# TECHNICAL NOTE

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# Italian Population Allele and Genotype Frequencies for the AmpliType® PM and the HLA-DQ-alpha Loci*

**ABSTRACT:** The distribution of six genetic loci analyzed by PCR using the commercial AmpliType® PM (PolyMarker) kit (Perkin Elmer, Norwalk, CT) was evaluated in 200 unrelated Italian individuals. The examined loci included: Group-specific component (Gc) (1), D7S8 (2), hemoglobin G gammaglobin (HBGG) (3), glycophorin A (GYPA) (4), low density lipoprotein receptor (LDLR) (5), and HLA DQ-alpha (6). The AmpliType PM Kit analysis is based on the reverse dot blot format and the results are interpreted by reading the pattern of blue dots which determine the alleles present at each locus. The population data collected allow the implementation of AmpliType PM into routine casework.

The use of polymerase chain reaction (PCR) for in vitro amplification of specific DNA sequences has made it possible to analyze samples which cannot be typed by Southern methods (7) including DNA from different sources, old and degraded DNA (8–9).

PCR and non-radioactive oligonucleotide probe typing have been used in population studies (10,11). These methods for analyzing DNA were first introduced with the Amplitype HLA DQ-alpha Kit (Roche Molecular System) and expanded with the Amplitype PM (PolyMarker Kit). The Amplitype HLA DQ-alpha Kit was the first PCR-based test applied to forensic analysis based on the reverse dot blot typing technology (12).

The PM Kit co-amplifies specific polymorphic regions of five genetic loci and HLA DQ-alpha in DNA extracted from biological samples (13). The examined loci include: Group-specific component (Gc) (chromosome 4), D7S8 (chromosome 7), hemoglobin G gammaglobin (HBGG) (chromosome 11), glycophorin A (GYPA) (chromosome 4), low density lipoprotein receptor (LDLR) (chromosome 19), and HLA DQ-alpha (chromosome 6).

Following amplification, typing of these loci is performed by

hybridization of the amplified PCR products to DNA probe strips containing sequence specific immobilized probes. The probes distinguish three alleles for Gc and HBGG, two for D7S8, GYPA, and LDLR and six for HLA DQ-alpha. This simple and rapid method can be used to analyze the allele and genotype frequencies in a population.

We present here the distribution of polymorphisms found in a representative Italian population that were determined using the PM kit.

## Material and Methods

### Populations Samples

Whole blood samples were collected from 200 unrelated individuals representative of all Italian regions. Samples were collected from at least five individuals from each region. Genomic DNA was extracted from 500 μL of whole blood with the phenol/chloroform procedure (14), and was quantified by spectrophotometry.

### PCR Amplification

Amplification by PCR was performed using 10 to 50 ng of human genomic DNA, following the protocols described in the Amplitype Package Insert. All the samples were amplified in the Perkin-Elmer DNA Thermal Cycler 480. Some samples were amplified in the GeneAmp PCR Systems 9600 (Perkin-Elmer) to evaluate different PCR instrument systems. Reproducible results were obtained using both of these thermal cyclers.

The PCR products generated using the AmpliType PM Kit were analyzed on a NuSieve 3:1 agarose gel (FMC, Rockland, ME) (Fig. 1).

### Typing of Loci

Amplified PM samples were typed with the AmpliType PM DNA Probe Strips following the manufacturer's protocol. The HLA DQ-alpha was characterized on a separate probe strip supplied in the AmpliType HLA DQ-alpha Kit (Roche Molecular System) under identical conditions.

