TECHNICAL NOTE

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Arab Population Data on the PCR-Based Loci: HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, and D1S80

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ABSTRACT: Allele and genotype frequencies for seven polymerase chain reaction (PCR)-based DNA genetic markers were determined in an Arab sample population. The loci analyzed were HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc and D1S80. Results were obtained from the first six loci using the AmpliType HLA-DQα DNA and AmpliType PM PCR Amplification and Typing Kits. The VNTR locus D1S80 PCR product was analyzed by polyacrylamide electrophoresis and silver staining. All loci meet Hardy-Weinberg expectations. The frequency data can be used in forensic analyses and paternity tests to estimate the frequency of a DNA profile in the Arab population.

KEYWORDS: pathology and biology, Arab, population databases, polymerase chain reaction, Hardy-Weinberg, HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, D1S80

The ability to type DNA from biological evidence samples has dramatically increased over the past years. Much of this progress has been driven by the discovery of new genetic polymorphisms among human DNA and the development of new procedures such as the polymerase chain reaction (PCR) [*1–8*]. PCR analysis can be used to amplify regions that contain specific sequence differences such as the HLA-DQA1 and PolyMarker (PM) loci and regions of length polymorphisms termed "amplified fragment length polymorphisms" (AMP-FLPs) [9], such as the D1S80 locus. The AmpliType PM PCR Amplification and Typing Kit (Perkin-Elmer Corporation, Norwalk, CT) enables the simultaneous amplification of the following six loci: HLA-DQA1, low-density-lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gammaglobulin (HBGG), D7S8, and group specific component (Gc). The last five loci make up the PM loci. All six loci are typed using reverse dot blot analysis in which amplified DNA hybridizes to allele specific oligonucleotide probes immobilized on nylon typing strips. Although all six loci are amplified simultaneously, the HLA-DQA1 PCR product is typed independently on nylon typing strips from the AmpliType HLA-DQα forensic DNA amplification and typing kit (Perkin Elmer).

The amplification of variable number of tandem repeat (VNTR) sequences by PCR has also been shown to be highly informative [*10*]. One of the polymorphic regions that can be amplified readily by the PCR is the VNTR which is found at locus D1S80. D1S80 is a VNTR which contains a 16 base pair core repeat and the alleles vary in size as a function of the number of repeat sequences contained within it. After amplification of the D1S80 alleles, the PCR products can be resolved and detected by polyacrylamide gel electrophoresis and silver staining.

It is desirable to collect allele/genotype frequency data from different populations so that the forensic scientist will be able to provide an estimate of the rarity of a genetic profile. This paper presents the first report of the allele/genotype frequency data for HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc and D1S80 loci in an Arab population sample.

Materials and Methods

Sample Preparation

Ninety-four blood samples were collected from unrelated Arabs who underwent a postmortem examination at the Leopold Greenberg Institute of Forensic Medicine in Tel Aviv. The Arabs were identified based on common first names and surnames, and all were Moslems. Thirty individuals came from the Gaza Strip, 34 from Judaea and Samaria, and 36 from Israel. Bloodstains were prepared on cotton gauze, air dried and stored at −20°C. DNA was extracted according to the method of Comey et al. [*11*]. The slot blot procedure described by Waye et al. [*12*], with the exception that a chemiluminescent assay was employed [*13*], was used to estimate the quantity of human DNA extracted from each sample. Generally, 2–5 ng of DNA were amplified in the PCR.

HLA-DQA1 and PolyMarker Typing

The extracted DNA samples were amplified for HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and Gc loci using the AmpliType PM PCR Amplification and Typing kit according to the manufacturer's

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protocol, except 16 μg of bovine serum albumin (BSA) (Sigma Catalog #3350) was added to the PCR [14]. Amplification was carried out in a Perkin-Elmer DNA Thermal Cycler 480. The HLA-DQA1 PCR product was derived from the PM multiplex amplification and were typed using the HLA-DQα typing strips obtained from the AmpliType HLA-DQα PCR Amplification and Typing Kit (Perkin-Elmer Corp.). Interpretation of the typing results was carried out while the strips were still wet.

