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Navajo, Pueblo, and Sioux Population Data on the Loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, and D1S80

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ABSTRACT: Navajo, Pueblo, and Sioux population databases were established for the loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, and D1S80. With the exception of HLA-DQA1, the loci appear to be almost as informative in the Native American population samples as for Caucasians, for identity testing purposes. HLA DQA1 is not as informative as the other loci, due to the high frequencies of the '3' and '4' alleles in these Native American groups. Except for GYPA in Navajos, the distribution of the genotype frequencies for the various loci meet Hardy Weinberg expectations. The deviation at the GYPA locus had no affect on generating statistical estimates. Also, there is little evidence for departures from expectations of independence of alleles across loci. The data demonstrate that estimates of multiple locus profile frequencies can be obtained from the Native American databases for identity testing purposes using the product rule under the assumption of independence. In addition, the Navajo, Pueblo, and Sioux databases were more similar to each other than to U.S. Caucasians and African Americans.

KEYWORDS: forensic science, Navajo, Pueblo, Sioux, population databases, PCR, Hardy-Weinberg expectations, linkage equilibrium

The use of the polymerase chain reaction (PCR) (1) and subsequent typing of the amplified products has become an extremely useful technology for genetically characterizing forensic biological specimens. The prevalent PCR-based genetic markers used in North American forensic laboratories are low density lipoprotein receptor (LDLR) (2), glycophorin A (GYPA) (3), hemoglobin G gammaglobin (HBGG) (4), D7S8 (5), group-specific component (Gc) (6) (PM loci), HLA-DQA1 (7,8), and D1S80 (9,10). Population data are now available for U.S. Caucasians, African Americans, Hispanics, and Asians. To date, no Native American population databases containing all seven PCR-based loci have been described. This paper provides population data for the seven loci in Navajo, Pueblo, and Sioux so that the forensic scientist can evaluate, when appropriate, the rarity of multiple locus DNA profiles in additional population groups.

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Materials and Methods

Sample Preparation

Samples consisting of blood, buccal swabs and hair were collected from 81 Navajo and 103 Pueblo individuals. United Blood Services, a statewide New Mexico blood collection agency, collected many of the blood samples by venipuncture during routine blood drives. The remainder of the samples were collected at the Native American pueblos and reservations by Department of Public Safety Crime Laboratory personnel. These blood samples were collected on sterile cotton by finger prick, hairs were manually plucked, and buccal swabs were collected by scraping the oral cavity with sterile cotton swabs. The types of biological samples collected at the pueblos and reservations were determined by personal preference of each individual donor. In addition, bloodstains from 79 known Sioux samples from adjudicated cases were collected by the FBI.

The DNA was extracted by the phenol-chloroform method according to the method of Comey et al. (11) and/or by chelex extraction methods according to Amplitype User Guide, Version 2 (Perkin Elmer Corporation, Norwalk, CT). The quantity of extracted DNA was estimated using the slot-blot procedure described by Budowle et al. (12). One-to-five ng of DNA were used for PCR.

Typing

The DNA samples were amplified and typed for the PM loci by using the AmpliType® PM PCR Amplification and Typing Kit (Perkin Elmer Corporation, Norwalk, CT) according to the manufacturer's protocol. The HLA-DQA1 locus was amplified and typed by using the Amplitype HLA-DQα Forensic DNA Amplification and Typing Kit (Perkin Elmer Corporation, Norwalk, CT). Amplification was carried out in a Perkin-Elmer DNA thermal cycler 480. The D1S80 locus was typed by vertical gel electrophoresis according to the method described by Budowle et al. (13).

Statistical Analysis

The frequency of each allele for each locus was calculated from the numbers of each genotype in the sample set. Unbiased estimates of expected heterozygosity were computed as described by Edwards et al. (14). Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (15–17), the likelihood ratio test (14,18,19), and the exact test (20). An inter-class correlation criterion (21) was used for detecting

disequilibrium between loci pairs. Independence among the seven PCR-based loci was determined by examining whether or not the observed variance of the number of heterozygous loci in the population sample is within its confidence interval under the assumption of independence (22,23).