## Results

### Population Data

Population samples were analyzed to determine the frequencies of the six genetic loci, amplified using the AmpliType PM kit.
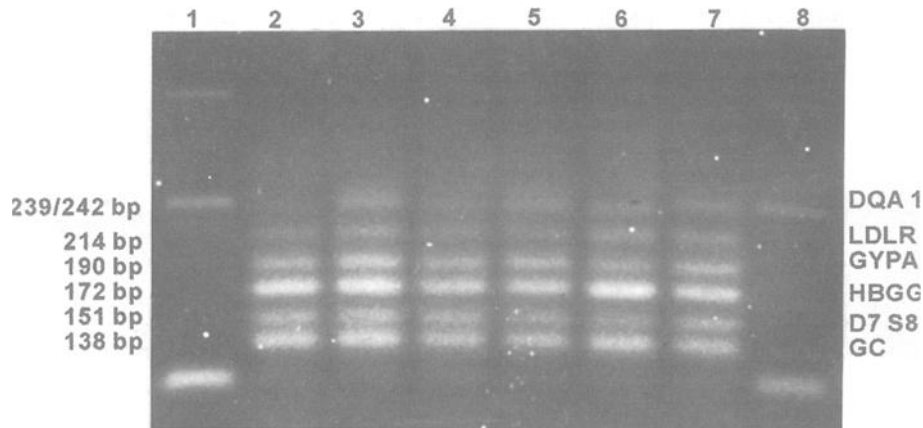
FIG. 1—*AmpliType PM PCR product gel. Lanes 1 and 8; 123 bp Ladder; lanes 2, 3, 4, 5, 6 and 7; 10 ng Genomic DNA.*

The allele and genotype frequencies for the five PM loci are shown in Tables 1 and 2. The allele and genotype frequencies of the HLA DQ-alpha that is co-amplified along with the PM markers are shown in Tables 3 and 4. The expected genotype frequencies (Tables 2 and 4) were calculated from allele frequencies on the basis of the Hardy-Weinberg equilibrium, and compared with the observed genotype values using chi-square test. In the Italian population, no significant differences between the observed and expected genotype frequencies were detected. This result is relevant to the use of the AmpliType PM markers in individual identification, supporting that genotype frequencies can be inferred from the allele frequencies. The power of discrimination values (Pd), calculated on the genotype data (15) for the five AmpliType PM genetic markers and HLA DQ-alpha are shown in Tables 2 and 4. In the Italian population the combined power of discrimination for the PM loci (including HLA DQ-alpha) is 0.9995.

In general, the genotype frequencies found in the Italian population are similar to those previously reported in Caucasians (16) (Tables 5 and 7).

In this study, the most common genotype at the LDLR, GYPA, HBGG and D7S8 loci was "AB," with a frequency of approximately 0.5 (Table 5). The most common genotypes at the "GC" and HLA DQ-alpha loci were "AC" and "CC" (0.325 and 0.31) and 1.2, 4 (0.185). The rarest observed genotypes were "BC" and "CC" for the HBGG locus and HLA DQ-alpha 1.3/2, 1.3/3, and 3/3 (1 out of 200 individuals).

A comparison of the four different populations (shown in Table

5) indicated that the genotype distributions were significantly different. In some cases, Italian versus U.S. Caucasian (16) at the locus D7S8 and Italian versus U.S. Southwestern Hispanic (16) at the loci GYPA, HBGG and D7S8 show significant deviations at the $p = 0.05$ level (Table 6). Table 8 shows that at the HLA DQ-alpha locus there are significant deviations at the $p = 0.05$ level between the Italian and other ethnic groups (16). In order to confirm these differences we used the shuffling method (1000 simulations) to compare allele counts, not genotype counts, because they provide a better sampling. The data suggest (Table 9), as expected, that U.S. Caucasians and U.S. Southeastern Hispanics generally are similar to Italians and that U.S. Southwestern Hispanic are not. The most notable difference is at the HLA DQ-alpha locus, because of the low frequency of the three allele in Italians. Further work is in progress to investigate the significance of this genotype variation.

**Discussion**

The AmpliType PM Kit utilized PCR amplification and reverse dot blot typing for performing genetic population studies and forensic analysis.

These techniques allow simultaneous amplification and typing of six genetic polymorphic loci. The power of discrimination obtained is very informative from a single sample aliquot. The discrimination power for these six markers in the Italian population is 99.95% (Table 4).

In our study, we examined 200 individuals, representative of different regions. We found that the observed genotype frequencies compared well to the expected genotype frequencies, on the bases of the Hardy-Weinberg equilibrium ($p < 0.05$). The close fit to the Hardy-Weinberg equilibrium is important for the implementation of this typing method into forensic casework analysis. The data supports that genotype frequencies can be reliably estimated from allele frequency data because our population is in a panmixis condition. The AmpliType PM Kit, substantially increases the power of discrimination available from a PCR-based test and makes this analysis a valuable tool for individual identification.

