TECHNICAL NOTE

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Polymarker, HLA-DQA1, and D1S80 Allele Frequency Data in Chamorro and Filipino Populations from Guam*

ABSTRACT: Allele frequencies were determined in sample populations of Chamorros and Filipinos from Guam at the loci LDLR, GYPA, HBGG, D7S8, Gc, HLA-DQA1, and D1S80. Variable number tandem repeat alleles at the D1S80 locus were detected by silver staining following electrophoresis of amplified products in polyacrylamide. Allelic products of the other loci were detected by reverse dot blot hybridization following a multiplex amplification procedure. All loci, in both sample populations, are highly polymorphic and meet Hardy-Weinberg expectations, except for the D1S80 locus in the Chamorro population sample ($p = 0.025$). An interclass correlation analysis detected only one marginally significant departure from independence out of a total of 42 pairwise comparisons of the seven loci for both data sets (LDLR/HBGG in Chamorros, $p = 0.048$). The Chamorro and Filipino allele frequency data are similar to each other at six of the seven loci with only a marginally significant difference at the HLA-DQA1 locus ($p = 0.049$).

KEYWORDS: Chamorro, Filipino, population databases, PCR, Hardy-Weinberg expectations, HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, D1S80

The use of the polymerase chain reaction (PCR) has facilitated typing of human identity testing genetic markers. PCR-based loci, such as low-density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gammaglobin (HBGG), D7S8, group-specific component (Gc) (PM loci), HLA-DQA1, and D1S80, are used routinely around the world (1–7). Currently, population data on these markers from Pacific Island populations are scant. This paper presents allele frequency data in Chamorros and Filipinos, the two largest population subgroups in Guam, for seven PCR-based loci. The data can be used to provide estimates of the frequency of a DNA profile in human identity testing cases in Guam and may prove useful for studying the ethnohistory of human populations.

Materials and Methods

Blood samples from unrelated individuals from two population groups—Chamorros and Filipinos—were collected from volunteer donors from Guam. Each of the Chamorro and Filipino donors claimed to have both parents of Chamorro descent or both parents of Filipino descent, respectively. Procedures for extraction and quantification of the DNA were described previously (8,9).

The PM and HLA-DQA1 loci were amplified and typed using the AmpliType® PM + DQA1 PCR Amplification and Typing Kit (Perkin Elmer Corporation, Norwalk, CT) according to the manufacturer's protocol. The D1S80 locus was typed according to the method described by Budowle et al. (2). Allele designations for the D1S80 locus were determined by comparison of the sample fragments with those of the allelic ladders.

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. (10). Possible divergence from Hardy-Weinberg expectations (HWE) was tested by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (11–14), the likelihood ratio test (10,12,15), and the exact test (16), based on 2000 shuffling experiments. An interclass correlation criterion (17) for two-locus associations was used for detecting disequilibrium between the loci.

A 2 × N contingency table exact test was used to generate a G-statistic (1000 shuffling experiments) (18,19) to test for homogeneity between sample populations. The program was kindly provided by R. Chakraborty (University of Texas School of Biomedical Sciences, Houston).

Results and Discussion

Allele frequency data for the PM, HLA-DQA1, and D1S80 loci in Chamorros and Filipinos are shown in Tables 1–3. All loci are highly polymorphic and meet HWE, except for the D1S80 locus in Chamorros ($p = 0.025$). The probability of discrimination (PD) and exclusion (PE) for the loci are displayed in Tables 4a and 4b. An interclass correlation test detected only one marginally significant departure from independence out of a total of 42 pairwise comparisons of the seven loci in the two data sets: LDLR/HBGG in Chamorros ($p = 0.048$).

The allele frequency data at the seven PCR-based loci in the two Guam population samples were compared with each other

TABLE 1—*Observed HLA-DQA1 allele frequencies in Chamorros and Filipinos from Guam.*

Allele	Chamorros[a] (N = 97)[c]	Filipinos[b] (N = 97)[c]
1.1	0.144	0.098
1.2	0.216	0.351
1.3	0.062	0.082
2	0.062	0.057
3	0.227	0.211
4.1	0.119	0.062
4.2/4.3	0.170	0.139

[a] Chamorros—observed homozygosity = 0.134; expected homozygosity (unbiased) = 0.166; HWE—homozygosity test (p = 0.404), likelihood ratio test (p = 0.276), exact test (p = 0.256).

[b] Filipinos—observed homozygosity = 0.237; expected homozygosity (unbiased) = 0.206; HWE—homozygosity test (p = 0.452), likelihood ratio test (p = 0.750), exact test (p = 0.752).

