TECHNICAL NOTE

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Hungarian Population Data on Seven PCR-Based Loci

REFERENCE: Budowle, B., Woller, J., Koons, B. W., Furedi, S., Errera, J. D., and Padar, Z., **"Hungarian Population Data on Seven PCR-Based Loci,"** *Journal of Forensic Sciences,* JFSCA, Vol. 41, No. 4, July 1996, pp. 667–670.

ABSTRACT: Hungarian population data for the loci LDLR, GYPA, HBGG, D7S8, Gc, HLA-DQA1, and D1S80 were generated. The genotype frequency distributions for the loci do not deviate from Hardy Weinberg expectations. Furthermore, there was little evidence for departures from expectations of independence between the loci. Using a test for homogeneity all the loci were similar between two Hungarian population samples and only the HLA-DQA1 locus was statistically different between Hungarians and US Caucasians. There generally would be little forensic differences, whether a Hungarian or a US Caucasian database was used, for estimating multiple locus profile frequencies for the seven PCR-based loci.

KEYWORDS: forensic science, DNA, Hungary, Population Databases, polymerase chain reaction, Hardy-Weinberg Equilibrium, HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, D1S80

The polymerase chain reaction (PCR)-based loci that predominately have been used in forensic analyses of biological materials in the United States are low density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gammaglobin (HBGG), D7S8, group-specific component (Gc) (PM loci), HLA-DQA1, and D1S80. For the use of these PCR-based genetic markers in identity testing, it is desirable to collect allele/genotype frequency data from relevant population(s) so that the forensic scientist can provide an estimate of the rarity of a multiple locus genetic profile. While substantial population data for the PM, HLA-DQA1, and D1S80 loci exist (1–18, unpublished data), there have been little population data generated on Eastern Europeans (17,18). This paper presents population data on seven PCR-based loci in a Hungarian sample population and compares that data with another Hungarian study and US Caucasians.

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This is publication number 95-13 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation.

Received for publication 17 July 1995; revised manuscript received 10 Oct. 1995; accepted for publication 22 Nov. 1995.

Materials and Methods

Sample Preparation

Whole blood samples were collected in EDTA Vacutainer tubes from 189 unrelated Hungarians and provided by the Institute of Forensic Sciences in Budapest, Hungary for study. The samples were air-dried on cotton cloth and extracted as described previously (19). The quantity of DNA in each sample was estimated using the slot-blot procedure described by Waye et al. (20). Generally, two-to-five ng of DNA were amplified by PCR.

Typing

The PM loci were typed using the AmpliType® PM PCR Amplification and Typing Kit (Perkin-Elmer Corporation, Norwalk, CT). The amplification conditions were those recommended by the manufacturer, except that 16 μg of bovine serum albumin (Sigma, catalog # 3350) were added to the PCR (21,22). Amplification was carried out in a Perkin-Elmer 9600 Thermal Cycler.

The population samples also were typed using the AmpliType® HLA-DQα Forensic DNA Amplification and Typing Kit (Perkin Elmer Corporation, Norwalk, CT) by following the manufacturer's recommended protocol. The HLA-DQα PCR product was derived from the PM multiplex amplification.

The DNA samples were typed for D1S80 according to the method of Budowle et al. (2).

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. (23). Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (24–26), the likelihood ratio test (23,27,28), and the exact test (29). An interclass correlation criterion (30) was used for detecting disequilibrium between loci. Independence across the PM markers, HLA-DQA1, and D1S80 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 (31,32).

A 2 × C contingency table exact test was used to generate a G-statistic (1000 shuffling experiments) (33,34) to test for homogeneity between the Hungarian sample populations and among the Hungarians and a US Caucasian population sample.

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Results and Discussion

The distributions of observed allele and genotype frequencies for the PM, HLA-DQA1, D1S80 loci in our Hungarian population sample are shown in Tables 1, 2, 4, 5, and 6. The genotype frequency distributions for all seven PCR-based loci do not deviate from HWE based on the homozygosity test, likelihood ratio test, and the exact test (Tables 3, 4, and 6).