*Acknowledgments*

TABLE 1—*Observed allele frequency distributions for the five PM loci in 200 unrelated Italians.*

| Allele | | Frequency |
|--------|--|-----------|
| LDLR | A | 0.47 |
| LDLR | B | 0.53 |
| GYPA | A | 0.55 |
| GYPA | B | 0.45 |
| HBGG | A | 0.44 |
| HBGG | B | 0.55 |
| HBGG | C | 0.01 |
| D7S8 | A | 0.54 |
| D7S8 | B | 0.46 |
| GC | A | 0.30 |
| GC | B | 0.16 |
| GC | C | 0.54 |

TABLE 2—*Observed and expected AmpliType® PM genotype frequencies in the Italian population.*

| Genotype | Observed | | Expected | | |
|---|---|---|---|---|---|
| | $n^a$ | freq. | n | freq. | $\chi^2$ |
| **LDLR** | | | | | |
| AA | 42 | 0.21 | 44.7 | 0.22 | 0.157 |
| AB | 105 | 0.525 | 99.7 | 0.50 | 0.282 |
| BB | 53 | 0.265 | 55.7 | 0.28 | 0.126 |
| | | | | Total | 0.565† |
| Total | 200 | | | | |
| **GYPA** | | | | | |
| AA | 58 | 0.29 | 59.4 | 0.30 | 0.033 |
| AB | 102 | 0.51 | 99.2 | 0.50 | 0.080 |
| BB | 40 | 0.20 | 41.4 | 0.20 | 0.048 |
| | | | | Total | 0.161† |
| Total | 200 | | | | |
| **HBGG** | | | | | |
| AA | 40 | 0.20 | 38.3 | 0.19 | 0.077 |
| AB | 93 | 0.465 | 96.25 | 0.48 | 0.110 |
| AC | 2 | 0.01 | 2.19 | 0.01 | * |
| BB | 63 | 0.315 | 60.5 | 0.30 | 0.103 |
| BC | 1 | 0.005 | 2.75 | 0.014 | * |
| CC | 1 | 0.005 | 0.03 | 0.0002 | * |
| | | | | Total | 0.479† |
| Total | 200 | | | | |
| **D7S8** | | | | | |
| AA | 62 | 0.31 | 57.2 | 0.286 | 0.395 |
| AB | 90 | 0.45 | 99.5 | 0.498 | 0.909 |
| BB | 48 | 0.24 | 43.2 | 0.216 | 0.523 |
| | | | | Total | 1.827† |
| Total | 200 | | | | |
| **GC** | | | | | |
| AA | 16 | 0.08 | 18.3 | 0.09 | 0.289 |
| AB | 24 | 0.12 | 19.66 | 0.09 | 0.957 |
| AC | 65 | 0.325 | 64.74 | 0.324 | 0.001 |
| BB | 8 | 0.04 | 5.28 | 0.264 | 1.400 |
| BC | 25 | 0.125 | 34.78 | 0.174 | 2.748 |
| CC | 62 | 0.31 | 57.25 | 0.286 | 0.395 |
| | | | | Total | 5.790† |
| Total | 200 | | | | |

Expected genotype frequencies were calculated assuming Hardy-Weinberg equilibrium from the allele frequencies in Table 1. The combined power of discrimination of PM without HLA-DQ-alpha was (PD1) = 99.54.

$n^a$ = Refers to the number of individuals (n = 200) in the database.
*All genotypes with less than 3 observations were pooled together (n observed = 4, n expected = 4.97 $\chi^2$ = 0.189).
†$\chi^2$ LDLR = 0.565, 0.25 < P < 0.50, df = 1.
†$\chi^2$ GYPA = 0.161, 0.10 < P < 0.25, df = 1.
†$\chi^2$ HBGG = 0.479, 0.25 < P < 0.50, df = 1.
†$\chi^2$ D7S8 = 1.827, 0.10 < P < 0.25, df = 1.
†$\chi^2$ GC = 5.79, 0.10 < P < 0.25, df = 3.

