR Profiler Kit.*

Locus	D3S1358	vWA	FGA	THO1	TPOX	CSF1PO	D5S818	D13S317	D7S820
Obs. Hz*	0.665	0.767	0.820	0.612	0.617	0.728	0.782	0.806	0.743
Exp. Hz†	0.701	0.798	0.863	0.670	0.628	0.719	0.790	0.814	0.761
PD‡	0.861	0.923	0.964	0.844	0.788	0.861	0.918	0.936	0.906
PIC§	0.647	0.765	0.846	0.618	0.560	0.674	0.756	0.785	0.722

* Observed heterozygosity.
† Expected heterozygosity (unbiased).
‡ Power of discrimination (26).
§ Polymorphism information content (27).

TABLE 4—*Comparison of probability of paternity exclusion (PE) values among several different ethnic populations.*

Locus	Japanese (This study) (n = 206)	African-American* (n = 195)	U.S. Caucasian* (n = 200)
D3S1358	0.4480	0.5260	0.5797
vWA	0.5955	0.6394	0.6170
FGA	0.7212	0.7202	0.7173
THO1	0.4221	0.5250	0.5418
TPOX	0.3632	0.5764	0.3589
CSF1PO	0.4832	0.6048	0.4854
D5S818	0.5831	0.5375	0.4554
D13S317	0.6244	0.4725	0.5940
D7S820	0.5414	0.5742	0.6307
Combined	0.9991	0.9996	0.9994

* Values cited from kit protocol.

Yellow loci (D5S818, D13S317 and D7S820) and lower in the Green I loci (TH01, TPOX and CSF1PO) than those of other populations. The combined PE value of Japanese also seems a little lower. The slightly lower combined PD and PE values in the Japanese population may suggest that Japanese people have long been isolated for both historical and geographical reasons, and also may be the result that the Japanese population may not be as old as the other two groups.

However, this method at these loci is also very useful for personal identification and paternity testing in the Japanese population because of the extremely high combined PD and PE values.

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Additional information and reprint requests:
Toshimichi Yamamoto
Department of Legal Medicine
Nagoya University School of Medicine
65 Tsuruma-cho, Showa-ku
Nagoya 466-8550
Japan