An analysis was performed to determine whether there were any detectable associations between any of the seven PCR-based loci. An inter-class correlation test analysis demonstrated that there was only one example for correlation between the alleles at any of the pairs of loci. The departure from expectation was between D1S80 and D7S8 loci ($P = 0.03$) (Table 7). The departure is statistically significant, but not highly significant. Regardless, there was only one example of a departure out of a total of 21 interclass correlation tests, which is approximately 5% of the comparisons. Thus, the amount of departures was no more than expected. The data suggest that overall there is little evidence for departures from independence for the seven PCR-based loci in the Hungarian sample population.

TABLE 1—*Observed genotype frequency distributions of PM loci in 182 unrelated Hungarians.*

Genotype	Frequency
LDLR AA	0.159
LDLR AB	0.516
LDLR BB	0.324
GYPA AA	0.324
GYPA AB	0.456
GYPA BB	0.220
HBGG AA	0.247
HBGG AB	0.489
HBGG BB	0.264
HBGG AC	0.000
HBGG BC	0.000
HBGG CC	0.000
D7S8 AA	0.467
D7S8 AB	0.390
D7S8 BB	0.143
Gc AA	0.071
Gc AB	0.071
Gc BB	0.011
Gc AC	0.352
Gc BC	0.154
Gc CC	0.341

TABLE 2—*Observed allele frequency distributions for PM loci in 182 unrelated Hungarians.*

Allele	Frequency
LDLR A	0.418
LDLR B	0.582
GYPA A	0.552
GYPA B	0.448
HBGG A	0.492
HBGG B	0.508
HBGG C	0.000
D7S8 A	0.662
D7S8 B	0.338
Gc A	0.283
Gc B	0.124
Gc C	0.593

TABLE 3—*Tests for independence on PM loci.*

LDLR	
Obs. Homozygosity	48.4%
Exp. Homozygosity*	51.2%
Homozygosity Test†	0.438
Likelihood Ratio Test†	0.441
Exact Test†	0.441
GYPA	
Obs. Homozygosity	54.4%
Exp. Homozygosity*	50.4%
Homozygosity Test†	0.282
Likelihood Ratio Test†	0.296
Exact Test†	0.296
HBGG	
Obs. Homozygosity	51.1%
Exp. Homozygosity*	49.9%
Homozygosity Test†	0.741
Likelihood Ratio Test†	0.890
Exact Test†	0.767
D7S8	
Obs. Homozygosity	61.0%
Exp. Homozygosity*	55.1%
Homozygosity Test†	0.112
Likelihood Ratio Test†	0.111
Exact Test†	0.111
Gc	
Obs. Homozygosity	42.3%
Exp. Homozygosity*	44.6%
Homozygosity Test†	0.535
Likelihood Ratio Test†	0.875
Exact Test*	0.944

*Expected homozygosity is an unbiased estimate.
†These values are probability values.

TABLE 4—*Distribution of observed HLA-DQA1 genotype frequencies in 180 unrelated Hungarians.*

Genotype	Frequency*
1.1–1.1	0.028
1.1–1.2	0.050
1.1–1.3	0.039
1.1–2	0.039
1.1–3	0.022
1.1–4	0.133
1.2–1.2	0.011
1.2–1.3	0.022
1.2–2	0.044
1.2–3	0.039
1.2–4	0.106
1.3–1.3	0.022
1.3–2	0.022
1.3–3	0.028
1.3–4	0.106
2–2	0.011
2–3	0.033
2–4	0.044
3–3	0.011
3–4	0.083
4–4	0.106

*Observed Homozygosity = 18.9%; Expected Homozygosity (unbiased) = 20.4%; HWE—Homozygosity Test ($P = 0.618$), Likelihood Ratio Test ($P = 0.861$), Exact Test ($P = 0.846$).