D1S80 Typing

The DNA was amplified using the D1S80 primers described by Kasai et al. [8]. Primer A is 28 base pairs in length (5'-GAAACTGGCCTCCAAACACTGCCCGCCG-3') and primer B

TABLE 1—*HLA-DQA1 observed allele frequencies in an Arab population sample.*

	Arab (N = 94)
Allele 1.1	0.085
Allele 1.2	0.138
Allele 1.3	0.165
Allele 2	0.096
Allele 3	0.138
Allele 4	0.378

N = The number of individuals in the database.
NOTE: Only 94 of 100 samples were typeable.

TABLE 2—*Distribution of observed HLA-DQA1 genotype frequencies in an Arab population sample.*

Genotype	Arab (N=94)
1.1–1.1	0.000
1.1–1.2	0.021
1.1–1.3	0.032
1.1–2	0.011
1.1–3	0.011
1.1–4	0.096
1.2–1.2	0.021
1.2–1.3	0.074
1.2–2	0.000
1.2–3	0.053
1.2–4	0.085
1.3–1.3	0.032
1.3–2	0.021
1.3–3	0.043
1.3–4	0.096
2–2	0.032
2–3	0.021
2–4	0.074
3–3	0.021
3–4	0.106
4–4	0.149

NOTE: a) Observed homozygosity = 0.255; expected homozygosity (unbiased) = 0.220; HWE - homozygosity test (p=0.413), likelihood ratio test (p=0.553), exact test (p=0.553). b) N refers to the number of individuals in the database.

TABLE 3—*Observed allele frequency distributions for PM loci of 94 Arab samples.*

Allele	LDLR	GYPA	HBGG	D7S8	Gc
A	0.457	0.617	0.388	0.649	0.218
B	0.543	0.383	0.585	0.351	0.271
C	NA	NA	0.027	NA	0.511

NOTE: NA = There is no C allele on the typing strips with the AmpliType PM PCR Amplification and Typing Kit for LDLR, GYPA and D7S8.

is 29 base pairs in length (5'-GTCTTGTTGGAGATG-CACGTGCCCCTTGC-3'). The DNA was amplified by PCR according to the protocol of Baechtel et al. [15]. The PCR was carried out in 50 μL reaction volumes containing 5 ng template DNA at a maximum, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 1 nmole of each of the four deoxyribonucleoside triphosphates, 12.5 pmoles of each primer, 8 μg BSA, and 2.5 units of Taq DNA polymerase. Amplifications were carried out in a Gene Amp PCR System 9600 (Perkin-Elmer Corp.) and were subjected to 27 cycles of denaturation at 95°C for 10 seconds, primer annealing at 67°C for 10 seconds and primer extension at 70°C for 30 seconds.

The amplification products were typed by electrophoretic separation in vertical polyacrylamide gels previously described by

TABLE 4—*Observed frequency distributions of PM loci genotypes in a sample of 94 unrelated Arabs.*

Genotype	LDLR[a]	GYPA[b]	HBGG[c]	D7S8[d]	Gc[e]
AA	0.202	0.383	0.181	0.447	0.021
AB	0.511	0.468	0.383	0.404	0.138
BB	0.287	0.149	0.383	0.149	0.085
AC	NA	NA	0.032	NA	0.255
BC	NA	NA	0.021	NA	0.234
CC	NA	NA	0.000	NA	0.266