A 2 × C contingency table exact test was used to generate a G-statistic (1000 shuffling experiments) (24,25) to test for homogeneity between population samples. The program was kindly provided by R. Chakraborty (University of Texas School of Biomedical Sciences, Houston, Texas).

Results and Discussion

The distributions of observed allele and genotype frequencies for the PM, HLA-DQA1, and D1S80 loci in the three Native American populations are shown in Tables 1, 2, 4, 5, and 6. The data demonstrate that the Navajo, Pueblo, and Sioux are highly polymorphic for these loci. Based on heterozygosity at each locus, except for HLA-DQA1, the Native Americans appear to be as polymorphic as Caucasians (13). Furthermore, except for GYPA in the Navajo population, the genotype frequency distributions for the loci do not deviate from HWE based on the homozygosity test, likelihood ratio test, and the exact test (Tables 3, 4, and 6). When using the Bonferroni correction (26) for multiple comparisons, the GYPA departure from HWE was no longer significant.

The deviation observed for GYPA in Navajos demonstrates that the effects of departures from HWE have little impact on forensic statistical estimates (27), particularly for the Navajo database. Table 7 displays the GYPA genotype frequency estimates derived when no assumption of independence is invoked, i.e., the counting method, compared with estimates derived using the assumption of HWE. The difference in frequency estimates by the two methods is marginal; moreover, the product rule for GYPA AA and BB yields a more conservative estimate than the counting method. Thus, there would be no wrongful assumption made if HWE were assumed for GYPA in Navajos.

An analysis was performed to determine whether or not there were any detectable associations between any of the seven PCR-based loci. An inter-class correlation test analysis comparing pairs of loci demonstrated that there were three examples of significant correlations between the alleles. These occurred between Gc/DQA1 and LDLR/D7S8 in the Pueblo population and between D1S80/LDLR in the Sioux population. There were no highly significant correlations (Table 8). After Bonferroni correction (26) and

TABLE 1—*Observed genotype frequency distributions of PM loci.*

	Navajo	Pueblo	Sioux
Genotype	(N = 81)[a]	(N = 103)[a]	(N = 64)[a]
LDLR AA	0.259	0.340	0.328
LDLR AB	0.556	0.524	0.453
LDLR BB	0.185	0.136	0.219
GYPA AA	0.494	0.563	0.547
GYPA AB	0.494	0.350	0.391
GYPA BB	0.012	0.087	0.063
HBGG AA	0.062	0.019	0.188
HBGG AB	0.506	0.388	0.469
HBGG BB	0.432	0.573	0.344
HBGG AC	0.000	0.010	0.000
HBGG BC	0.000	0.010	0.000
HBGG CC	0.000	0.000	0.000
D7S8 AA	0.457	0.262	0.156
D7S8 AB	0.358	0.505	0.500
D7S8 BB	0.185	0.233	0.344
Gc AA	0.012	0.000	0.000
Gc AB	0.037	0.107	0.047
Gc BB	0.148	0.097	0.063
Gc AC	0.049	0.097	0.250
Gc BC	0.346	0.447	0.297
Gc CC	0.407	0.252	0.344

[a]N refers to the number of individuals in the database.

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

	Navajo	Pueblo	Sioux
Allele	(N = 81)[a]	(N = 103)[a]	(N = 64)[a]
LDLR A	0.537	0.602	0.555
LDLR B	0.463	0.398	0.445
GYPA A	0.741	0.738	0.742
GYPA B	0.259	0.262	0.258
HBGG A	0.315	0.218	0.422
HBGG B	0.685	0.772	0.578
HBGG C	0.000	0.010	0.000
D7S8 A	0.636	0.515	0.406
D7S8 B	0.364	0.485	0.594
Gc A	0.056	0.102	0.148
Gc B	0.340	0.374	0.234
Gc C	0.604	0.524	0.617

[a]N refers to the number of individuals in the database.

