TECHNICAL NOTE

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Allele Frequencies of Six STR Loci in Argentine Populations*

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ABSTRACT: Allele frequencies of six short tandem repeat (STR) loci were determined in a Caucasian urban sample of La Plata city and three Amerindian sample populations of Argentina. Allele frequencies showed differences between urbans and Amerindians, and among Amerindians as well. The degree of genetic differentiation of subpopulations was mainly due to the Amerindian contribution. Mapuche, Mocovi, and pooled Amerindian populations showed little evidence of HW disequilibrium, and association of alleles. In the urban sample, there is no evidence of population substructuring. Forensic probabilities of exclusion and matching showed high differences between the population groups. Finally, La Plata sample did not show differences with Caucasians from other geographic regions.

KEYWORDS: forensic science, DNA typing, Argentina, Caucasian, Amerindian, population genetics, THO1, CSF1PO, TPOX, F13B, F13A01, FESFPS, allele frequencies, STR loci, Argentine Caucasian and Amerindian populations, forensic probabilities

The Argentine population comprises predominantly European descendants with two to five generations born in the country. This structure is particularly valid for the largest urban areas of Buenos Aires, Cordoba, Mendoza, and Santa Fe provinces. The genetic composition of Argentine population is also influenced by genetic mixture with native aborigine populations. Although the Amerindian component has not the same quantitative occurrence than the one observed for other South American countries, it constitutes a well defined section of our people composition (1). In Argentina, the city of La Plata is an urban center surrounded by important industrial settlements and a wide university area which attract immigration from other provinces. For this reason, during the last 40 years, La Plata has almost doubled its population not only due to the natural increase of its inhabitants but also to immigration. This report presents allele frequency data of the STR loci HUMTH01, HUMTPOX, HUMF13B, HUMF13A01, HUMCSF1PO, and HUMFESFPS for an urban sample of La Plata city and three samples of the major Argentine Amerindian populations. The survey was performed by PCR amplification. The level of variation among and within all four populations and the potential usefulness of these loci for paternity and identification testing were also examined. Moreover, to test the genetic homogeneity of the current La Plata population, it was compared with Caucasian samples from other geographic populations.

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Materials and Methods

Population Sample

Population studies were carried out on 100 unrelated donors of the La Plata city and three Argentine Amerindian populations comprising 50 Mapuche individuals, 36–50 Mocovies, and 50 Wichis. The three Amerindian samples were also analyzed as a group referred to pooled Amerindians. The urban sample includes workers undergoing blood testing for job admission, suspects and victims from forensic cases, and laboratory personnel. Mapuche sample belongs to localities of Cerro Policía and Aguada Guzmán, province of Río Negro, South Argentina. The two other Amerindian groups inhabit the North of Argentina: the Wichis, proceding from Santa Victoria, province of Salta, and the Mocovies, from Colonia Dolores, in Santa Fe.

DNA Analysis

Genomic DNA was isolated from 20 μL blood samples by Chelex 100 resin (BioRad, CA) according to Walsh P et al. (2). DNA samples were amplified in monoplex PCR reactions each containing a set of commercial primers from the Gene Print-STR System, (Promega Corp., Madison, WI) for HUMTH01, HUMCSF1PO, HUMTPOX, HUMF13B, HUMF13A01, and HUMFESFPS loci. Each PCR mixture included 50 ng of DNA extract; 1,5 mM $MgCl_2$; 200 μM each dATP, dGTP, dTTP; 10 μM dCTP; 0.08 μCi α-^{32}P-dCTP (NEN, Dupont, Boston, MA), 0.45 units of Taq polymerase (Promega Corp.) in a final volume of 10 μL of 1 × Taq buffer. Amplifications were carried out in a PTC-100 programmable thermocycler (MJ Research, Inc., Walertown, MA) following cycling conditions devised by Promega Corp. for each primer set. PCR products were electrophoresed through 6% polyacrylamide, 7 M urea on a Sequi-Gen cell (Bio-Rad, Hercules, CA) and the alleles detected by autoradiography. Alleles assignment was made by size comparison with appropriate allelic ladders from the Promega Corp. STR kits.