[c] N = number of individuals in the database.

TABLE 2—*Observed allele frequency distributions for PM loci.*

Allele	Chamorros[a] (N^c = 97)	Filipinos[b] (N^c = 96)
LDLR A	0.232	0.292
LDLR B	0.768	0.708
GYPA A	0.505	0.500
GYPA B	0.495	0.500
HBGG A	0.227	0.182
HBGG B	0.773	0.818
HBGG C	0.000	0.000
D7S8 A	0.459	0.542
D7S8 B	0.541	0.458
Gc A	0.258	0.224
Gc B	0.423	0.474
Gc C	0.320	0.302

[a] Chamorros/LDLR—observed homozygosity = 0.680; expected homozygosity (unbiased) = 0.642; HWE—homozygosity test (p = 0.428), likelihood ratio test (p = 0.382), exact test (p = 0.382).

Chamorros/GYPA—observed homozygosity = 0.526; expected homozygosity (unbiased) = 0.498; HWE—homozygosity test (p = 0.577), likelihood ratio test (p = 0.687), exact test (p = 0.687).

Chamorros/HBGG—observed homozygosity = 0.629; expected homozygosity (unbiased) = 0.648; HWE—homozygosity test (p = 0.702), likelihood ratio test (p = 0.595), exact test (p = 0.775).

Chamorros/D7S8—observed homozygosity = 0.557; expected homozygosity (unbiased) = 0.501; HWE—homozygosity test (p = 0.271), likelihood ratio test (p = 0.313), exact test (p = 0.313).

Chamorros/Gc—observed homozygosity = 0.423; expected homozygosity (unbiased) = 0.344; HWE—homozygosity test (p = 0.102), likelihood ratio test (p = 0.457), exact test (p = 0.413).

[b] Filipinos/LDLR—observed homozygosity = 0.583; expected homozygosity (unbiased) = 0.585; HWE—homozygosity test (p = 0.979), likelihood ratio test (p = 1.000), exact test (p = 1.000).

Filipinos/GYPA—observed homozygosity = 0.458; expected homozygosity (unbiased) = 0.497; HWE—homozygosity test (p = 0.444), likelihood ratio test (p = 0.540), exact test (p = 0.540).

Filipinos/HBGG—observed homozygosity = 0.698; expected homozygosity (unbiased) = 0.700; HWE—homozygosity test (p = 0.959), likelihood ratio test (p = 1.000), exact test (p = 1.000).

Filipinos/D7S8—observed homozygosity = 0.479; expected homozygosity (unbiased) = 0.501; HWE—homozygosity test (p = 0.671), likelihood ratio test (p = 0.680), exact test (p = 0.680).

Filipinos/Gc—observed homozygosity = 0.385; expected homozygosity (unbiased) = 0.363; HWE—homozygosity test (p = 0.644), likelihood ratio test (p = 0.621), exact test (p = 0.560).

[c] N = number of individuals in the database.

TABLE 3—*D1S80 allele frequencies in Chamorros and Filipinos from Guam.*

Allele	Chamorros[a] (N = 99)[c]	Filipinos[b] (N = 97)[c]
17	0.000	0.005
18	0.111	0.160
19	0.005	0.000
20	0.000	0.005
21	0.061	0.052
22	0.010	0.026
23	0.000	0.000
24	0.424	0.258
25	0.040	0.036
26	0.015	0.005
27	0.030	0.010
28	0.020	0.041
29	0.015	0.010
30	0.152	0.227
31	0.106	0.139
32	0.005	0.015
37	0.000	0.000
38	0.005	0.000
39	0.000	0.005
>41	0.000	0.005

[a] Observed homozygosity = 0.253; expected homozygosity (unbiased) = 0.230; HWE—homozygosity test (p = 0.593), likelihood ratio test (p = 0.032), exact test (p = 0.025).

[b] Observed homozygosity = 0.196; expected homozygosity (unbiased) = 0.165; HWE—homozygosity test (p = 0.419), likelihood ratio test (p = 0.912), exact test (p = 0.820).

[c] N = number of individuals.

TABLE 4a—*Power of discrimination (PD) and probability of exclusion (PE) values for the Chamorro sample population.*

Locus	PD (Obs)[a]	PD (Exp)[b]	PE
1 D1S80	0.90705030	0.92388926	0.58869996
2 LDLR	0.52269104	0.52218272	0.14641508
3 GYPA	0.63683707	0.62497343	0.18748671
4 HBGG	0.51525136	0.51694083	0.14461148
5 D7S8	0.64512701	0.62328215	0.18664686
6 Gc	0.81709002	0.80563865	0.35984595
7 HLA-DQA1	0.93591242	0.94868752	0.65966331
Total	0.99996751	0.99997525	0.95676138

[a] PD was calculated using observed genotype frequencies.
[b] PD was calculated using expected genotype frequencies.