TABLE 3—*Tests for HWE on PM loci.*

	Navajo	Pueblo	Sioux
LDLR	(N = 81)	(N = 103)	(N = 64)
Obs. Homozygosity	44.4%	47.6%	54.7%
Exp. Homozygosity[a]	50.0%	51.8%	50.2%
Homozygosity Test[b]	0.320	0.386	0.474
Likelihood Ratio Test[b]	0.377	0.397	0.599
Exact Test[b]	0.377	0.397	0.599
GYPA			
Obs. Homozygosity	50.6%	65.1%	60.9%
Exp. Homozygosity[a]	61.4%	61.1%	61.4%
Homozygosity Test[b]	0.047	0.414	0.936
Likelihood Ratio Test[b]	0.014	0.470	1.000
Exact Test[b]	0.014	0.343	1.000
HBGG			
Obs. Homozygosity	49.4%	59.2%	53.1%
Exp. Homozygosity[a]	56.6%	64.2%	50.8%
Homozygosity Test[b]	0.191	0.294	0.714
Likelihood Ratio Test[b]	0.136	0.168	0.790
Exact Test[b]	0.175	0.136	0.790
D7S8			
Obs. Homozygosity	64.2%	49.5%	50.0%
Exp. Homozygosity[a]	53.4%	49.8%	51.4%
Homozygosity Test[b]	0.051	0.954	0.825
Likelihood Ratio Test[b]	0.052	1.000	0.804
Exact Test[b]	0.052	1.000	1.000
Gc			
Obs. Homozygosity	56.8%	35.0%	40.6%
Exp. Homozygosity[a]	48.1%	42.2%	45.4%
Homozygosity Test[b]	0.118	0.135	0.447
Likelihood Ratio Test[b]	0.259	0.124	0.213
Exact Test[a]	0.124	0.245	0.367

[a]Expected homozygosity is an unbiased estimate.
[b]These values are probability values.

TABLE 4—*Distribution of observed HLA-DQα genotype frequencies.*

Genotype	Navajo[a] (*N* = 81)[d]	Pueblo[b] (*N* = 103)[d]	Sioux[c] (*N* = 79)[d]
1.1-1.1	0.000	0.010	0.000
1.1-1.2	0.000	0.000	0.013
1.1-1.3	0.000	0.000	0.000
1.1-2	0.000	0.000	0.000
1.1-3	0.025	0.000	0.013
1.1-4	0.148	0.097	0.051
1.2-1.2	0.000	0.000	0.013
1.2-1.3	0.000	0.000	0.000
1.2-2	0.000	0.000	0.000
1.2-3	0.000	0.000	0.025
1.2-4	0.025	0.019	0.038
1.3-1.3	0.000	0.000	0.000
1.3-2	0.000	0.000	0.000
1.3-3	0.000	0.019	0.013
1.3-4	0.025	0.010	0.000
2-2	0.000	0.000	0.000
2-3	0.000	0.010	0.025
2-4	0.000	0.058	0.013
3-3	0.062	0.010	0.266
3-4	0.210	0.194	0.380
4-4	0.506	0.573	0.152

[a]Navajo—Observed Homozygosity = 0.568; Expected Homozygosity (unbiased) = 0.541; HWE—Homozygosity Test (P = 0.626), Likelihood Ratio Test (P = 0.438), Exact Test (P = 0.654).

[b]Pueblo—Observed Homozygosity = 0.592; Expected Homozygosity (unbiased) = 0.599; HWE − Homozygosity Test (P = 0.897), Likelihood Ratio Test (P = 0.368), Exact Test (P = 0.418).

[c]Sioux—Observed Homozygosity = 0.430; Expected Homozygosity (unbiased) = 0.398; HWE − Homozygosity Test (P = 0.560), Likelihood Ratio Test (P = 0.562), Exact Test (P = 0.354).

