TECHNICAL NOTE

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The Slovenian Population Data on the PCR Based Loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, and D1S80

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ABSTRACT: Allele frequencies for the loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, and D1S80 were determined for a sample population of unrelated individuals from Slovenia. All loci meet Hardy-Weinberg expectations, except the loci GYPA ($p = 0.041$) and D1S80 ($p = 0.009$). There is little evidence for association of alleles among the seven loci. Only one out of 21 pairwise comparisons demonstrated departures from independence (HLA-DQA1/HBGG, $p = 0.008$). The allelic frequency data generally are similar to that of U.S. Caucasians.

KEYWORDS: forensic science, Slovenia, population database, HLA-DQA1, polymarker, D1S80, Hardy-Weinberg expectations, linkage equilibrium, polymerase chain reaction

Amplification of specific polymorphic regions of DNA by polymerase chain reaction (PCR) is widely used in forensic genetic identity testing of biological material or individuals. The first commercially-available kit for forensic identity testing was based on PCR at the HLA-DQA1 locus and detection using non-radioactive labeled oligonucleotide probes in a reverse dot-blot technique. An additional kit for human identity testing, also based on PCR and reverse dot-blot typing, is the AmpliType PM kit. The PM kit enables amplification of five polymorphic loci simultaneously. These loci are: low density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G-gamma globin (HBGG), D7S8, and group specific component (GC). Another class of DNA markers are the variable number tandem repeat (VNTR) loci which also can be assayed using the PCR. The D1S80 locus is widely used, consists of a 16-base repeat sequence, and can be typed using a commercial kit. After amplification, the D1S80 alleles can be separated and detected by polyacrylamide gel electrophoresis with subsequent silver staining.

For the use of these PCR-based genetic markers in the human identification testing, it is desirable to collect allele/genotype frequency data from relevant population(s) so that a valid estimate of the frequency of the multilocus profile can be provided. This paper presents allele/genotype frequency data for the HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, and D1S80 loci in a Slovenian population sample.

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Materials and Methods

Sample Preparation—Buccal swabs were taken from unrelated individuals from casework. The DNA was extracted using the Chelex method (1).

PCR amplification and typing was performed using the AmpliType HLA-DQA1 and AmpliType PM Amplification and Typing kit (Perkin Elmer Corporation, Norwalk, CT) and a Perkin Elmer 480 thermal cycler according to the manufacturer's recommendations. The DNA samples were also typed for the D1S80 locus by using the AmpliFLP D1S80 Amplification and Typing kit (Perkin Elmer Corporation, Norwalk, CT). This amplification was carried out in a Perkin Elmer GeneAmp 9600 thermal cycler. D1S80 amplification products were analyzed using the Gene-Amp Detection Gel High Resolution Gel Concentrate and Loading Buffer (Perkin Elmer Corporation, Norwalk, CT) and the GIBCO BRL Model SA 32 apparatus (0.8 mm comb and spacers) and GelFix (Serva). The gels were run and analyzed followed by silver staining according to the manufacturer's recommendations.

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. (2). Possible divergence from Hardy-Weinberg expectations (HWE) was tested by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (3–6) and the exact test (7), based on 2000 shuffling experiments. An interclass correlation criterion (8) for two-locus associations was used for detecting disequilibrium between the loci.

Results and Discussion

The distributions of observed allelic frequencies for the seven PCR-based loci are shown in Tables 1–3. All loci were highly polymorphic, and all loci except GYPA ($p = 0.041$) and D1S80 ($p = 0.009$), meet HWE. After employing the Bonferroni correction (9) for the number of loci analyzed (i.e., 7 loci per database), these de-

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TABLE 1—*PM allele frequencies in a Slovenian sample population (n = 401).*

Locus/Allele	Frequency
LDLR A*	0.397
LDLR B	0.603
GYPA A†	0.570
GYPA B	0.430
HBGG A‡	0.545
HBGG B	0.450
HBGG C	0.005
D7S8 A§	0.670
D7S8 B	0.330
Gc A‖	0.315
Gc B	0.118
Gc C	0.566

* Observed Homozygosity = 0.506; Expected Homozygosity (unbiased) = 0.521; HWE - Homozygosity Test ($p = 0.559$) and Exact Test ($p = 0.593$).