Statistical Approach

Since PCR amplification detects both alleles at these loci, the allelic frequencies were calculated by simple gene counting (3). Possible divergence from Hardy-Weinberg equilibrium (HWE) was computed by: i) X^2 test as in Deka R et al. 1992 (4); ii) exact test as number of distinct homozygous and heterozygous genotypes (5); and iii) G^2 likelihood ratio test (6). The level of significance in ii) and iii) tests was determined by simulation model, according to Chakraborty R et al. (1991) (5). Gene diversity was computed as described in Gill and Evett (7). Detection of nonrandom association of alleles was tested by the variance observed in the number of heterozygous loci (s_k^2 statistic) and its 95% confidence intervals (8). The degree of genetic differentiation was measured by F_{ST} statistic analysis (9) for each of the three following groups: Amerindian plus urban, Amerindian and Caucasian populations. To estimate the level of genetic differentiation in the Caucasian population, we employed data from North American (10–13), German (14), French (15), Swiss (16), and Austrian Caucasians (17). The potential application of the six STR loci for forensic identification and paternity testing was assayed by computing the average power of exclusion (Pex) and the probability of matching (PM) (18,19).

Results and Discussion

Table 1 shows the allelic frequency distribution for each one of the six loci analyzed in the four population samples. Frequencies of some alleles are clearly variable among the population groups. This

TABLE 1—*Sample size, allele frequencies and Hardy-Weinberg tests of six loci in Argentine populations.*

		HUMTH01		HUMCSF1PO		HUMTPOX		HUMF13B		HUMF13A01		HUMFESFPS	
Population	Allele	Nc	Freq	Nc	Freq	Nc	Freq	Nc	Freq	Nc	Freq	Nc	Freq
Urban	3.2	–	–	–	–	–	–	–	–	31	0.155	–	–
	4	–	–	–	–	–	–	–	–	13	0.065	–	–
	5	–	–	–	–	–	–	–	–	28	0.140	–	–
	6	52	0.260	–	–	–	–	22	0.110	55	0.275	–	–
	7	45	0.225	–	–	–	–	4	0.020	59	0.295	2	0.010
	8	25	0.125	1	0.005	106	0.530	43	0.215	2	0.010	2	0.010
	9	27	0.135	3	0.015	20	0.100	54	0.270	2	0.010	1	0.005
	9.3	45	0.225	–	–	–	–	–	–	–	–	–	–
	10	6	0.030	52	0.260	12	0.060	76	0.380	–	–	43	0.215
	11	–	–	57	0.285	52	0.260	1	0.005	–	–	97	0.485
	12	–	–	66	0.330	10	0.050	–	–	1	0.005	41	0.205
	13	–	–	20	0.100	–	–	–	–	2	0.010	11	0.055
	14	–	–	1	0.005	–	–	–	–	–	–	3	0.015
	15	–	–	–	–	–	–	–	–	4	0.020	–	–
	16	–	–	–	–	–	–	–	–	2	0.010	–	–
N=100	17	–	–	–	–	–	–	–	–	1	0.005	–	–
Exact test			0.402		0.684		0.761		0.100		0.231		0.481
$G^2_{(df)}$			$15.2_{(15)}$		$12.0_{(18)}$		$9.2_{(21)}$		$19.8_{(15)}$		$38.4_{(42)}$		$28.5_{(21)}$
Likelihood			0.439		0.846		0.988		0.179		0.631		0.127
Mapuche	3.2	–	–	–	–	–	–	–	–	24	0.240	–	–
	4	–	–	–	–	–	–	–	–	14	0.140	–	–
	5	–	–	–	–	–	–	–	–	16	0.160	–	–
	6	53	0.530	–	–	–	–	4	0.040	11	0.110	–	–
	7	29	0.290	–	–	–	–	–	–	33	0.330	–	–
	8	1	0.010	–	–	49	0.490	5	0.050	–	–	2	0.020
	9	5	0.050	10	0.100	3	0.030	39	0.390	–	–	–	–
	9.3	12	0.120	–	–	–	–	–	–	–	–	–	–
	10	–	–	22	0.220	4	0.040	51	0.510	–	–	17	0.170
	11	–	–	30	0.300	22	0.220	1	0.010	–	–	52	0.520
	12	–	–	32	0.320	21	0.210	–	–	–	–	26	0.260
	13	–	–	5	0.050	1	0.010	–	–	–	–	3	0.030
	14	–	–	1	0.010	–	–	–	–	2	0.020	–	–
	15	–	–	–	–	–	–	–	–	–	–	–	–
	16	–	–	–	–	–	–	–	–	–	–	–	–
N=50	17	–	–	–	–	–	–	–	–	–	–	–	–
Exact test			0.580		0.821		0.722		0.613		0.540		0.910
$G^2_{(df)}$			$25.2_{(15)}$		$13.2_{(18)}$		$6.3_{(21)}$		$4.9_{(15)}$		$20.4_{(42)}$		$7.2_{(21)}$
Likelihood			0.025		0.778		0.999		0.993		0.997		0.998
Mocovi	3.2	–	–	–	–	–	–	–	–	28	0.333	–	–
	4	–	–	–	–	–	–	–	–	11	0.131	–	–
	5	–	–	–	–	–	–	–	–	25	0.298	–	–
	6	19	0.190	–	–	–	–	2	0.024	7	0.083	–	–
	7	51	0.510	–	–	2	0.024	–	–	12	0.143	–	–
	8	6	0.060	1	0.010	34	0.405	16	0.191	–	–	2	0.028
	9	2	0.020	4	0.041	8	0.095	27	0.321	–	–	–	–
	9.3	22	0.220	–	–	–	–	–	–	–	–	–	–
	10	–	–	32	0.326	–	–	39	0.464	–	–	13	0.180
	11	–	–	23	0.235	18	0.214	–	–	–	–	36	0.500
	12	–	–	32	0.327	22	0.262	–	–	–	–	11	0.153
	13	–	–	6	0.061	–	–	–	–	–	–	10	0.139
	14	–	–	–	–	–	–	–	–	1	0.012	–	–
	15	–	–	–	–	–	–	–	–	–	–	–	–
	16	–	–	–	–	–	–	–	–	–	–	–	–