NA = There is no C allele on the typing strips with the AmpliType PM PCR Amplification and Typing Kit for LDLR, GYPA and D7S8.
[a]LDLR: observed homozygosity = 0.489, expected homozygosity (unbiased) = 0.501, HWE-homozygosity test (p = 0.822), likelihood ratio test (p = 0.840), exact test (p = 0.840).
[b]GYPA: observed homoqygosity = 0.532, expected homozygosity (unbiased) = 0.525, HWE-homozygosity test (p = 0.891), likelihood ratio test (p = 1.000), exact test (p = 1.000).
[c]HBGG: observed homozygosity = 0.564, expected homozygosity (unbiased) = 0.491, HWE-homozygosity test (p = 0.159), likelihood ratio test (p = 0.315), exact test (p = 0.309).
[d]D7S8: observed homoqygosity = 0.596, expected homozygosity (unbiased) = 0.542, HWE-homozygosity test (p = 0.295), likelihood ratio test (p = 0.360), exact test (p = 0.261).
[e]Gc: observed homozygosity = 0.372, expected homozygosity (unbiased) = 0.379, HWE-homozygosity test (p = 0.900), likelihood ratio test (p = 0.385), exact test (p = 0.415).

TABLE 5—*G statistic (p-values) test for homogeneity based on PM loci allele distribution in Arab, U.S. Caucasian and African American population groups.*

	LDLR	GYPA	HBGG	D7S8	Gc
Arab v. African American	$<10^{-3}$	0.005	$<10^{-3}$	0.467	$<10^{-3}$
Arab v. Caucasian	0.915	0.508	0.044	0.512	$<10^{-3}$
African American v. Caucasian	$<10^{-3}$	0.158	$<10^{-3}$	0.120	$<10^{-3}$

Budowle et al. [16]. The gels were allowed to polymerize overnight at room temperature. Four μL of amplified DNA sample were mixed with 2 μL loading buffer (25 μg xylene cyanol, 25 μg bromophenol blue, and 4 g sucrose per 10 mL of 120 mM Tris-formate, pH 9.0). The entire sample volume (6 μL) was inserted into a gel well. Adjacent to each sample was an allelic ladder [15]. Separation was carried out with settings of 1000 V, 200 mA and 50 W at ambient temperature. The amplified alleles and the ladder on the gel were revealed by silver staining according to previously described procedures [9,15]. Allele designations were determined by visual comparison with fragments in the allelic ladder.

Statistical Analysis

Allelic frequencies of the 7 loci analyzed were calculated from the numbers of each genotype in the sample set (that is, the gene count method). Unbiased estimates of expected heterozygosity were computed as described by Edwards et al. [17]. The expected numbers of distinct homozygous and heterozygous genotypes and their standard error (SE) were calculated according to the method described by Chakraborty et al. [18,19]. Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies [18,20,21], the log likelihood ratio test criterion [17,19,22], and the exact test [23]. An interclass correlation criterion [24] was used for detecting potential disequilibrium between loci. Independence among more than two loci was determined by examining whether or not the observed variance of the number of heterozygous loci in the population sample was outside its confidence interval under the assumption of independence [25,26].

A 2 × C contingency table exact test was used to generate a G-statistic (1000 shuffling experiments) [27,28] to test for homogeneity for the allele frequency distributions between Arab population sample and U.S. Caucasians and African Americans. The program was kindly provided by R. Chakraborty (University of Texas School of Biomedical Sciences, Houston, Texas).

TABLE 7—*Two-locus interclass correlation test for HLA-DQA1, PM and D1S80 loci for 94 unrelated Arabs.*

	Arab (N = 94)
LDLR/GYPA	0.520
LDLR/HBGG	0.225
LDLR/D7S8	0.281
LDLR/Gc	0.506
LDLR/DQA1	0.813
LDLR/D1S80	0.391
GYPA/HBGG	0.621
GYPA/D7S8	0.566
GYPA/Gc	0.740
GYPA/DQA1	0.058
GYPA/D1S80	0.182
HBGG/D7S8	0.407
HBGG/Gc	0.421
HBGG/DQA1	0.025
HBGG/D1S80	0.629
D7S8/Gc	0.703
D7S8/DQA1	0.590
D7S8/D1S80	0.933
Gc/DQA1	0.349
Gc/D1S80	0.244
DQA1/D1S80	0.343

NOTE: N = The number of individuals in the database.

