TECHNICAL NOTE

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Chilean Population Data on Ten PCR-Based Loci

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ABSTRACT: Allele frequencies for ten PCR-based loci LDLR, GYPA, HBGG, D7S8, Gc, HLA-DQA1, D1S80, CSF1PO, TPOX and THO1 were determined in unrelated Chileans from Santiago. All loci except HBGG and THO1 meet Hardy-Weinberg expectations. There is little evidence for association of alleles among the ten loci. Only 3 out of 45 pairwise comparisons demonstrated departures from independence. The allelic frequency data are similar to other comparable data.

KEYWORDS: forensic science, Chile, population database, short tandem repeats, Hardy-Weinberg expectations, linkage equilibrium, polymerase chain reaction, DNA typing

The use of the polymerase chain reaction (PCR) in DNA typing of humans has provided a powerful tool for forensic and paternity testing analyses. The PCR-based technologies offer several advantages over the restriction fragment length polymorphism typing method, such as short analysis times, the ability to type degraded samples, and the potential for automating the process. Moreover, several PCR-based commercial kits are available to facilitate typing of genetic markers (1–3).

In order to apply DNA analysis for human identity testing in Chile, a sample population of unrelated individuals from the capital, Santiago, were typed for the loci LDLR (4), GYPA (5), HBGG (6), D7S8 (7), Gc (8), HLA-DQA1 (9), D1S80 (10,11), CSF1PO (12,13), TPOX (14), and HUMTHO1 (12,14,15). The data describe allele frequencies for these ten loci.

Materials and Methods

Sample Preparation: 30 uL of whole blood samples from 132 unrelated individuals from Santiago were deposited on filter papers and allowed to dry at ambient temperature. The DNA was extracted by the organic method described by Comey, et al. (16). The quantity of extracted DNA was estimated using the slot-blot hybridization procedure described by Waye, et al. (17) and Budowle, et al. (18).

Typing: Approximately 1–5 ng of DNA were used in each PCR. The DNA was amplified and typed for the Polymarker (PM) loci LDLR, GYPA, HBGG, D7S8, and Gc using the AmpliType PM PCR Amplification and Typing Kit (Perkin Elmer, Norwalk CT). The HLA-DQA1 locus was amplified and typed using the AmpliType DQα Typing Kit (Perkin-Elmer, Norwalk CT). The primers for the D1S80 locus were those described by Kasai, et al. (10). Amplification and typing of the D1S80 locus was carried out according to the methods described by Budowle, et al. (19). The loci CSF1PO, TPOX, and THO1 were analyzed using the Gene Print Kit (Promega Corp., Madison, WI) and visualized by silver staining (19).

Statistical analysis: the frequency of each allele for each locus was calculated from the numbers of each genotype in the sample set (i.e., the gene count method). Unbiased estimates of expected heterozygosity were computed as described by Edwards et al. (12). Possible divergence from Hardy-Weinberg expectations (HWE) was tested by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (20–23), the likelihood ratio test (12,21,24), and the exact test (25), based on 2000 shuffling experiments. An interclass correlation criterion (26) for two-locus associations was used for detecting disequilibrium between the STR loci. Independence across the ten PCR-based loci also was determined by examining whether or not the observed variance of the number of heterozygous loci in the population sample is outside its confidence interval under the assumption of independence (27,28).

A 2 $\times$ N contingency table exact test was used to generate a G-statistic (2000 shuffling experiments) (29,30) to test for homogeneity between sample populations. The program for this analysis was kindly provided by R. Chakraborty (University of Texas School of Biomedical Sciences, Houston, Texas).

Results and Discussion

The distributions of observed allelic frequencies for the ten loci are shown in Tables 1–4. All loci were highly polymorphic. All loci meet HWE except for the loci HBGG and THO1. The departures from HWE were significant but not highly significant.

An inter-class correlation test analysis detected three departures from independence in 45 pair-wise comparisons of the ten loci (D7S8/Gc, $p = 0.029$; D7S8/D1S80, $p = 0.029$; and HLA-DQA1/D1S80, $p = 0.018$). This number of pair-wise departures is not substantially more than would be expected (6.7% observed versus 5.0% expected). In addition, an alternate method that analyzes all ten loci at one time was used to detect deviation from expectation when applying the product rule to derive a multiple loci

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TABLE 1—*PM allele frequencies in a Chilean sample population (*N = *129).*

Locus/Allele	Frequency
LDLR A*	0.547
LDLR B	0.453
GYPA A†	0.574
GYPA B	0.426
HBGG A‡	0.434
HBGG B	0.531
HBGG C	0.035
D7S8 A§	0.671
D7S8 B	0.329
Gc A‖	0.229
Gc B	0.167
Gc C	0.605

*Observed Homozygosity = 0.481; Expected Homozygosity (unbiased) = 0.502; HWE—Homozygosity Test (p = 0.621), Likelihood Ratio Test (p = 0.608), and Exact Test (p = 0.730).

†Observed Homozygosity = 0.426; Expected Homozygosity (unbiased) = 0.509; HWE—Homozygosity Test (p = 0.061), Likelihood Ratio Test (p = 0.073), and Exact Test (p = 0.073).

‡Observed Homozygosity = 0.465; Expected Homozygosity (unbiased) = 0.470; HWE—Homozygosity Test (p = 0.919), Likelihood Ratio Test (p = 0.028), and Exact Test (p = 0.045).