TABLE 1—*(Continued)*

		HUMTH01		HUMCSF1PO		HUMTPOX		HUMF13B		HUMF13A01		HUMFESFPS	
Population	Allele	Nc	Freq	Nc	Freq	Nc	Freq	Nc	Freq	Nc	Freq	Nc	Freq
N=50*	17	–	–	–	–	–	–	–	–	–	–	–	–
Exact test			0.010		0.262		0.142		0.721		0.573		0.061
G^2 (df)			$16.2_{(15)}$		$16.5_{(18)}$		$6.6_{(21)}$		$2.6_{(15)}$		$17.9_{(42)}$		$17.7_{(21)}$
Likelihood			0.367		0.559		0.998		0.999		0.999		0.668
Wichi	3.2	–	–	–	–	–	–	–	–	27	0.270	–	–
	4	–	–	–	–	–	–	–	–	9	0.090	–	–
	5	–	–	–	–	–	–	–	–	49	0.490	–	–
	6	13	0.130	–	–	2	0.020	–	–	6	0.060	–	–
	7	77	0.770	–	–	–	–	–	–	9	0.090	–	–
	8	–	–	–	–	21	0.210	1	0.010	–	–	–	–
	9	3	0.030	1	0.010	1	0.010	65	0.650	–	–	–	–
	9.3	7	0.070	–	–	–	–	34	0.340	–	–	–	–
	10	–	–	29	0.290	–	–	–	–	–	–	15	0.150
	11	–	–	18	0.180	39	0.390	–	–	–	–	65	0.650
	12	–	–	51	0.510	37	0.370	–	–	–	–	11	0.110
	13	–	–	1	0.010	–	–	–	–	–	–	9	0.090
	14	–	–	–	–	–	–	–	–	–	–	–	–
	15	–	–	–	–	–	–	–	–	–	–	–	–
	16	–	–	–	–	–	–	–	–	–	–	–	–
N=50	17	–	–	–	–	–	–	–	–	–	–	–	–
Exact test			0.732		0.730		0.576		0.800		0.191		0.352
G^2 (df)			$6.1_{(15)}$		$6.9_{(18)}$		$6.4_{(21)}$		$2.3_{(15)}$		$16.2_{(42)}$		$10.5_{(21)}$
Likelihood			0.978		0.991		0.999		0.999		0.999		0.971

N = sample size, * H01 (N=50), 1PO (N=49), POX, 13B, A01 (N=42), FPS (N=36).
Nc = chromosome number, Freq = allelic frequencies.
Exact test = chi square test (1 df) based observed/expected homozygosity.
Likelihood G^2 test based observed/expected genotype.

is particularly evident for allele number seven at HUMTH01 locus, which shows a frequency of 77% in the Wichi population, and 22.5% in the Caucasian urban sample of La Plata.