[d]N refers to the number of individuals in the database.

TABLE 5—*HLA-DQα observed allele frequencies.*

	Navajo (*N* = 81)	Pueblo (*N* = 103)	Sioux (*N* = 79)
Allele 1.1	0.086	0.058	0.038
Allele 1.2	0.012	0.010	0.051
Allele 1.3	0.012	0.015	0.006
Allele 2	0.000	0.034	0.019
Allele 3	0.179	0.121	0.494
Allele 4	0.710	0.762	0.392

the fact that there were three examples of deviation out of a total of 63 interclass correlation tests, which is approximately 5% of the comparisons (the amount of deviation expected), the data support that the seven PCR-based loci meet expectations of independence in all three Native American sample populations. As an additional test for association, independence among the seven loci was evaluated by examining whether or not the observed variance (s_k^2) of the number of heterozygous loci in the population sample is within its confidence interval under the assumption of independence using the procedure described by Brown et al. (22). There was no evidence of association for the seven loci in the three Native American sample populations using the s_k^2 criterion ($s_k^2{}_{NAVAJO}$ = 1.858, 95% confidence interval of variance is 1.111–2.089; $s_k^2{}_{PUEBLO}$ = 1.563, 95% confidence interval of variance is 1.142–1.993; $s_k^2{}_{SIOUX}$ = 1.896, 95% confidence interval of variance is 1.046–2.218).

TABLE 6—*D1S80 allele frequencies.*

Allele	Navajo[a] (*N* = 72)[d]	Pueblo[b] (*N* = 93)[d]	Sioux[c] (*N* = 60)[d]
14	0.000	0.000	0.000
15	0.000	0.000	0.000
16	0.049	0.108	0.050
17	0.000	0.005	0.000
18	0.153	0.253	0.358
19	0.104	0.027	0.067
20	0.000	0.005	0.008
21	0.042	0.011	0.008
22	0.000	0.011	0.017
23	0.000	0.000	0.000
24	0.215	0.301	0.258
25	0.153	0.091	0.042
26	0.000	0.000	0.067
27	0.000	0.022	0.000
28	0.028	0.027	0.017
29	0.111	0.000	0.000
30	0.042	0.059	0.025
31	0.076	0.075	0.067
32	0.000	0.000	0.017
33	0.000	0.000	0.000
34	0.000	0.000	0.000
35	0.000	0.000	0.000
36	0.000	0.000	0.000
37	0.000	0.000	0.000
38	0.000	0.000	0.000
39	0.000	0.000	0.000
40	0.000	0.000	0.000
41	0.000	0.000	0.000
>41[e]	0.028	0.005	0.000

[a]Navajo—Observed Homozygosity = 0.194; Expected Homozygosity (unbiased) = 0.123; HWE − Homozygosity Test (P = 0.067), Likelihood Ratio Test (P = 0.750), Exact Test (P = 0.554).

[b]Pueblo—Observed Homozygosity = 0.183; Expected Homozygosity (unbiased) = 0.181; HWE − Homozygosity Test (P = 0.972), Likelihood Ratio Test (P = 0.559), Exact Test (P = 0.715).

[c]Sioux—Observed Homozygosity = 0.233; Expected Homozygosity (unbiased) = 0.208; HWE − Homozygosity Test (P = 0.625), Likelihood Ratio Test (P = 0.211), Exact Test (P = 0.380).

[d]N refers to the number of individuals in the database.

[e]All alleles migrating slower than the largest allele in the ladder (that is, allele #41) are placed in the >41 allele class.

TABLE 7—*Navajo GYPA genotype frequencies estimated using the product rule and the counting method.*

Genotype	Product rule	Counting method
AA	0.549	0.494
AB	0.384	0.494
BB	0.067	0.012

The Native American population data for the seven loci generally are significantly different from Caucasian and African American data (13). Out of 35 tests for homogeneity between the loci in each of the Native American groups compared with U.S. Caucasians and African Americans only five were not significantly different (data not shown). However, the Native American groups shared more loci with similar allele frequency distributions among themselves that were not statistically significant (Table 9). As expected, there were more loci that were similar between the Navajo and Pueblo than either Native American sample population compared with Sioux.