§Observed Homozygosity = 0.558; Expected Homozygosity (unbiased) = 0.557; HWE—Homozygosity Test (p = 0.969), Likelihood Ratio Test (p = 1.000), and Exact Test (p = 1.000).

‖Observed Homozygosity = 0.411; Expected Homozygosity (unbiased) = 0.444; HWE—Homozygosity Test (p = 0.455), Likelihood Ratio Test (p = 0.168), and Exact Test (p = 0.201).

frequency estimate (27,28). There was no evidence of association among the ten loci assessed in this Chilean population sample using the s_k^2 criterion (s_k^2 = 1.852, 95% confidence interval of variance is 1.692–2.765). The loci HBGG and THO1 reside close to each other on chromosome 11 (at location 11p15.5). However, there was no evidence of departures from the expectation of independence between these two loci (p = 0.841). The results indicate that there is little evidence for detectable gametic phase disequilibrium among the ten loci.

Because of previous data that the general population in Chile is composed predominately of Caucasians and Aboriginal Indians (31), the allele distributions at each locus in this study were compared with sample populations from United States Caucasians and southwestern Hispanics (Table 5). The Chileans and southwestern Hispanics were statistically similar at 9 of the 10 loci, while United States Caucasians were similar at only 5 of the 10 loci. Because of these similarities in allele frequencies between the Chileans and southwestern Hispanics, there would be no anticipated substantial differences in DNA profile frequency estimates if either sample

TABLE 2—*HLA-DQA1 allele frequencies in a Chilean sample population (*N = *130).*

Locus/Allele	Frequency
HLA-DQA1 1.1	0.127
HLA-DQA1 1.2	0.085
HLA-DQA1 1.3	0.031
HLA-DQA1 2	0.123
HLA-DQA1 3	0.235
HLA-DQA1 4	0.400

*Observed Homozygosity = 0.215; Expected Homozygosity (unbiased) = 0.252; HWE—Homozygosity Test (p = 0.342), Likelihood Ratio Test (p = 0.716), and Exact Test (p = 0.862).

TABLE 3—*D1S80 locus allele frequencies in a Chilean sample population (*N = *132).*

Allele	Frequency
16	0.004
17	0.000
18	0.269
19	0.000
20	0.008
21	0.011
22	0.030
23	0.004
24	0.311
25	0.080
26	0.004
27	0.000
28	0.049
29	0.042
30	0.076
31	0.095
32	0.004
33	0.000
34	0.008
35	0.000
36	0.008

*Observed Homozygosity = 0.220; Expected Homozygosity (unbiased) = 0.192; HWE—Homozygosity Test (p = 0.423), Likelihood Ratio Test (p = 0.160), and Exact Test (p = 0.089).

population was used as a reference database for the ten PCR-based loci.

In conclusion, a Chilean database has been developed for the loci LDLR, GYPA, HBGG, D7S8, Gc, HLA-DQA1, D1S80, CSF1PO, TPOX, and THO1. The data suggest that the frequency of a multiple locus profile can be estimated by the application of the product rule. Generally, the allele distributions of these ten loci are similar to southwestern Hispanics.

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TABLE 4—*CSF1PO, TPOX, and THO1 loci allele frequencies in a Chilean sample population (*N = *132).*

Allele	CSF1PO*	TPOX†	THO1‡
6			0.309
7		0.004	0.221
8		0.473	0.076
9	0.011	0.087	0.099
9.3			0.267
10	0.330	0.038	0.023
11	0.280	0.303	0.004
12	0.326	0.091	
13	0.045	0.004	
14	0.008		

*Observed Homozygosity = 0.326; Expected Homozygosity (unbiased) = 0.293; HWE—Homozygosity Test (p = 0.406), Likelihood Ratio Test (p = 0.402), and Exact Test (p = 0.522).

†Observed Homozygosity = 0.333; Expected Homozygosity (unbiased) = 0.331; HWE—Homozygosity Test (p = 0.951), Likelihood Ratio Test (p = 0.278), and Exact Test (p = 0.452).

‡Observed Homozygosity = 0.313; Expected Homozygosity (unbiased) = 0.229; HWE—Homozygosity Test (p = 0.023), Likelihood Ratio Test (p = 0.023), and Exact Test (p = 0.021).

TABLE 5—G *statistic test (*p *values ± SD) for homogeneity allele distributions at ten PCR-based loci between a Chilean sample population and either United States Caucasians or United States southwestern Hispanics.*

Locus	Chile/U.S. Caucasian[a]	Chile/S.W. Hispanic[b]
LDLR	0.041 ± 0.004	0.714 ± 0.010
GYPA	0.859 ± 0.008	0.081 ± 0.006
HBGG	0.057 ± 0.005	0.154 ± 0.011
D7S8	0.192 ± 0.009	0.841 ± 0.008
GC	0.686 ± 0.010	0.212 ± 0.009
HLA-DQA1	0.049 ± 0.005	0.612 ± 0.011
D1S80	$<10^{-3}$	0.056 ± 0.005
CSF1PO	0.260 ± 0.014	0.278 ± 0.014
TPOX	0.011 ± 0.002	0.097 ± 0.007
THO1	$<10^{-3}$	0.006 ± 0.002

*Data from Budowle, et al. (2,32).
†Data from Budowle, et al. (2,32).

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