The results of HWE tests are depicted in Table 1. The exact test of heterozygosity indicates disequilibrium at HUMTH01 (p = 0.010) locus for Mocovies. In the pooled Amerindian sample, this test indicates disequilibrium at HUMTH01 (p = 0.022), HUMTPOX (P = 0.042), and HUMF13A01 (P = 0.038) loci (not shown). The test based on the number of distinct homozygous and heterozygous genotypes detected an excess of *10–10* homozygous individuals (observed = 4, expected = 1) at HUMFESFPS locus for the Mocovi population. The G^2 test, which seems to be more appropriate for small samples, detected deviations from HWE at HUMTH01 (P = 0.025) for Mapuches, and HUMTH01 (P = 0.032) and HUMCSF1PO (p = 0.049) for the pool of Amerindians. Departures from HWE may be caused by factors such as population substructuring, sampling size, nonrandom mating, or disturbing forces like admixture, genetic drift, selection, endogamy, etc. (5,20,21). Our data showed 8 HWE departures out of the 4–5 possible random deviations, considering the 90 comparison and α = 0.05. Deviations observed in the pooled Amerindian sample were expected and they can be explained by the Whalund's principle (22). Other HW disequilibrium might be caused by sampling error although in Amerindian populations a biological effect could also be assumed.

Population substructuring can be detected either as deviation from Hardy-Weinberg equilibrium or as gametic phase equilibrium (21). Since the loci examined here are located in different chromosomes, we could expect no association of their alleles. In spite of this, Sk^2 results show disequilibrium in the pairs CSF1PO/FESFPS (Sk^2 = 0.520, Cl 95% = 0.517) for Mapuche population; CSF1PO/F13B (Sk^2 = 0.628, Cl 95% = 0.478) CSF1PO/F13A01 (Sk^2 = 0.539, Cl 95% = 0.479) and F13B/FESFPS (Sk^2 = 0.533, Cl 95% = 0.523) for Mocovies; and TH01/CSF1PO (Sk^2 = 0.527, Cl 95% = 0.501), CSF1PO/F13B (Sk^2 = 0.575, Cl 95% = 0.513) CSF1PO/F13A01 (SK^2 = 0.446, Cl 95% = 0.427), CSF1PO/FESFPS (SK^2 = 0.520, Cl 95% = 0.505) and F13B/F13A01 (SK^2 = 0.510, Cl 95% = 0.482) for the pool of Amerindian samples. The allelic association found in our samples might be interpreted as the consequence of heterogeneity within each of these populations. In this context, the level of non-Amerindian admixture found in Indian populations (23,24) could possibly explain these findings, though the size of the sample should not rule out. With the purpose of evaluating whether the HW and gametic phase disequilibrium found in the aborigine samples have any effect on forensic statistics, we compared the estimated genotype frequencies by applying the counting method as in Scholl S et al. 1996 (25), which can be used when no assumption of independence is presumed. The results indicated that such disequilibrium has little impact on the genotype frequencies and does not seem to be relevant for forensic purposes. On the other hand, all tests performed with the Caucasian urban sample of La Plata showed that it is homogeneous and provided no evidence of population substructuring. These results also support the use of observed allele frequencies to estimate genotype frequencies, whereas the product rule might be applied to estimate the frequencies of a multilocus profile.

Tables 2 and 3 show gene diversity values h for the different loci, the level of variation among and within the groups, and the F_{ST} values for each polymorphic marker. The expected heterozygosity values for each locus varied in all four populations and no evidence of locus characteristic profile was detected. Accordingly, for La Plata sample, HUMTH01 (h = 0.796) was the most variable locus while HUMTPOX was the less variable one (h = 0.635). Conversely, for the Wichi population, the lowest value corresponded to the HUMTH01 (h = 0.384) locus and the highest one to HUMTPOX (h = 0.678). The genetic diversity (Fst) of

TABLE 2—*Gene diversity analysis of six STR loci in four Argentine population samples.*

	Gene Diversity (h)				Source of Variation in the Four Populations			Source of Variation in Amerindian		
Locus	Urban	Mapuche	Mocovi	Wichi	Within	Among	F_{ST}	Within	Among	F_{ST}
TH01	0.796 ± 0.040	0.618 ± 0.069	0.652 ± 0.067	0.384 ± 0.069	0.649	0.073	0.101	0.551	0.074	0.118
CSF1PO	0.732 ± 0.044	0.746 ± 0.062	0.725 ± 0.064	0.624 ± 0.069	0.712	0.011	0.015	0.698	0.014	0.019
TPOX	0.635 ± 0.048	0.664 ± 0.067	0.715 ± 0.070	0.678 ± 0.066	0.661	0.037	0.053	0.679	0.028	0.039
F13B	0.724 ± 0.045	0.584 ± 0.069	0.640 ± 0.074	0.462 ± 0.074	0.626	0.035	0.053	0.558	0.031	0.052
F13A01	0.789 ± 0.041	0.776 ± 0.059	0.753 ± 0.067	0.668 ± 0.067	0.754	0.043	0.054	0.810	0.031	0.038
FESFPS	0.673 ± 0.047	0.632 ± 0.068	0.675 ± 0.078	0.534 ± 0.071	0.635	0.009	0.014	0.607	0.011	0.018
Mean	0.725 ± 0.058	0.670 ± 0.069	0.693 ± 0.041	0.558 ± 0.109			0.048			0.047

