TECHNICAL NOTE

Marios A. Cariolou,[1] Ph.D.; Panayiotis Manoli,[1] T.E.I.; Maria Christophorou,[1] Evy Bashiardes,[1] M.Phil.; Alexandros Karagrigoriou,[2] Ph.D.; and Bruce Budowle,[3] Ph.D.

Greek Cypriot Allele and Genotype Frequencies for Amplitype® PM-DQA1 and D1S80 Loci

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ABSTRACT: A sample from the Greek Cypriot population was typed at seven forensically important PCR-based loci: LDLR, GYPA, HBGG, D7S8, GC, HLA-DQA1, and D1S80. The results showed that all loci meet Hardy-Weinberg expectations and that there is no evidence for association of alleles between loci. Allelic frequency distributions at all loci, except HLA-DQA1 and two D1S80 alleles, were similar to those of U.S. Caucasians. Greek Cypriot population databases have been created and can be used for forensic analyses to estimate the frequency of a multiple locus DNA profile.

KEYWORDS: forensic science, DNA typing, Cyprus, LDLR, HBGG, GYPA, D7S8, GC, HLA-DQA1, D1S80, population genetics, polymerase chain reaction

DNA identification procedures for civil and forensic investigations were introduced in Cyprus within the past three years. The population of the island of Cyprus is estimated to be 714,000 (1991 census) with an approximate ethnic composition of 80% Greek Cypriots (which includes Armenians, Maronites, and Latins), 18% Turkish Cypriots, and 2% foreign residents. We report here, for the first time, population genetic data for the human leukocyte antigen (HLA) DQA1, PolyMarker (LDLR, GYPA, HBGG, D7S8, GC), and D1S80 loci for the Greek Cypriots. These data may be used in criminal and civil law investigations.

Materials and Methods

Blood samples were collected in EDTA vacutainer tubes, after informed consent, from 107 unrelated Greek Cypriot individuals. DNA was isolated from purified leukocytes using the organic extraction procedure described by Budowle et al. (1). The quantity of DNA in each sample was estimated using the QuantiBlot Kit (Perkin-Elmer Corporation, Norwalk, CT). Two to five nanograms of isolated DNA were amplified by PCR.

The PolyMarker (PM) and HLA-DQA1 loci were amplified and typed on strips using the Amplitype PM-DQA1 Amplification Typing Kit (Perkin-Elmer Corporation, Norwalk, CT). D1S80 amplification was carried out using the primers and amplification protocol described by Kasai et al. (2). PCR products were subsequently separated on a 6% polyacrylamide gel as described by Budowle et al. (3), with the exception that a continuous system was utilized ($0.5\times$ TBE = 45 mM Tris, 45 mM boric acid, 1 mM EDTA) and bisacrylamide was used as the crosslinker. Alleles were identified by comparison with a D1S80 allelic ladder (Perkin-Elmer Corporation, Norwalk, CT) run adjacent to the unknown, amplified population samples.

The frequency of each allele for each locus was calculated from the numbers of each genotype in the sample population. The computed unbiased estimates of expected heterozygosity, divergence from Hardy-Weinberg expectations (HWE) using the unbiased estimate of the expected homozygote/heterozygote frequencies, the likelihood ratio test, the exact test, disequilibrium between pairs of loci using an inter-class correlation criterion, and independence among the seven PCR-based loci were determined as described previously by Budowle et al. (4). A 2×2 contingency table exact test was used to generate a G-statistic (2000 shuffling experiments) (5,6) to test for homogeneity between our Greek Cypriot sample and United States Caucasians. The program for the statistical analyses was kindly provided by R. Chakraborty (University of Texas, School of Biomedical Sciences, Houston, TX).

Results and Discussion

The distribution of observed allele and genotype frequencies for the PM, HLA-DQA1, and D1S80 loci in a population sample of 107 unrelated Greek Cypriots is displayed in Tables 1, 2, and 3. Hardy-Weinberg Expectations (HWE), based on the homozygosity test, likelihood ratio test, and the exact test, were met for all seven PCR-based loci.