† Observed Homozygosity = 0.459; Expected Homozygosity (unbiased) = 0.509; HWE - Homozygosity Test ($p = 0.044$), and Exact Test ($p = 0.041$).

‡ Observed Homozygosity = 0.514; Expected Homozygosity (unbiased) = 0.499; HWE - Homozygosity Test ($p = 0.553$), and Exact Test (p = 0.852).

§ Observed Homozygosity = 0.554; Expected Homozygosity (unbiased) = 0.557; HWE - Homozygosity Test ($p = 0.893$), and Exact Test ($p = 0.906$).

‖ Observed Homozygosity = 0.424; Expected Homozygosity (unbiased) = 0.433; HWE - Homozygosity Test ($p = 0.705$), and Exact Test ($p = 0.496$).

TABLE 2—*HLA-DQA1 allele frequencies in a Slovenian sample population (n = 353).*

Locus/Allele	Frequency
HLA-DQA1 1.1	0.173
HLA-DQA1 1.2	0.202
HLA-DQA1 1.3	0.064
HLA-DQA1 2	0.122
HLA-DQA1 3	0.110
HLA-DQA1 4	0.330

NOTE: Observed Homozygosity = 0.213; Expected Homozygosity (unbiased) = 0.210; HWE - Homozygosity Test ($p = 0.848$), and Exact Test ($p = 0.463$).

TABLE 3—*D1S80 locus allele frequencies in a Slovenian sample population (n = 291).*

Allele	Frequency
16	0.005
17	0.002
18	0.213
19	0.002
20	0.026
21	0.017
22	0.057
23	0.012
24	0.364
25	0.065
26	0.014
27	0.012
28	0.038
29	0.047
30	0.012
31	0.079
32	0.009
33	0.002
34	0.003
36	0.003
37	0.017
39	0.002
40	0.002

NOTE: Observed Homozygosity = 0.192; Expected Homozygosity (unbiased) = 0.196; HWE - Homozygosity Test ($p = 0.883$), and Exact Test ($p = 0.009$).

TABLE 4—*Power of discrimination/probability of exclusion.*

Locus	PD (Obs)*	PD (Exp)†	PE
1 HLA-DQA1	0.92613154	0.92620832	0.59273595
2 LDLR	0.60663802	0.61360128	0.18203006
3 GYPA	0.59213562	0.61998178	0.18503843
4 HBGG	0.63663783	0.63012290	0.19281309
5 D7S8	0.58998389	0.59128250	0.17229507
6 Gc	0.72916213	0.73607921	0.29071825
7 D1S80	0.93534559	0.94192844	0.64073649
Total	0.99996908	0.99997489	0.95377977

* Based on observed genotypes.
† Based on expected genotypes.

partures were no longer considered significant. Furthermore, the observed homozygosity for GYPA and D1S80 was lower than expected values (Tables 1 and 3). Thus, the data do not support that substantial population substructure exists in our Slovenian sample population. The PD and PE for the seven loci are displayed in Table 4.

An inter-class correlation test analysis detected one departure from independence in 21 pair-wise comparisons of the seven loci (HLA-DQA1/HBGG, $p = 0.008$). The results indicate that there is little evidence for detectable gametic phase disequilibrium among the seven loci.

While allele frequencies between the Slovenian data and other Caucasians, such as those reported for the United States (10,11) are generally similar, the allele distributions at the loci HLA-DQA1 ($p = 0.001$), Gc ($p = 0.034$), and D1S80 ($p = 0.004$) were significantly different compared with U.S. Caucasians. For the HLA-DQA1 locus allele 3 is the allele that is different between Slovenians and U.S. Caucasians ($f = 0.110$ versus $f = 0.216$, respectively); for the Gc locus, allele B differs ($f = 0.118$ versus $f = 0.172$); and for the D1S80 locus, allele 37 differs ($f = 0.0.017$ versus 0.001, respectively). However, little difference in seven locus DNA profile frequency estimates would be anticipated using either reference population (data not shown).

In conclusion, a database for the loci HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, and D1S80 is available for Slovenians. The data can be used to estimate multilocus profile frequencies.

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