TABLE 3—*Observed allele frequency distributions for HLA-DQ-alpha locus in 200 unrelated Italians.*

| Allele | Frequency |
|---|---|
| 1.1 | 0.175 |
| 1.2 | 0.190 |
| 1.3 | 0.0425 |
| 2 | 0.130 |
| 3 | 0.0525 |
| 4 | 0.410 |

TABLE 4—*Observed and expected AmpliType® HLA-DQ-alpha genotype frequencies in the Italian population.*

| Genotype | Observed | | Expected | | |
|---|---|---|---|---|---|
| | $n^a$ | freq. | n | freq. | $\chi^2$ |
| **HLA-DQ-alpha** | | | | | |
| 1.1, 1.1 | 5 | 0.025 | 6.13 | 0.03 | 0.207 |
| 1.1, 1.2 | 7 | 0.035 | 13.30 | 0.066 | 2.984 |
| 1.1, 1.3 | 4 | 0.02 | 2.98 | 0.015 | 0.353 |
| 1.1, 2 | 11 | 0.055 | 9.10 | 0.045 | 0.397 |
| 1.1, 3 | 6 | 0.03 | 3.68 | 0.018 | 1.471 |
| 1.1, 4 | 32 | 0.16 | 28.7 | 0.143 | 0.379 |
| 1.2, 1.2 | 10 | 0.05 | 7.22 | 0.036 | 1.07 |
| 1.2, 1.3 | 2 | 0.01 | 3.23 | 0.016 | * |
| 1.2, 2 | 8 | 0.04 | 9.88 | 0.049 | 0.358 |
| 1.2, 3 | 2 | 0.01 | 3.99 | 0.02 | * |
| 1.2, 4 | 37 | 0.185 | 31.16 | 0.156 | 1.094 |
| 1.3, 1.3 | 2 | 0.01 | 0.36 | 0.0018 | * |
| 1.3, 2 | 1 | 0.005 | 2.21 | 0.111 | * |
| 1.3, 3 | 1 | 0.005 | 0.89 | 0.0045 | * |
| 1.3, 4 | 5 | 0.025 | 6.97 | 0.035 | 0.557 |
| 2, 2 | 5 | 0.025 | 3.38 | 0.017 | 0.776 |
| 2, 3 | 3 | 0.015 | 2.73 | 0.0137 | 0.027 |
| 2, 4 | 19 | 0.095 | 21.32 | 0.1066 | 0.252 |
| 3, 3 | 1 | 0.005 | 0.55 | 0.0028 | * |
| 3, 4 | 7 | 0.035 | 8.61 | 0.043 | 0.301 |
| 4, 4 | 32 | 0.16 | 33.62 | 0.168 | 0.078 |
| | | | | Total | 10.748† |
| Total | 200 | | | | |

Expected genotype frequencies were calculated assuming Hardy-Weinberg equilibrium from the allele frequencies in Table 1. The combined power of discrimination of PM with HLA-DQ-alpha was (PD2) = 99.95.
$n^a$ = Refers to the number of individuals (n = 200) in the database.
*All genotypes with less than 3 observations were pooled together (n observed = 9, n expected = 11.23 $\chi^2$ = 0.443).
†$\chi^2$ HLA-DQ-alpha = 10.748, 0.25 < P < 0.50, df = 10.

TABLE 5—*PM genotype distribution in Italian Caucasian, U.S. Caucasian, and U.S. Hispanic populations.*

