TECHNICAL NOTE

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Fixed Bin Population Data for the VNTR Loci D1S7, D2S44, D4S139, D5S110, D10S28, and D14S13 in a Population Sample from Rio De Janeiro, Brazil

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ABSTRACT: Fixed bin frequencies for the VNTR loci D1S7, D2S44, D4S139, D5S110, D10S28, and D14S13 were determined in a Rio de Janeiro sample population. The data were generated by RFLP analysis of *Hae*III-digested genomic DNA and chemiluminescent detection. The six VNTR loci meet Hardy-Weinberg expectations, and there is no evidence for association of alleles between the VNTR loci. The frequency data can be used in forensic analyses and paternity tests to estimate the frequency of a DNA profile in the general Brazilian population.

KEYWORDS: forensic science, DNA typing, gene frequency, population genetics, Hardy-Weinberg Equilibrium, restriction fragment length polymorphism, D1S7, D2S44, D4S139, D5S110, D10S28, D14S13, Rio de Janeiro, Brazil

At the level of DNA, polymorphic loci have been used to construct a genetic map of the human genome (1). These mapping efforts have identified medically important genes and genetic markers for population genetic studies, such as the variable number of tandem repeat (VNTR) loci. The VNTR loci are highly polymorphic in humans and can be typed reliably from a number of different tissue sources (2,3). The ability to perform human identity testing is facilitated by analyses of VNTR loci. In order to estimate the rarity of a DNA profile, some general population data are required. Except for the D4S139 locus (4), no VNTR locus population data, generated by the restriction fragment length (RFLP) method, are available for the Rio de Janeiro area. This paper describes RFLP population data on the VNTR loci D1S7, D2S44, D4S139, D5S110, D10S28, and D14S13 in a sample of Rio de Janeiro population using the restriction enzyme *Hae*III and chemiluminescence detection.

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Materials and Methods

Sample Preparation and Typing

The source of the DNA samples were from unrelated and non-black individuals from Rio de Janeiro. DNA, from peripheral blood samples, was extracted non-organically, digested with *Hae*III, separated by electrophoresis in 0.8% agarose gels, Southern transferred to nylon membranes (Byodine A), and hybridized to alkaline phosphatase-conjugated probes, according to the protocol supplied with the ACES 2.0 plus Kit (Life Technologies—GibcoBRL, Gaithersburg, MD). The probes MS1 (for the D1S7 locus), PH30 (for the D4S139 locus), and LH1 (for the D5S110 locus) were purchased from Life Technologies—GibcoBRL (Gaithersburg, MD). The probe TBQ7 (for the D10S28 locus) was supplied by the Promega Corporation (Madison, WI). The probe CMM101 (for the D14S13 locus) was kindly provided by Dr. Arthur J. Eisenberg, University of North Texas Health Science Center, Fort Worth, TX. The estimated base pair sizing of the digested DNA fragments was performed by comparison to the GibcoBRL molecular weight marker (i.e., sizing ladder), using a scanner and SigmaGel/Jandel software (San Rafael, CA).

Statistical Analysis

The fragment size data were sorted into fixed bins according to the method of Budowle et al. (5). Possible divergence from Hardy-Weinberg expectations (HWE) was determined by the likelihood ratio test and the exact test (6,8,9). An interclass correlation criterion for two-locus associations was used for detecting disequilibrium between the VNTR loci (10). Independence across the six VNTR loci also was determined by examining whether the observed variance of the number of heterozygous loci in the population sample is outside its confidence interval under the assumption of independence (11,12).

Results and Discussion

This is the first report on the distribution of bin frequencies for six VNTR loci in the general Brazilian population. The 31 fixed bin frequency distributions for the D1S7, D2S44, D4S139, D5S110, D10S28, and D14S13 loci are shown in Table 1. All loci are highly polymorphic, and there is no evidence for departure from HWE

