

Frequency of the hypervariable DNA loci D18S849, D3S1744, D12S1090 and D1S80 in a mixed ancestry population of Chilean blood donors

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ABSTRACT. Blood donors (N = 150) at San José Hospital (Santiago, Chile) were typed for one VNTR locus (D1S80) and three STR loci (D18S849, D3S1744, D12S1090). A questionnaire was used to determine the socioeconomic level of the donors, because it is known that some genetic markers (e.g., the ABO and Rh groups) are differentially distributed between different socioeconomic strata. This methodology revealed that two of the three socioeconomic strata distinguishable in Santiago were present in our sample of blood donors, with stratum II representing the middle strata and stratum III the low strata. Allele frequency was determined for each locus and socioeconomic stratum, and it was found that the allele distributions of each locus in socioeconomic strata II and III were statistically similar. All loci conformed to the Hardy-Weinberg law and there was no evidence for association between the alleles of the four loci, allelic frequencies being similar to those found in North American Hispanic populations. The results support the view that the analysis of these loci may have useful applications in population genetics as well as in identity tests.

Key words: DNA polymorphisms, Chilean population, STR, VNTR, DNA typing, Forensic science

INTRODUCTION

In Chile, allele frequencies for genetic markers are known to vary according to socioeconomic strata. For example, the A allele of the ABO blood group and the “d” allele are more frequent in the highest stratum, which has more genes of European origin, while native Amerindian genes are more prevalent in the lowest socioeconomic stratum (Valenzuela et al., 1987). The usefulness of genetic markers for identity testing and paternity analysis in Chile thus depends on knowing the allele frequencies in the different socioeconomic strata, but while the frequencies of the classical genetic markers are known (Valenzuela et al., 1987; Valenzuela, 1988) there have been no studies on socioeconomic class differences in allele frequencies using DNA markers.

The present study describes the allele frequencies for one VNTR locus (D1S80) and three STR loci (D18S849, D3S1744, D12S1090) in an urban population of Northern Santiago, and compares these frequencies with data published in the literature and unpublished data from the Santiago forensic medicine service (Jorquera and Budowle, 1998).

MATERIAL AND METHODS

Blood samples (5 ml) were collected by venous puncture in tubes containing ACD from 150 unrelated blood donors at the San José Hospital blood bank whose catchment area is the Northern part of Santiago. DNA was extracted by an organic method described by Comey et al. (1994), primers for the D1S80 locus were those described by Kasai et al. (1990) and amplification and typing of the D1S80 were carried out by methods described by Budowle et al. (1995). The loci D18S849, D3S1744 and D12S1090 were analyzed using the Multiplex I kit (Lifecodes Corp.) and the alleles resolved by denaturing polyacrylamide gel electrophoresis according to the manufacturer's protocol.

Each blood donor also responded to a socioeconomic classification questionnaire that was used to assign the donor to a socioeconomic strata based on power, prestige, occupation, educational level, income, life style, housing-district and other criteria (Sepúlveda, 1960).

The Amerindian (Matson et al., 1967; Llop and Rothhammer, 1988) and Caucasian (Campillo, 1976) ethnic composition was estimated from the percentage of aboriginal admixture (Bernstein, 1931), using ABO and Rh (anti-D serum) systems as genetic markers.

Allele frequencies at the four DNA loci were calculated by the gene counting method (Li, 1976) and the unbiased estimates of expected heterozygosity (H) as described by Edwards et al. (1992). Standard errors of allele frequencies were calculated as the square root of the variance of a binomial distribution. Possible divergence from Hardy-Weinberg equilibrium expectations was tested for by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (Nei and Roychoudhury, 1974; Nei, 1978; Chakraborty et al., 1988, 1991), using the likelihood ratio test (Chakraborty et al., 1991; Edwards et al., 1992; Weir, 1992), and an exact test (Guo and Thompson, 1992). An interclass correlation criterion (Karlin et al., 1981) for two-locus associations was used for detecting disequilibrium between the alleles at different loci (Chakraborty et al., 1993). The power of exclusion for each locus was determined according to the method of Weiner (1968).

RESULTS AND DISCUSSION

The questionnaire revealed two socioeconomic strata, stratum II (the middle socioeconomic strata) with about 23.7% aboriginal admixture and stratum III (low socioeconomic strata)

with about 37.1% of aboriginal admixture.