An inter-class correlation test analysis and a variance test, performed to determine whether there were any detectable associations between pair-wise comparisons of the seven loci tested, demonstrated that there was no evidence for departures from independence for these loci in Greek Cypriots (highest P-value = 1.000; lowest P-value = 0.065; P level for the analysis was set

[1]Director, DNA ID Lab, biologist, visiting student, molecular biologist, respectively, Cyprus Institute of Neurology and Genetics, DNA Identification Laboratory, Nicosia, Cyprus.

[2]Associate professor, Department of Mathematics and Statistics, University of Cyprus, Nicosia, Cyprus.

[3]Unit Chief, Forensic Science Research and Training Center, FBI Academy, Quantico, VA.

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TABLE 1—*Observed genotype frequency distributions for PM loci in 107 unrelated Greek Cypriots.*

Genotype	LDLR		GYPA		HBGG		D7S8		GC	
	#	Freq.	#	Freq.	#	Freq.	#	Freq.	#	Freq.
AA	24	0.224	36	0.337	27	0.252	42	0.393	11	0.103
AB	56	0.524	56	0.523	50	0.468	52	0.486	11	0.103
BB	27	0.252	15	0.140	23	0.215	13	0.121	3	0.028
AC	...	NA	...	NA	3	0.028	...	NA	35	0.327
BC	...	NA	...	NA	4	0.037	...	NA	21	0.196
CC	...	NA	...	NA	0	0.000	...	NA	26	0.243

#= number of individuals; Freq. = Frequency; NA = Not Applicable (only two alleles). Allele frequencies: LDLR-A = 0.486, LDLR-B = 0.514; GYPA-A = 0.598, GYPA-B = 0.402; HBGG-A = 0.500, HBGG-B = 0.467, HBGG-C = 0.033; D7S8-A = 0.636, D7S8-B = 0.364; GC-A = 0.318, GC-B = 0.178, GC-C = 0.504.

TABLE 2—*Observed HLA-DQA1 genotype frequencies in 107 unrelated Greek Cypriots*

Genotype	#	Frequency*
1.1, 1.1	3	0.028
1.1, 1.2	15	0.140
1.1, 1.3	1	0.009
1.1, 2	2	0.019
1.1, 3	6	0.056
1.1, 4.1	11	0.103
1.2, 1.2	10	0.093
1.2, 1.3	2	0.019
1.2, 2	3	0.028
1.2, 3	7	0.065
1.2, 4.1	19	0.178
1.2, 4.2/4.3	1	0.009
1.3, 3	2	0.019
1.3, 4.1	4	0.037
2, 3	3	0.028
2, 4.1	2	0.019
3, 4.1	8	0.075
4.1, 4.1	8	0.075

= number of individuals. *Observed homozygosity = 19.6%; Expected homozygosity (unbiased) = 22.8%; HWE-Homozygosity test (P = 0.427); Likelihood ratio test (P = 0.910); Exact test (P = 0.964). Allele frequencies: 1.1 = 0.192; 1.2 = 0.313; 1.3 = 0.042; 2 = 0.047; 3 = 0.121; 4.1 = 0.280; 4.2/4.3 = 0.005.

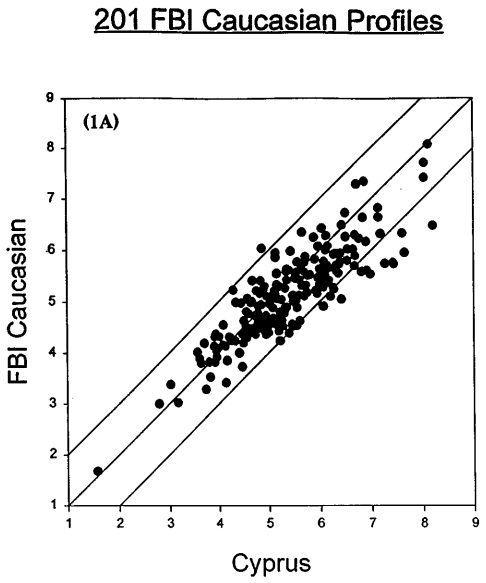
TABLE 3—*D1S80 observed allele frequencies in 107 unrelated Greek Cypriots.*