| PM Genotype | | Italian Caucasian (n = 200) | USA Caucasian* (n = 148) | USA Southeastern Hispanic* (n = 94) | USA Southwestern Hispanic* (n = 96) |
|---|---|---|---|---|---|
| LDLR | AA | 0.210 | 0.176 | 0.191 | 0.313 |
| LDLR | AB | 0.525 | 0.554 | 0.447 | 0.500 |
| LDLR | BB | 0.265 | 0.270 | 0.362 | 0.188 |
| GYPA | AA | 0.290 | 0.351 | 0.330 | 0.448 |
| GYPA | AB | 0.510 | 0.466 | 0.404 | 0.417 |
| GYPA | BB | 0.200 | 0.182 | 0.266 | 0.135 |
| HBGG | AA | 0.200 | 0.223 | 0.160 | 0.135 |
| HBGG | AB | 0.465 | 0.493 | 0.521 | 0.365 |
| HBGG | AC | 0.010 | 0.000 | 0.011 | 0.052 |
| HBGG | BB | 0.315 | 0.277 | 0.266 | 0.046 |
| HBGG | BC | 0.005 | 0.000 | 0.043 | 0.042 |
| HBGG | CC | 0.005 | 0.007 | 0.000 | 0.000 |
| D7S8 | AA | 0.310 | 0.358 | 0.340 | 0.458 |
| D7S8 | AB | 0.450 | 0.514 | 0.489 | 0.448 |
| D7S8 | BB | 0.240 | 0.128 | 0.170 | 0.094 |
| GC | AA | 0.080 | 0.054 | 0.053 | 0.083 |
| GC | AB | 0.120 | 0.074 | 0.181 | 0.083 |
| GC | AC | 0.325 | 0.331 | 0.266 | 0.292 |
| GC | BB | 0.040 | 0.047 | 0.043 | 0.063 |
| GC | BC | 0.125 | 0.176 | 0.181 | 0.208 |
| GC | CC | 0.310 | 0.318 | 0.277 | 0.271 |

*USA population data Budowle et al. (16).

TABLE 6—*Comparison of PM genotype distribution in Italian Caucasian, U.S. Caucasian and U.S. Hispanic populations (2-way R × C contingency table).*

| Population | Locus | $\chi^2$ | P |
|---|---|---|---|
| Italian/U.S. Caucasian | LDLR | 0.655 (NS) | 0.75 < P < 0.90 |
| Italian/U.S. Caucasian | GYPA | 1.481 (NS) | 0.25 < P < 0.50 |
| Italian/U.S. Caucasian | HBGG | 3.032 (NS) | 0.50 < P < 0.75 |
| Italian/U.S. Caucasian | D7S8 | 6.819* | 0.025 < P < 0.05 |
| Italian/U.S. Caucasian | GC | 4.215 (NS) | 0.50 < P < 0.75 |
| Italian/U.S. Southeastern Hispanic | LDLR | 2.910 (NS) | 0.10 < P < 0.25 |
| Italian/U.S. Southeastern Hispanic | GYPA | 3.094 (NS) | 0.10 < P < 0.25 |
| Italian/U.S. Southeastern Hispanic | HBGG | 7.267 (NS) | 0.10 < P < 0.25 |
| Italian/U.S. Southeastern Hispanic | D7S8 | 1.830 (NS) | 0.25 < P < 0.50 |
| Italian/U.S. Southeastern Hispanic | GC | 4.714 (NS) | 0.25 < P < 0.50 |
| Italian/U.S. Southwestern Hispanic | LDLR | 4.504 (NS) | 0.05 < P < 0.10 |
| Italian/U.S. Southwestern Hispanic | GYPA | 7.429* | 0.010 < P < 0.025 |
| Italian/U.S. Southwestern Hispanic | HBGG | 15.09* | P = 0.01 |
| Italian/U.S. Southwestern Hispanic | D7S8 | 11.19* | P = 0.005 |
| Italian/U.S. Southwestern Hispanic | GC | 5.037 (NS) | 0.25 < P < 0.50 |

*Significant deviations at P = 0.05 level.
NS = Not significant.