TABLE 6—*D1S80 allele frequencies in a sample of 94 unrelated Arabs.*

Allele	Frequency
16	0.000
17	0.005
18	0.147
19	0.000
20	0.000
21	0.049
22	0.060
23	0.000
24	0.418
25	0.043
26	0.038
27	0.000
28	0.076
29	0.071
30	0.011
31	0.022
32	0.011
33	0.000
34	0.022
35	0.000
36	0.005
37	0.000
38	0.005
39	0.000
40	0.000
41	0.000
>41[a]	0.016

[a]All alleles migrating slower than the largest allele in the ladder (i.e. allele 41) are placed in the >41 allele class.

Observed homozygosity =0.228, expected homozygosity (unbiased) = 0.214, HWE-homozygosity test (p= 0.739), likelihood ratio test (p=0.353), exact test (p= 0.459).

Results and Discussion

The distribution of observed allele and genotype frequencies for HLA-DQA1 in the Arab population sample are shown in Tables 1 and 2. The observed heterozygosity for the locus is 74.5%. The genotype frequency does not deviate from HWE on the basis of the homozygosity test, likelihood ratio test and the exact test. The Arab HLA-DQA1 data are significantly different from U.S. Caucasian and African American databases [15,29] on basis of the G statistic (P-value $<10^{-3}$).

The PM loci analysis is a multiplex amplification and typing approach with readily typeable alleles. This is the first report of allele and genotype distributions of the PM loci for an Arab population sample (Tables 3,4). The five PM loci ranged in observed heterozygosity from 40.4% (for D7S8) to 62.8% (for Gc) in the Arab population sample. There is no detectable deviation from HWE for the PM loci based on the homozygosity test, log likelihood ratio test, and the exact test (Table 5). Significant differences in allele frequencies between the different major population groups might be expected. However, the allele distributions were similar among all three populations for D7S8. Arabs and Caucasians were also similar at the LDLR and GYPA loci. Arabs and African Americans were not similar at any other loci other than D7S8.

For the first time, the distribution of allele and genotype frequencies in an Arab population sample are being reported at the D1S80 locus (Table 6). There were 22 different nominal alleles observed in our population sample of 94 people. The observed heterozygosity for D1S80 in the Arab sample population is 77.2%. A test for independence for the alleles within a locus, based on the number of distinct heterozygote and homozygote genotypes was performed. There was no deviation from expected values. Six different homozygote and 28 different heterozygote genotypes were observed, while the expected number of homozygote and heterozygote classes were 3.5 ± 1.2 and 30.8 ± 3.6, respectively. Additionally, the distribution of D1S80 genotypes does not deviate from HWE based on the homozygosity test, log likelihood ratio test and the exact test (Table 6). Arab D1S80 population data are statistically different when compared to U.S. Caucasian and African American population groups (P-value $<10^{-3}$).

Estimating the frequency of a DNA profile containing all seven loci by the direct count method with a database of 94 individuals is generally not practical because of the limited size of the database. A more meaningful estimate of a multiple locus profile frequency would be obtained using the product rule. Except for GYPA and Gc (which are both on chromosome 4), the other loci in the study are on different chromosomes and, therefore, it might be expected that their frequencies would be independent. Nevertheless, analyses were performed to determine whether or not there were any detectable correlations between HLA-DQA1, PM and D1S80 loci. An interclass correlation test analysis demonstrated that in all cases except one (HBGG/DQA1 – $P = 0.025$) there is no evidence for correlation between the alleles of the paired loci (Table 7). The amount of deviation, that is, one out of the twenty-one comparisons (4.8%) is no more than expected.

In conclusion, an Arab population database has been established for seven PCR-based polymorphic loci of which six can be analyzed in a multiplex fashion. The distribution of the genotype frequencies and alleles for the various loci meet expectations of independence. The data demonstrate that estimates of multiple-locus profile frequencies can be obtained for identity testing purposes using the product rule under the assumption of independence.