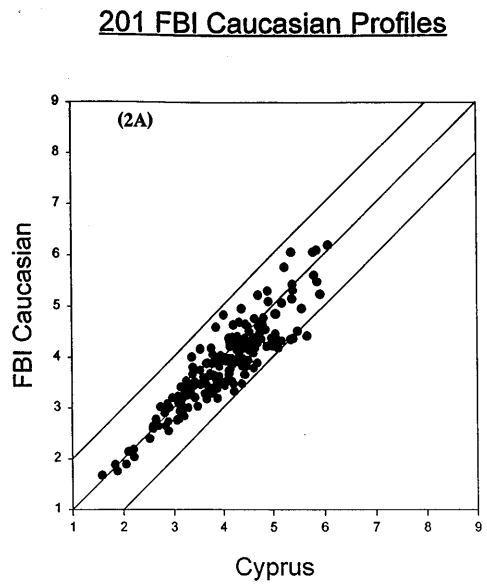
Allele	Frequency*
17	0.014
18	0.178
19	0.014
20	0.005
21	0.037
22	0.079
23	0.005
24	0.393
25	0.051
26	0.019
27	0.056
28	0.075
29	0.042
30	0.014
31	0.019

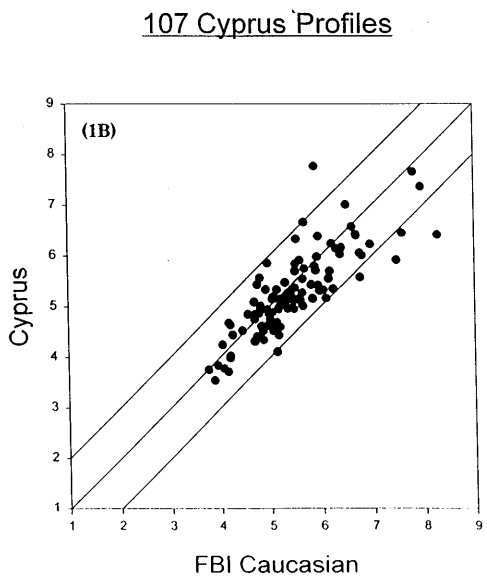
*Observed homozygosity = 15%; Expected homozygosity (unbiased) = 20.4%; Homozygosity test (P = 0.162); Likelihood test (P = 0.196); Exact tests (P = 0.347).

at 0.05; observed variance of heterozygous loci = 1.573; 95% confidence interval for variance is 1.178 to 1.968).

The allele frequencies of the seven PCR-based loci in our Greek Cypriot database were compared with another Caucasian population database (3,4). The Cypriots and U.S. Caucasians were statistically similar at 5 of the 7 loci. The populations were different at the HLA-DQA1 and D1S80 loci ($p < 10^{-3}$). The HLA-DQA1 1.2, 2, and 3 allele frequencies were notably different between Cypriots and U.S. Caucasians (0.313 versus 0.176; 0.047 versus 0.118; and 0.122 versus 0.216, respectively). In fact, the HLA-DQA1 1.2 allele frequency observed in our Cypriot sample population is one of the highest reported for Caucasians, while the HLA-DQA1 2 allele frequency is one of the lowest reported among Caucasians. Although many of the allele frequencies at the D1S80