TABLE 7—*HLA-DQ-alpha genotype distribution in Italian Caucasian, U.S. Caucasian and U.S. Hispanic populations.*

| HLA-DQ-alpha Genotype | Italian Caucasian (n = 200) | USA Caucasian* (n = 148) | USA Southeastern Hispanic* (n = 94) | USA Southwestern Hispanic* (n = 96) |
|---|---|---|---|---|
| 1.1, 1.1 | 0.025 | 0.014 | 0.064 | 0.021 |
| 1.1, 1.2 | 0.035 | 0.068 | 0.064 | 0.052 |
| 1.1, 1.3 | 0.02 | 0.007 | 0.011 | 0.000 |
| 1.1, 2 | 0.055 | 0.041 | 0.053 | 0.031 |
| 1.1, 3 | 0.03 | 0.054 | 0.032 | 0.042 |
| 1.1, 4 | 0.16 | 0.047 | 0.074 | 0.115 |
| 1.2, 1.2 | 0.05 | 0.020 | 0.032 | 0.021 |
| 1.2, 1.3 | 0.01 | 0.000 | 0.021 | 0.010 |
| 1.2, 2 | 0.04 | 0.061 | 0.032 | 0.031 |
| 1.2, 3 | 0.01 | 0.068 | 0.064 | 0.083 |
| 1.2, 4 | 0.185 | 0.115 | 0.064 | 0.052 |
| 1.3, 1.3 | 0.01 | 0.014 | 0.011 | 0.000 |
| 1.3, 2 | 0.005 | 0.007 | 0.032 | 0.000 |
| 1.3, 3 | 0.005 | 0.014 | 0.032 | 0.031 |
| 1.3, 4 | 0.025 | 0.027 | 0.043 | 0.021 |
| 2, 2 | 0.025 | 0.020 | 0.043 | 0.010 |
| 2, 3 | 0.015 | 0.041 | 0.043 | 0.021 |
| 2, 4 | 0.095 | 0.047 | 0.074 | 0.083 |
| 3, 3 | 0.005 | 0.061 | 0.032 | 0.042 |
| 3, 4 | 0.035 | 0.135 | 0.149 | 0.198 |
| 4, 4 | 0.16 | 0.142 | 0.032 | 0.135 |

*USA population data Budowle et al. (16).

TABLE 8—*Comparison of HLA-DQ-alpha genotype distribution in Italian Caucasian, U.S. Caucasian and U.S. Hispanic populations. (2-way R × C contingency table).*

| Population | Locus | $\chi^2$ | P |
|---|---|---|---|
| Italian/U.S. Caucasian | HLA-DQ-alpha | 55.346* | P < 0.005 |
| Italian/U.S. Southeastern Hispanic | HLA-DQ-alpha | 56.020* | P < 0.005 |
| Italian/U.S. Southwestern Hispanic | HLA-DQ-alpha | 54.691* | P < 0.005 |

*Significant deviations at P = 0.05 level.

TABLE 9—*Comparison between AmpliType PM and HLA-DQ-alpha alleles distribution in Italian Caucasian, U.S. Caucasian and U.S. Hispanic populations.*

| Populations | Locus | P-Value |
|---|---|---|
| Italian/U.S. Caucasian | LDLR | 0.721 |
| Italian/U.S. Caucasian | GYPA | 0.4111 |
| Italian/U.S. Caucasian | HBGG | 0.695 |
| Italian/U.S. Caucasian | D7S8 | 0.034* |
| Italian/U.S. Caucasian | Gc | 0.466† |
| Italian/U.S. Caucasian | HLA-DQ-alpha | <10⁻³† |
| Italian/U.S. Southeastern Hispanic | LDLR | 0.206 |
| Italian/U.S. Southeastern Hispanic | GYPA | 0.721 |
| Italian/U.S. Southeastern Hispanic | HBGG | 0.413 |
| Italian/U.S. Southeastern Hispanic | D7S8 | 0.311 |
| Italian/U.S. Southeastern Hispanic | Gc | 0.165 |
| Italian/U.S. Southeastern Hispanic | HLA-DQ-alpha | <10⁻³† |
| Italian/U.S. Southwestern Hispanic | LDLR | 0.034* |
| Italian/U.S. Southwestern Hispanic | GYPA | 0.013* |
| Italian/U.S. Southwestern Hispanic | HBGG | 0.005† |
| Italian/U.S. Southwestern Hispanic | D7S8 | 0.002† |
| Italian/U.S. Southwestern Hispanic | Gc | 0.339 |
| Italian/U.S. Southwestern Hispanic | HLA-DQ-alpha | <10⁻³† |

*Significant deviations.
†Highly significant deviations.
Data from FBI Laboratory.

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