Allele frequencies and their standard errors were calculated from the genotypes of individuals for the two strata and the total population (Table 1). The allele distributions at each

Table 1. Allele frequencies and their standard errors at three STR loci and a VNTR locus in low and middle socio-economic strata in a population of Chilean blood donors.

Locus	Allele	Allele frequencies and standard errors		
		Middle strata	Low strata*	Total
D12S1090	9	0.077 ± 0.052	0.072 ± 0.015	0.070 ± 0.014
	10	0.000 ± 0.000	0.017 ± 0.008	0.015 ± 0.006
	11	0.000 ± 0.000	0.048 ± 0.012	0.041 ± 0.011
	12	0.077 ± 0.052	0.062 ± 0.014	0.064 ± 0.013
	13	0.038 ± 0.038	0.017 ± 0.008	0.018 ± 0.007
	14	0.000 ± 0.000	0.024 ± 0.009	0.021 ± 0.008
	15	0.000 ± 0.000	0.014 ± 0.007	0.015 ± 0.006
	16	0.000 ± 0.000	0.007 ± 0.005	0.006 ± 0.004
	17	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003
	18	0.000 ± 0.000	0.021 ± 0.008	0.018 ± 0.007
	19	0.038 ± 0.038	0.051 ± 0.013	0.047 ± 0.011
	20	0.154 ± 0.071	0.065 ± 0.014	0.073 ± 0.014
	21	0.154 ± 0.071	0.051 ± 0.013	0.061 ± 0.013
	22	0.038 ± 0.038	0.110 ± 0.018	0.105 ± 0.017
	23	0.077 ± 0.052	0.089 ± 0.017	0.088 ± 0.015
	24	0.000 ± 0.000	0.072 ± 0.015	0.070 ± 0.014
	25	0.077 ± 0.052	0.072 ± 0.015	0.079 ± 0.015
	26	0.077 ± 0.052	0.103 ± 0.018	0.102 ± 0.016
	27	0.077 ± 0.052	0.051 ± 0.013	0.053 ± 0.012
	28	0.077 ± 0.052	0.024 ± 0.009	0.026 ± 0.009
	29	0.038 ± 0.038	0.017 ± 0.008	0.018 ± 0.007
	31	0.000 ± 0.000	0.007 ± 0.005	0.006 ± 0.004
32	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003	
N		26	292	342
D3S1744	14	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003
	15	0.115 ± 0.063	0.108 ± 0.018	0.108 ± 0.017
	16	0.039 ± 0.038	0.098 ± 0.017	0.099 ± 0.017
	17	0.077 ± 0.052	0.172 ± 0.022	0.166 ± 0.021
	18	0.462 ± 0.098	0.331 ± 0.027	0.337 ± 0.026
	19	0.077 ± 0.052	0.155 ± 0.021	0.148 ± 0.020
	20	0.154 ± 0.071	0.105 ± 0.018	0.108 ± 0.017
	21	0.039 ± 0.038	0.024 ± 0.009	0.023 ± 0.008
	22	0.039 ± 0.038	0.003 ± 0.003	0.009 ± 0.005
	N		26	296
D18S849	13	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003
	14	0.039 ± 0.038	0.034 ± 0.010	0.032 ± 0.010
	15	0.423 ± 0.097	0.255 ± 0.025	0.270 ± 0.025
	16	0.385 ± 0.095	0.389 ± 0.028	0.391 ± 0.027
	17	0.115 ± 0.063	0.232 ± 0.024	0.218 ± 0.023
	18	0.039 ± 0.038	0.081 ± 0.016	0.081 ± 0.015
	19	0.000 ± 0.000	0.007 ± 0.005	0.006 ± 0.004
N		26	298	324
D1S80	17	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003
	18	0.286 ± 0.085	0.240 ± 0.024	0.247 ± 0.023
	19	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003
	20	0.036 ± 0.035	0.026 ± 0.009	0.028 ± 0.009
	21	0.071 ± 0.049	0.030 ± 0.010	0.031 ± 0.009
	22	0.000 ± 0.000	0.026 ± 0.009	0.025 ± 0.008
	23	0.000 ± 0.000	0.013 ± 0.007	0.014 ± 0.006
	24	0.286 ± 0.085	0.332 ± 0.027	0.317 ± 0.025
	25	0.000 ± 0.000	0.066 ± 0.014	0.058 ± 0.012
	26	0.071 ± 0.049	0.007 ± 0.005	0.011 ± 0.006
	27	0.000 ± 0.000	0.010 ± 0.006	0.011 ± 0.005
	28	0.036 ± 0.035	0.049 ± 0.012	0.050 ± 0.011
	29	0.071 ± 0.049	0.023 ± 0.009	0.025 ± 0.008
	30	0.071 ± 0.049	0.046 ± 0.012	0.053 ± 0.012
	31	0.036 ± 0.035	0.086 ± 0.016	0.092 ± 0.015
	32	0.000 ± 0.000	0.007 ± 0.005	0.006 ± 0.004
	33	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003
	34	0.000 ± 0.000	0.020 ± 0.008	0.017 ± 0.007
	36	0.036 ± 0.035	0.003 ± 0.003	0.006 ± 0.004
	37	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003
39	0.000 ± 0.000	0.003 ± 0.003	0.003 ± 0.003	
N		28	304	360