TABLE 4b—*Power of discrimination (PD) and probability of exclusion (PE) values for the Filipino sample population.*

Locus	PD (Obs)[a]	PD (Exp)[b]	PE
1 D1S80	0.94781592	0.95051659	0.66766021
2 LDLR	0.56944444	0.57029442	0.16391481
3 GYPA	0.60156250	0.62500000	0.18750000
4 HBGG	0.46332465	0.46292983	0.12684211
5 D7S8	0.61046007	0.62324580	0.18662893
6 Gc	0.79058160	0.79332213	0.34633656
7 HLA-DQA1	0.92847274	0.92915035	0.60194124
Total	0.99997197	0.99997637	0.95828050

[a] PD was calculated using observed genotype frequencies.
[b] PD was calculated using expected genotype frequencies.

TABLE 5—*G-statistic test (p values) for homogeneity in various populations.*

Populations	LDLR	GYPA	HBGG	D7S8	Gc	DQA1	D1S80
Chamorros/Filipinos[a]	0.218	1.000	0.314	0.103	0.556	0.049	0.052
Chamorros/Chinese[b]	1.000	0.046	0.730	0.009	0.707	0.032	0.057
Chamorros/Koreans[c]	0.075	0.136	0.165	0.275	0.199	0.002	$<10^{-3}$
Filipinos/Chinese[b]	0.211	0.034	0.154	0.373	0.430	0.001	0.038
Filipinos/Korean[c]	0.002	0.927	0.007	0.578	0.256	$<10^{-3}$	$<10^{-3}$

[a] Data from current study.
[b] Data from Huang and Budowle (5,6).
[c] Data from Woo and Budowle (7).

(Table 5). The two groups were statistically similar at six of the seven loci, with only one marginally significant difference at the HLA-DQA1 locus ($p = 0.049$). Allele frequencies in the two populations groups also were compared with U.S. Caucasian (1,3) and Oriental (5–7) population data. Allele frequencies at most loci in the Pacific Island populations were statistically different compared with those in U.S. Caucasians, the exceptions being: Chamorros/U.S. Caucasians at the HLA-DQA1 locus ($p = 0.199$), and Filipinos/U.S. Caucasians at the GYPA ($p = 0.402$) and the D7S8 ($p = 0.104$) loci. The Chamorro and Filipino allelic data were more similar to Chinese (5,6) and Korean (7) data (Table 5) than to U.S. Caucasians.

Chamorros are believed to have migrated to Guam from the Philippines and Malaysia approximately 3500 years ago (20). Plato and Cruz (20) showed that the allele frequencies for blood group systems and haptoglobin for Chamorros and Filipinos are similar. The data on the seven PCR-based markers in our current study also suggest that Chamorros and Filipinos are similar. The consistency between the Filipino and Chamorro groups implies greater similarity between these island populations than to major continental population groups (e.g., Caucasian and Asian). This observation may be expected because of the origin of Chamorros and potential admixture from Filipinos who immigrated subsequently to Guam. The protein-based and DNA-based population data also suggest that Caucasian (i.e., Spanish from the colonial era) admixture in the two major island populations of Guam is small. For forensic identity typing purposes, either the Chamorro or the Filipino databases could be used for human identity purposes. Irrespective of which database is used, little difference would be obtained for the estimate of the rarity of a DNA profile. These data support the position of the National Research Council Report (21) that for forensic identity testing where a specific population group does not have a database (e.g., Yapese), allele frequencies from a closely related group (e.g., Chamorros or Filipinos) can be applied.

In conclusion, databases for two Pacific Island populations, Chamorros and Filipinos, have been established for the PCR-based loci LDLR, GYPA, HBGG, D7S8, Gc, HLA-DQA1, and D1S80. The seven loci are highly polymorphic, the distribution of the genotype frequencies for the various loci generally meet HWE, and there is little evidence for departures from expectations of independence of alleles between loci. The data demonstrate that (1) estimates of multiple-locus profile frequencies can be obtained from either Guam database for identity testing purposes using the product rule under the assumption of independence, and (2) Chamorros and Filipinos are genetically similar at the loci analyzed in the current study. The data also may be useful for ethnohistory studies.

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