TABLE 8—*Two locus inter-class correlation test for HLA-DQA1, PM, and D1S80 loci.*

	Navajo	Pueblo	Sioux
LDLR/GYPA	0.619	0.521	0.186
LDLR/HBGG	1.000	0.820	1.000
LDLR/D7S8	0.201	0.036[a]*	0.912
LDLR/Gc	0.431	0.736	0.272
LDLR/DQA1	1.000	0.659	0.317
GYPA/HBGG	0.695	0.257	0.299
GYPA/D7S8	0.794	0.381	0.555
GYPA/Gc	0.520	0.066	0.842
GYPA/DQA1	0.059	0.181	0.883
HBGG/D7S8	0.172	0.816	0.383
HBGG/Gc	0.215	0.347	0.258
HBGG/DQA1	0.769	0.966	0.202
D7S8/Gc	0.353	0.885	0.472
D7S8/DQA1	0.183	0.641	0.492
Gc/DQA1	0.135	0.028*	0.965
D1S80/LDLR	0.258	0.126	0.039*
D1S80/GYPA	0.805	0.614	0.950
D1S80/HBGG	0.364	0.484	0.874
D1S80/D7S8	0.148	0.730	0.901
D1S80/Gc	0.288	0.811	0.398
D1S80/DQA1	0.118	0.794	0.556

[a]* = deviation at $P = 0.05$ level; With Bonferroni correction the level of rejection is $P = 0.0008$.

TABLE 9—*G statistic test (P values) for homogeneity on PM, HLA-DQA1, and D1S80 allele distributions.*

Locus	Navajo/Pueblo	Navajo/Sioux	Pueblo/Sioux
LDLR	0.254	0.809	0.435
GYPA	1.000	1.00	1.000
HBGG	0.052	0.062	$<10^{-3}$
D7S8	0.023	$<10^{-3}$	0.055
Gc	0.153	0.010	0.026
HLA-DQA1	0.064	$<10^{-3}$	$<10^{-3}$
D1S80	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$

There exist substantial population data on GYPA (known as MN) and Gc based on protein polymorphisms. While some Native American data on MN exist, no statistical comparisons of our data with MN protein population data were made because of known technical limitations of the antisera used for detecting M and N antigens (28). However, our navajo Gc data were compared with protein-based Gc subtyping data on Navajo described by Kamboh and Ferrell (29). There was no significant difference between the Gc allele frequency distributions ($P = 0.372$).

In conclusion, extant data demonstrate that general population groups (that is, U.S. Caucasians, African Americans, etc.) are appropriate as reference groups for forensic identity testing (30–32). Therefore, it was anticipated that subgroup population data on the PM, HLA-DQA1, and D1S80 loci would not be necessary for estimating DNA profile frequencies. However, it is desirable to generate some subgroup population data on Native American groups to demonstrate the degree of polymorphism these subgroups may contain in forensically relevant loci. Navajo, Pueblo, and Sioux population databases have been established for seven PCR-based polymorphic loci. With the exception of HLA DQA1, the loci appear to be almost as informative in the Native American population samples as in Caucasians for identity testing purposes. HLA DQA1 is not as informative as the other loci, due to the high frequencies of the '3' and '4' alleles in these Native American groups. The distribution of the genotype frequencies for the loci meet HWE, and there is little evidence for departures from expectations of independence of alleles across loci. The data demonstrate that estimates of multiple locus profile frequencies can be obtained from the Native American databases for identity testing purposes using the product rule under the assumption of independence. Furthermore, the Navajo, Pueblo, and Sioux databases appear more similar to each other than to U.S. Caucasians and African Americans.

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