N refers to the number of chromosomes sampled. * Acuña et al., 2000.

locus in strata II and III were statistically similar ($P > 0.05$; χ^2 test). All loci were highly polymorphic and the blood donor database showed no evidence of departure from Hardy-Weinberg equilibrium (Table 2). There was no evidence of association between the loci using the interclass correlation test (Table 3).

Table 2. Summary of Hardy-Weinberg test results for a population of Chilean blood donors.

		D12S1090	D3S1744	D18S849	D1S80
Observed homozygosity	(%)	8.20	19.80	29.90	20.60
Expected homozygosity (unbiased)	(%)	6.50	19.40	27.90	18.00
Homozygosity test *	($\frac{0}{1}$)	0.372	0.912	0.556	0.371
Likelihood test *	($\frac{0}{1}$)	0.679	0.450	0.883	0.204
Exact test *	($\frac{0}{1}$)	0.582	0.564	0.885	0.275

*These values are probability values.

Table 3. Karlin correlation test for a population of Chilean blood donors.

Loci	P value
D12S1090 / D3S1744	0.483
D12S1090 / D18S849	0.060
D12S1090 / D1S80	0.648
D3S1744 / D18S849	0.139
D3S1744 / D1S80	0.370
D18S849 / D1S80	0.070

Comparisons of the distribution frequencies for all loci between the blood donor database and data from the Santiago forensic medicine service were made using the χ^2 homogeneity test, but, as shown in Figure 1, no significant difference was observed between these two Chilean samples for any of the four loci. Figure 1 also compares the Chilean population and four ethnically different populations (US Black, US White, US Hispanic, US Oriental).

Table 4 shows the probability of exclusion for paternity tests; the D12S1090 locus showed one of the highest powers of exclusion (0.8625) in our sample in relation to the other loci. The combined power of exclusion was 0.9908.

Figures 2 and 3 show the frequencies of the different alleles of the four hypervariable DNA loci in different populations. After combining allele classes to avoid low expected values, a χ^2 homogeneity test for allele frequency distribution between the populations was performed. Most alleles were observed in all populations, with the most frequent alleles usually not differing for each population.

For the D18S849 locus the most frequent alleles for all five populations were 15, 16 and 17, with frequencies in the range of 13.6-41.7%. Our results for D18S849 in blood donors are very similar to those of US Hispanics (User's Manual; Lifecodes Corp.).

The frequencies of different D3S1744 locus alleles in the five populations were similar, with allele 18 predominating. The χ^2 test showed significant differences between the Chilean sample and US Black and US Oriental populations (User's Manual; Lifecodes Corp.).

The allele distributions for the D12S1090 locus were bimodal and very similar for the five populations, but a χ^2 homogeneity test for allele frequency distribution between the Chilean population and US Black, US White and US Oriental populations showed significant differences for this locus (User's Manual; Lifecodes Corp.).

Figure 1. Comparison of allele frequencies of four hypervariable loci in blood samples from Northern Santiago blood donors and blood samples from the Santiago forensic medicine service.