References

[1] Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., and Arnheim, N., "Enzymatic Amplification of Beta-Globulin Genomic Sequences and Restriction Analysis for Diagnosis of Sickle Cell Anemia," *Science*, Vol. 230, 1985, pp. 1350–1354.

[2] Gyllensten, U. B. and Erlich H. A., "Generation of Single-Stranded DNA by the Polymerase Chain Reaction and its Application to Direct Sequencing of the HLA-DQα Locus," *Proceedings of the National Academy of Sciences*, USA, Vol. 85, 1988, pp. 7652–7656.

[3] Yamamoto, T., Davis, C. G., Brown, M. S., Schneider, W. J., Casey, M. L., Goldstein, J. L., and Russell, D. W., "The Human LDL Receptor: A Cysteine-Rich Protein with Multiple Alu Sequences in its mRNA," *Cell*, Vol. 39, 1984, pp. 27–38.

[4] Siebert, P. D. and Fukuda, M., "Molecular Cloning of a Human Glycophorin B cDNA: Nucleotide Sequence and Genomic Relationship to Glycophorin A," *Proceedings of the National Academy of Sciences*, USA, Vol. 84, 1987, pp. 6735–6739.

[5] Slightom, J. L., Blechl, A. E., and Smithies, O., "Human Fetal G Gamma- and A Gamma-Globin Genes: Complete Nucleotide Sequences Suggest That DNA Can Be Exchanged Between These Duplicated Genes," *Cell*, Vol. 21, 1980, pp. 627–638.

[6] Horn, G. T., Richards, B., Merrill, J. J., and Klinger, K. W., "Characterization and Rapid Diagnostic Analysis of DNA Polymorphisms Closely Linked to the Cystic Fibrosis Locus," *Clinical Chemistry*, Vol. 36, 1990, pp. 1614–1619.

[7] Yang, F., Brune, J. L., Naylor, S. L., Apples, R. L., and Naberhaus, K. H., "Human Group-Specific Component (Gc) Is a Member of the Albumin Family," *Proceedings of the National Academy of Sciences, USA*, Vol. 82, 1985, pp. 7994–7998.

[8] Kasai, K., Nakamura, Y., and White, R., "Amplification of a Variable Number of Tandem Repeat (VNTR) Locus (pMCT118) by the Polymerase Chain Reaction (PCR) and Its Application to Forensic Science," *Journal of Forensic Sciences*, Vol. 35, 1990, pp. 1196–1200.

[9] Budowle, B., Chakraborty, R., Giusti, A. M., Eisenberg, A. J., and Allen, R. C., "Analysis of the VNTR Loci D1S80 by the PCR followed by High Resolution PAGE," *American Journal of Human Genetics*, Vol. 48, 1991, pp. 137–144.

[10] Kloosterman, A. D., Budowle, B., and Daselarr, P., "PCR-Amplification and Detection of the Human D1S80 VNTR Locus: Amplification Conditions, Population Genetics, and Application in Forensic Analysis," *International Journal of Legal Medecine*, Vol. 105, 1993, pp. 257–264.

[11] Comey, C. T., Koons, B. W., Presley, K. W., Smerick, J. B., Sobieralski, C. A., Stanley, D. M., and Baechtel, F. S., "DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis," *Journal of Forensic Sciences*, Vol. 39, 1994, pp. 1254-1269.

[12] Waye, J. S., Presley, L., Budowle, B., Shutler, G. G., and Fourney, R. M., "A Simple Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts," *Biotechniques*, Vol. 7, 1989, pp. 852–855.

[13] Budowle, B., Baechtel, F. S., Comey, C. T., Giusti, A. M., and Klevan, L., "Simple Protocols for Typing Forensic Biological Evidence: Chemiluminescent Detection for Human DNA Quantitation and RFLP Analyses and Manual Typing of PCR Amplified Polymorphisms," *Electrophoresis* (in press).