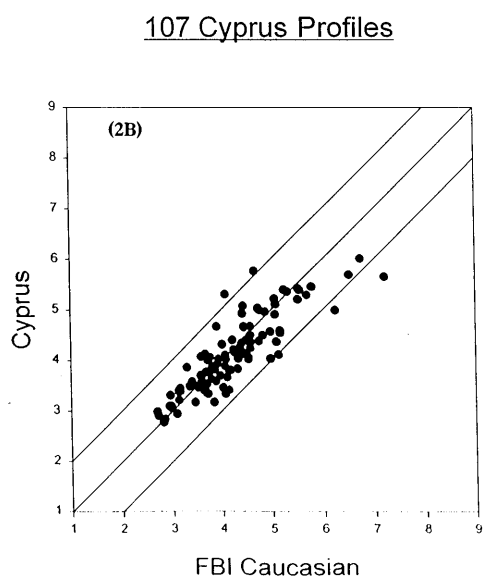


FIG. 1—*Scatter plot comparisons of Cypriot and U.S. Caucasian databases using seven locus profile frequency estimates. The inverse of the frequency estimated for the target profiles is plotted on a logarithmic scale to assess forensic significance between the databases. The* x- *and* y-*axes of each scatter plot are labeled with each population used in the comparison. (1A) 201 U.S. Caucasian target profiles; (1B) 107 Cypriot target profiles.*

FIG. 2—*Scatter plot comparisons of Cypriot and U.S. Caucasian databases using six locus profile frequency estimates. The inverse of the frequency estimated for the target profiles is plotted on a logarithmic scale to assess forensic significance between the databases. The* x- *and* y-*axes of each scatter plot are labeled with each population used in the comparison. (2A) 201 U.S. Caucasian target profiles; (2B) 107 Cypriot target profiles.*

locus were not substantially different between the Cypriots and the U.S. Caucasians, the 27 and 31 alleles did show differences (0.056 versus 0.007 and 0.019 versus 0.072, respectively). The differences observed for the DQA1 and D1S80 alleles above may be due to genetic differences, random drift, or selection of particular D1S80/HLA-DQA1 alleles, or sampling differences.

Recently the allele frequencies of another polymorphic gene (not used for forensic identity testing), apolipoprotein E, were found to differ in Greek Cypriots when compared with 46 different populations around the world (7). Greek Cypriot apolipoprotein E2 and E4 allele frequencies were demonstrated to be among the lowest in the world. In addition, the apolipoprotein E4 allele frequency declined as one traveled from north to south European regions such as Cyprus. Thus, for some genetic markers the allele frequencies in the Cypriot population may be substantially different compared with other Caucasian populations. However, for the genetic markers analyzed in our study, the majority were similar between Cypriots and U.S. Caucasians.

Although differences in allele frequencies were observed at only 2 of the 7 forensically important loci, the possible impact of different allele frequencies on estimates of the rarity of a multiple locus profile using either the Cypriot or U.S. Caucasian database was considered. To compare databases, scatter plots were generated using population data from both populations (Figs. 1 and 2) (8,9).

The inverse of the frequency estimated for the target profiles (Fig. 1*A* and 1*B*) was plotted on a logarithmic (base 10) scale for evaluation of forensic significance between the two reference populations. The data demonstrated that when a profile was rare in one sample population it also was rare in the other. Thus, there was a high correlation in multiple locus profile frequency estimates using either Caucasian database as a reference. However, there were some profiles that differ in frequency by more than ten-fold. To determine whether or not the HLA-DQA1 locus was contributing to the breadth of the plot, the locus was removed from the calculation of a multiple locus profile frequency (i.e., only six loci were used for calculations instead of seven), and the scatter plots were generated again (Fig. 2*A* and 2*B*). As expected, because the HLA-DQA1 locus was excluded from the calculation, all frequencies became slightly more common, and the breadth of the scatter plot was reduced.

Summary

In summary, a Greek Cypriot database has been established for seven PCR-based polymorphic loci. The distributions of the genotype frequencies for the various loci meet HWE, and there is no evidence for departures from expectations of independence of alleles across loci. The Greek Cypriot sample population is similar to U.S. Caucasians for all PM loci and the majority of D1S80 alleles. Frequency differences were noted between two D1S80 and three HLA-DQA1 alleles. Scatter plot analyses, however, suggest that target profiles from Caucasians would have very few instances where differences for DNA profile frequency estimates in Caucasian databases are substantial, and that the HLA-DQA1 locus can have an effect on the differences in frequency estimates. Finally, estimates of multiple locus profile frequencies can be obtained from the Greek Cypriot database for identity testing purposes using the product rule under the assumption of independence.

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Additional information and reprint requests:
Marios A. Cariolou, Ph.D.
Director, DNA Identification Laboratory
CING, P.O.Box 3462, 1683 Nicosia, Cyprus