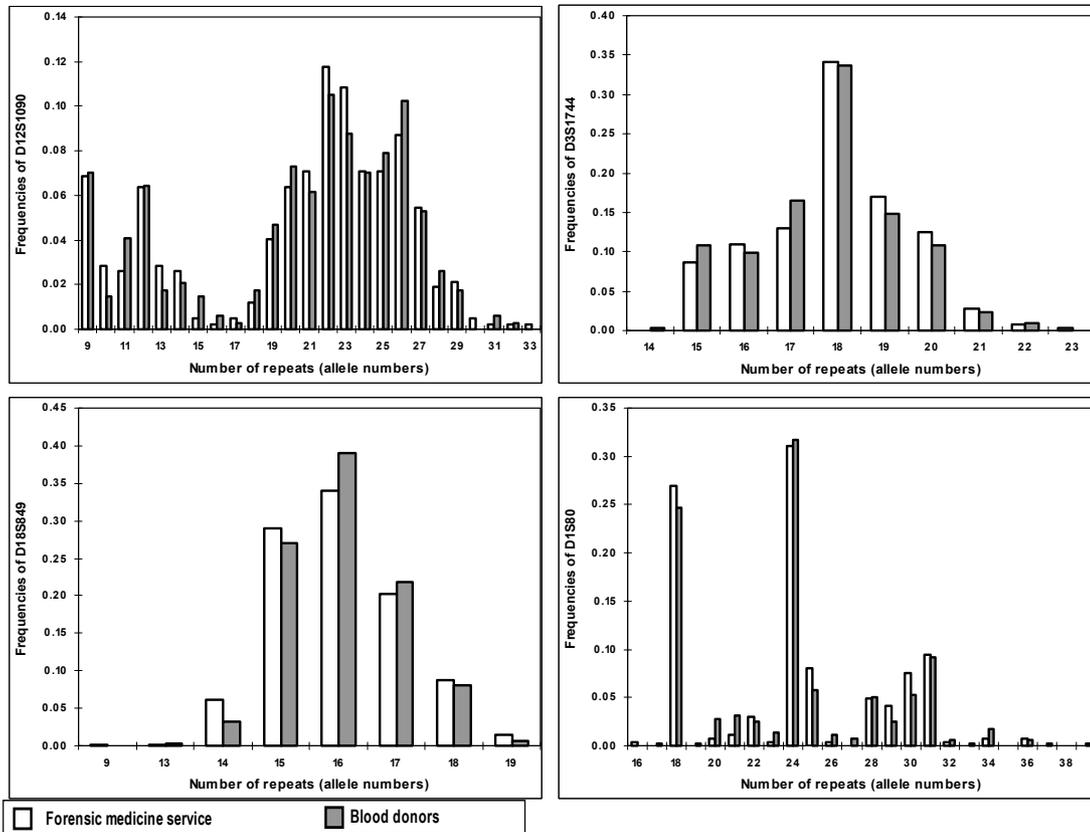


Table 4. Probability of exclusion results for a population of Chilean blood donors.

Loci	P.E.
D12S1090	0.86251463
D3S1744	0.62345420
D18S849	0.47567844
D1S80	0.65916137
Total	0.99074832

P.E., Probability of exclusion.

Comparisons of D1S80 allele distribution between populations (Figure 2) using the χ^2 test showed no significant differences between the Chilean population sample when compared with Southwestern US Hispanics and US Hispanics pooled (Zago et al., 1996; Huckenbeck et al., 1997).

The research presented in this paper shows that the hypervariable DNA loci investigated are distinguishable from other Caucasian (Spanish), Black and Oriental populations, but that the D3S1744 locus is indistinguishable from the Caucasian population. All the loci studied are indistinguishable from USA Hispanic populations (Figures 2 and 3). This study provides the first database for DNA markers in low and middle socioeconomic strata in a

Figure 2. Comparison of allele frequencies of three STRs in five ethnically different populations.