TABLE 1—*Fixed bin frequencies for several VNTR loci in Rio De Janeiro, Brazil.*

Bin	Size (bp)	D1S7	D2S44	D4S139	D5S110	D10S28	D14S13
1	1–639	0.001	0.002	0.000	0.002	0.001	0.000
2	640–772	0.000	0.006	0.000	0.001	0.003	0.000
3	773–871	0.001	0.005	0.000	0.002	0.001	0.000
4	872–963	0.003	0.002	0.000	0.000	0.005	0.000
5	964–1077	0.006	0.010	0.000	0.003	0.042	0.001
6	1078–1196	0.002	0.015	0.000	0.004	0.039	0.001
7	1197–1352	0.007	0.044	0.000	0.007	0.039	0.001
8	1353–1507	0.006	0.057	0.000	0.017	0.051	0.007
9	1508–1637	0.008	0.111	0.001	0.031	0.081	0.019
10	1638–1788	0.008	0.106	0.002	0.029	0.083	0.010
11	1789–1924	0.008	0.091	0.000	0.031	0.079	0.124
12	1925–2088	0.020	0.064	0.000	0.055	0.075	0.081
13	2089–2351	0.027	0.085	0.013	0.078	0.093	0.065
14	2352–2522	0.024	0.044	0.003	0.041	0.030	0.038
15	2523–2692	0.024	0.024	0.004	0.056	0.027	0.021
16	2693–2862	0.027	0.043	0.011	0.062	0.028	0.054
17	2863–3033	0.029	0.075	0.011	0.056	0.035	0.027
18	3034–3329	0.053	0.084	0.011	0.077	0.044	0.027
19	3330–3674	0.052	0.065	0.022	0.089	0.057	0.033
20	3675–3979	0.052	0.025	0.030	0.075	0.032	0.021
21	3980–4323	0.056	0.023	0.035	0.062	0.038	0.043
22	4324–4821	0.068	0.005	0.082	0.057	0.064	0.016
23	4822–5219	0.048	0.001	0.059	0.029	0.014	0.016
24	5220–5685	0.052	0.002	0.062	0.037	0.008	0.011
25	5686–6368	0.075	0.005	0.114	0.028	0.002	0.001
26	6369–7241	0.083	0.007	0.152	0.026	0.001	0.001
27	7242–8452	0.078	0.000	0.125	0.026	0.001	0.011
28	8453–10093	0.064	0.001	0.095	0.011	0.004	0.000
29	10094–11368	0.032	0.000	0.042	0.003	0.000	0.011
30	11369–12829	0.029	0.000	0.038	0.000	0.000	0.001
31	12830–25000	0.052	0.000	0.088	0.004	0.001	0.001
Number of chromosomes		1278	1230	1264	1222	1250	186
Individuals—single band		16	31	18	16	34	03
HWE/Likelihood ratio (p =)		0.897	0.993	0.291	0.933	0.306	0.729
HWE/Exact test (p =)		0.802	0.990	0.175	0.918	0.275	0.678

for any of the six loci based on the likelihood ratio test (6–8) and the exact test (9) (Table 1).

An interclass correlation test (10) analysis demonstrated that there is no detectable evidence for correlation between the alleles at any of the pairs of loci (Table 2). An alternate method that addresses all six VNTR loci at one time was used for testing for detectable deviation from expectation. The test examines whether or not the observed variance (s_k^2) of the number of heterozygous loci in a population sample is outside its confidence interval under the assumption of independence using the procedure described by Brown et al. (11,12). There was no evidence of association for the six loci using the s_k^2 criterion (s_k^2 = 0.469, 95% confidence interval of variance is 0.261–0.646).

TABLE 2—*Two locus inter-class correlation test for the VNTR loci in unrelated Brazilians.*

Loci Pair	Two-Sided Probability
D1S7/D2S44	0.068
D1S7/D4S139	0.540
D1S7/D5S110	0.370
D1S7/D10S28	0.459
D1S7/D14S13	0.421
D2S44/D4S139	0.833
D2S44/D5S110	0.249
D2S44/D10S28	0.667
D2S44/D14S13	0.927
D4S139/D5S110	0.271
D4S139/D10S28	0.665
D4S139/D14S13	0.102
D5S110/D10S28	0.893
D5S110/D14S13	0.502
D10S28/D14S13	0.575

Population data on the six VNTR loci in United States Caucasians were compared with our Brazilian data (13,14). There were very few instances in which substantial differences in fixed bin frequencies at any of the loci between the two population samples were observed. It would not be meaningful for forensic or paternity applications to compare statistically these databases with a test for homogeneity because of sampling variance and measurement biases between laboratories. Moreover, for even moderately large sample sizes, standard contingency table analysis exhibits extreme sensitivity to small perturbations and frequently results in a rejection of the null hypothesis of no difference, even if the difference is of little consequence (15). However, the binned data were compared as described by Chow et al. (16) and Huang and Budowle (17). Bins containing a minimum of five chromosomes were compared. Based on a ratio of bin frequencies (the larger divided by the smaller frequency), there were only six examples in which the ratio was greater than two-fold, and only one of these ratios exceeded three-fold. Two of the six examples were at the D14S13

locus, which contains data on 93 individuals only, in the Brazilian population sample. Thus, even though the ethnic make-up of the Brazilian and United States Caucasian populations is different, the data demonstrate that there would be little difference in a multiple locus profile frequency estimate, using either database, under the assumption of independence.

In conclusion, this report provides fixed bin frequency data for six VNTR loci for the Rio de Janeiro population. The results strongly support the conclusion that multiple locus VNTR DNA profiles are rare events. The data are consistent with the notion that there would be no anticipated forensic significance, whether a general or a regional population data base was used, to convey an estimate of the rarity of a DNA profile in a major group (18).

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