TABLE 5—*HLA-DQA1 observed allele frequencies in 180 unrelated Hungarians.*

Allele	Frequency
1.1	0.169
1.2	0.142
1.3	0.131
2	0.103
3	0.114
4	0.342

TABLE 6—*D1S80 observed allele frequencies in 189 unrelated Hungarians.*

Allele	Frequency
16	0.003
17	0.003
18	0.265
19	0.000
20	0.021
21	0.029
22	0.037
23	0.013
24	0.368
25	0.045
26	0.013
27	0.008
28	0.058
29	0.050
30	0.005
31	0.056
32	0.003
33	0.000
34	0.003
35	0.000
36	0.011
37	0.008
>41	0.003

Observed Homozygosity = 23.8%; Expected Homozygosity (unbiased) = 21.8%; HWE—Homozygosity Test ($P = 0.492$), Likelihood Ratio Test ($P = 0.062$), Exact Test ($P = 0.128$).

As an additional test for association, independence among the seven loci was evaluated by examining whether 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. (31). There was no evidence of association for the seven loci described in this Hungarian sample population using the s_k^2 criterion (s_k^2 = 1.663; 95% confidence interval of variance is 1.262–1.871).

There were no significant differences in allele frequencies for the loci LDLR, GYPA, HBGG, D7S8, Gc, HLA-DQA1, and D1S80 between our Hungarian sample population and the one described by Woller et al. (17,18). Moreover, only the HLA-DQA1 locus was different statistically between the US Caucasians (2,3) and Hungarians (Table 8). The HLA-DQA1 alleles 1.3 and 2 occur at frequencies of 0.041 and 0.216, respectively, in the US Caucasian sample population, and these were the two alleles that were noticeably different between Hungarians and US Caucasians. Thus, overall an Eastern European population sample is similar to US Caucasians. There would be no anticipated substantial differences in DNA profile frequency estimates, if either sample population were used as a reference database (data in preparation).

In conclusion, a Hungarian population database has been established for seven PCR-based polymorphic loci. The distribution of the genotype frequencies for the various 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 Hungarian database for identity testing purposes using the product rule under the assumption of independence. Additionally, the two different Hungarian databases are similar statistically for the PCR-based loci and the Hungarian samples are similar to US Caucasians for all PCR-based loci, except the HLA-DQA1 locus.

TABLE 7—*Two locus inter-class correlation test for HLA-DQA1, PM, and D1S80 loci for unrelated Hungarians.*

	Hungarians
LDLR/GYPA	0.383
LDLR/HBGG	0.274
LDLR/D7S8	0.278
LDLR/Gc	0.414
LDLR/DQA1	0.776
GYPA/HBGG	0.280
GYPA/D7S8	0.779
GYPA/Gc	0.244
GYPA/DQA1	0.736
HBGG/D7S8	0.914
HBGG/Gc	0.960
HBGG/DQA1	0.853
D7S8/Gc	0.353
D7S8/DQA1	0.914
Gc/DQA1	0.570
D1S80/LDLR	0.498
D1S80/GYPA	0.146
D1S80/HBGG	0.341
D1S80/D7S8	0.031*
D1S80/Gc	0.488
D1S80/DQA1	0.051

*= deviation at $P = 0.05$ level.

TABLE 8—*G statistic test (P values) for homogeneity on PM, HLA-DQA1, D1S80 allele distributions between Hungarians and between a US Caucasian sample population.**

Locus	Hungarian/Hungarian	Hungarian/Hungarian/ US Caucasian
LDLR	0.938 ± 0.008	0.646 ± 0.015
GYPA	0.299 ± 0.015	0.533 ± 0.016
HBGG	0.618 ± 0.015	0.537 ± 0.016
D7S8	0.134 ± 0.011	0.235 ± 0.013
Gc	0.783 ± 0.013	0.393 ± 0.015
HLA-DQA1	0.551 ± 0.016	$<10^{-3}$
D1S80	0.874 ± 0.011	0.414 ± 0.016

*Population data from this study, Woller et al. (17,18), and Budowle et al. (2,3).

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