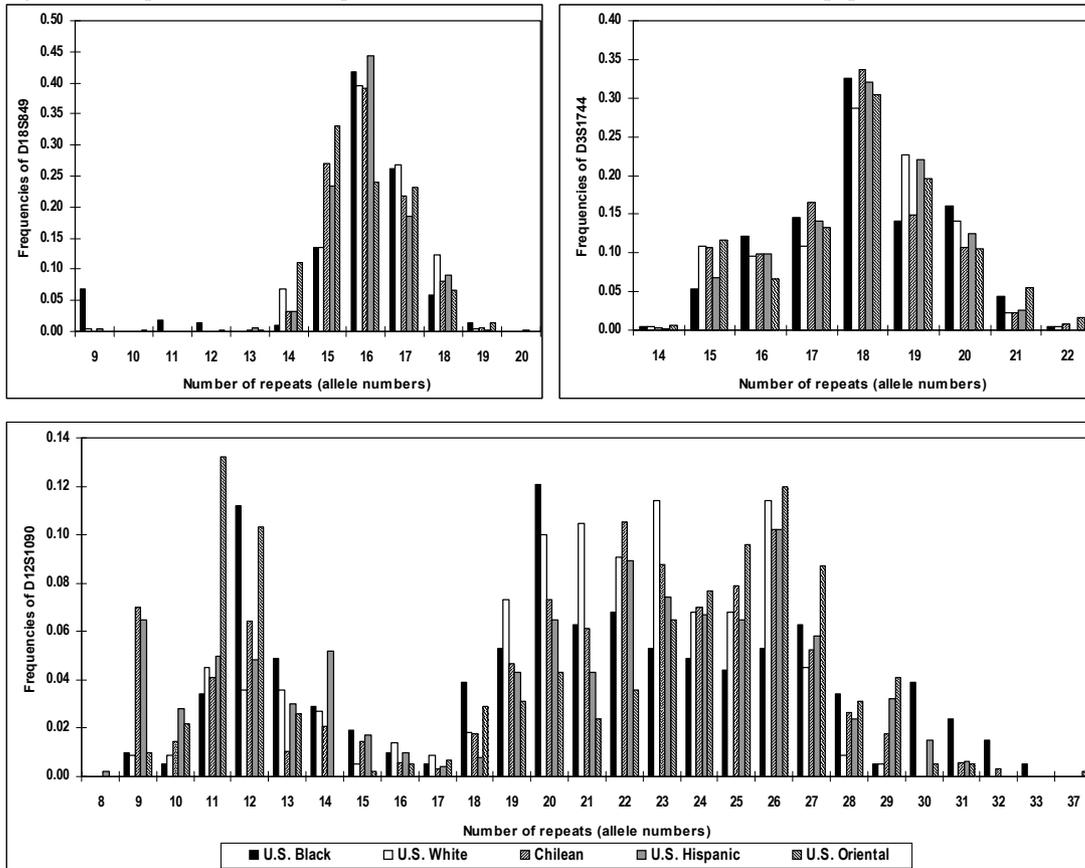
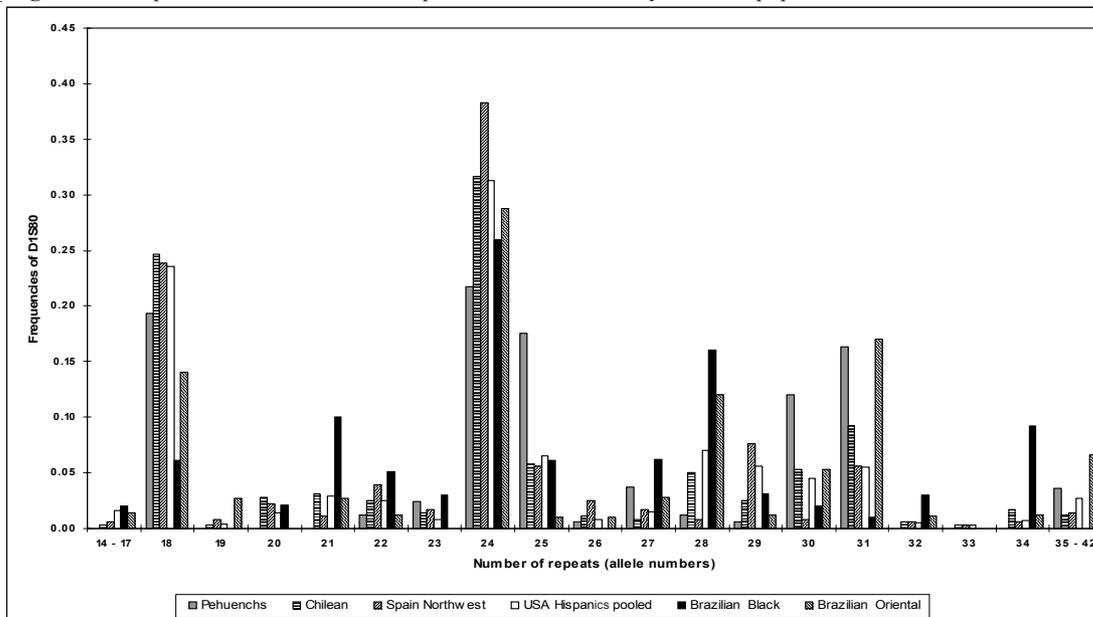


Figure 3. Comparison of D18S0 allele frequencies in six ethnically different populations.



Chilean population. The results presented indicate that the analysis of these loci may have useful applications in population genetics as well as in identity tests.

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