[14] Hochmeister, M. N., Budowle, B., Jung, J., Borer, U. V., Comey, C. T., and Dirnhofer, R., "PCR-Based Typing of DNA Extracted from Cigarette Butts," *International Journal of Legal Medicine*, Vol. 104, 1991, pp. 229–233.

[15] Baechtel, F. S., Smerick, J. B., Presley, K. W., and Budowle, B., "Multigenerational Amplification of a Reference Ladder for Alleles at Locus D1S80," *Journal of Forensic Sciences*, Vol. 38, 1993, 1176–1182.

[16] Budowle, B., Baechtel, F. S., Smerick, J. B., Presley, K. W., Giusti, A. M., Parsons, G., Alevy M. C. and Chakraborty, R., "D1S80 Population Data in African Americans, Caucasians, Southeastern Hispanics, and Orientals," *Journal of Forensic Sciences*, Vol. 40, 1995.

[17] Edwards, A., Hammond, H., Jin, L., Caskey, C. T., and Chakraborty, R., "Genetic Variation at Five Trimeric and Tetrameric Repeat Loci in Four Human Population Groups," *Genomics*, Vol. 12, 1992, pp. 241–253.

[18] Chakraborty, R., Smouse, P. E., and Neel, J. V., "Population Amalgamation and Genetic Variation: Observations on Artificially Agglomerated Tribal Populations of Central and South America," *American Journal of Human Genetics*, Vol. 43, 1988, pp. 709–725.

[19] Chakraborty, R., Fornage, M., Guegue, R., and Boerwinkle, E., "Population Genetics of Hypervariable Loci: Analysis of PCR Based VNTR Polymorphism Within a Population," T. Burke, G. Dolf, A. J. Jeffreys, R. Wolff, Eds., *DNA Fingerprinting: Approaches and Applications*, Birkhauser Verlag, Berlin, 1991, pp. 127–143.

[20] Nei, M. and Roychoudhury, A. K., "Sampling Variances of Heterozygosity and Genetic Distance," *Genetics*, Vol. 76, 1974, pp. 379–390.

[21] Nei, M., "Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals," *Genetics 89*, 1978, pp. 583–590.

[22] Weir, B. S., "Independence of VNTR Alleles Defined by Fixed Bins," *Genetics*, Vol. 130, 1992, pp. 873–887.

[23] Guo, S. W. and Thompson, E. A., "Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles," *Biometrics*, Vol. 48, 1992, pp. 361–372.

[24] Karlin, S., Cameron, E. C., and Williams, P. T., "Sibling and Parent-Offspring Correlation Estimation with Variable Family Size," *Proceedings of the National Academy of Sciences U.S.A.*, Vol. 78, 1981, pp. 2664–2668.

[25] Brown, A. H. D., Feldman, M. W., and Nevo, E., "Multilocus Structure of Natural Populations of Hordeum Spontaneum," *Genetics*, Vol. 96, 1980, pp. 523–536.

[26] Chakraborty, R., "Detection of Nonrandom Association of Alleles

from the Distribution of the Number of Heterozygous Loci in a Sample," *Genetics*, Vol. 108, 1984, pp. 719–731.

[27] Roff, D. A. and Bentzen, P., "The Statistical Analysis of Mitochondrial DNA Polymorphisms: χ^2 and the Problem of Small Samples," *Molecular Biology and Evolution*, Vol. 6, 1989, pp. 539–545.

[28] Sokal, R. R. and Rohlf, F. J., *Biometry*, 2nd Edition. W. H. Freeman and Company, San Francisco, 1981.

[29] Budowle, B., Lindsey, J. A., DeCou, J. A., Koons, B. W., Giusti, A. M., and Comey, C. T., "Validation and Population Studies of the Loci LDLR, GYPA, HBGG, D7S8, and Gc (PM loci), and HLA-DQα Using a Multiplex Amplification and Typing Procedure," *Journal of Forensic Sciences*, Vol. 40, 1995, pp. 45–54.

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