TABLE 3—*Gene diversity analysis of the six STR loci in Caucasian population samples from different sources.*

	Gene Diversity (h) in Caucasian Samples				Source of Variation		
Locus	1*	2	3	4	Within	Among	F_{ST}
TH01	0.796 ± 0.040	0.783 ± 0.029†	0.773 ± 0.052‖	0.776 ± 0.030**	0.780	0.002	0.003
CSF1PO	0.732 ± 0.044	0.734 ± 0.031†	0.722 ± 0.036‖	0.722 ± 0.045††	0.727	0.001	0.001
TPOX	0.635 ± 0.048	0.618 ± 0.035†	0.585 ± 0.057‖	0.587 ± 0.049††	0.609	0.001	0.002
F13B	0.724 ± 0.045	0.715 ± 0.031‡	0.735 ± 0.031¶	0.711 ± 0.084‡‡	0.708	0.002	0.002
F13A01	0.789 ± 0.041	0.730 ± 0.034§	0.726 ± 0.035‖	0.768 ± 0.027**	0.751	0.004	0.005
FESFPS	0.673 ± 0.047	0.700 ± 0.034§	0.647 ± 0.041‖	0.690 ± 0.034**	0.688	0.006	0.010

* Data from La Plata sample.
† Data from U.S. Caucasian sample (Budowle B et al. 1997).
‡ Data from U.S. Caucasian sample (Promega Manual, 1997).
§ Data from U.S. Caucasian sample (Hammond H et al. 1994).
‖ Data from German Caucasians (Puers et al. (1993).
¶ Data from Nishimura D and Murray J, 1992.
** Data from French Caucasians (Pftzeinger H et al. 1994).
†† Data from Swiss Caucasians (Hochmeister M et al. 1994).
‡‡ Data from Austrian Caucasians (Klintschar M and Crevenna R 1996).

TABLE 4—*Statistical parameters of medical/legal interest.*

Locus	Statistic	Urban	Mapuche	Mocovi	Wichi	Amerindian
THO1	Pex	0.595	0.361	0.404	0.209	0.369
	PM	0.088	0.200	0.184	0.418	0.202
CSF1PO	Pex	0.486	0.514	0.477	0.351	0.423
	PM	0.130	0.128	0.128	0.222	0.134
TPOX	Pex	0.394	0.417	0.466	0.387	0.449
	PM	0.191	0.162	0.128	0.186	0.132
F13B	Pex	0.479	0.304	0.361	0.187	0.303
	PM	0.123	0.268	0.203	0.398	0.252
F13A01	Pex	0.592	0.566	0.528	0.428	0.554
	PM	0.073	0.096	0.112	0.174	0.094
FESFPS	Pex	0.431	0.375	0.441	0.318	0.384
	PM	0.157	0.212	0.170	0.244	0.196

Pex (Power of exclusion), PM (Power of matching).

subpopulations ranged from 0.014 for HUMFESFPS locus to 0.101 for HUMTH01 locus, with an average of 0.048 (Table 2). For Amerindian F_{ST} values, the average is 0.047. Thus, the degree of genetic differentiation for the four samples analyzed is mainly due to the Amerindian contribution. On the other hand, the results of gene diversity (*h*) analysis in Caucasian samples showed homogeneity among the populations with F_{ST} values ranging 0.001–0.010 (Table 3). These findings suggest that although Argentine population is mixed (26), the level of admixture in the urban sample of La Plata is not enough to anticipate differences in DNA profile frequency when compared with other Caucasian populations.

Statistic data of medical/legal interest are presented in Table 4. Combined probability of exclusion calculation is 0.985 for La Plata sample, 0.966 for Mapuches, 0.972 for Mocovies, 0.90 for Wichis, and 0.962 for the pool of Amerindians. The combined matching probability ranged from ≅1/3400 individuals for Wichis to 1/325,000 for Urbans, ≅1/44,000 for Mapuches, 1/86,000 for Mocovies, and 1/60,000 for the pooled Amerindians. The high interpopulation variability registered in this study justifies the need for

local populations studies that may corroborate the relative efficiency of a genetic marker for these populations.

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