**TECHNICAL NOTE**

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# Italian Population Data on the Polymarker System and on the Five Short Tandem Repeat Loci CSF1PO, TPOX, TH01, F13B, and vWA

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**ABSTRACT:** A population study on five short tandem repeat (STR) loci and five sequence specific polymorphism loci was performed on unrelated Italian Caucasians. Separation and detection of the amplified STR fragments were carried out by high resolution vertical denaturing polyacrylamide gel electrophoresis (PAGE) and silver staining, respectively. The sequence specific loci were analyzed using the AmpliType PM Typing Kit (Perkin Elmer, Foster City, CA). All loci, except Gc ($p = 0.031$), meet Hardy-Wienberg expectations. In addition, there is no evidence for association of alleles between pairs of loci. The combined power of discrimination for the five STR loci is 0.9999862 and for the PM loci is 0.99503. The results suggest that these loci may be useful for human identification cases in Italy.

**KEYWORDS:** forensic science, population genetics, DNA typing, HumvWA31, HumF13B, HumTH01, HumTPOX, HumCSF1PO, LDLR, GYPA, HBGG, D7S8, Gc, Italy

Many forensic laboratories worldwide are evaluating and implementing highly polymorphic DNA loci, whose polymorphism derive from tetrameric tandem repeated core sequences (i.e., short tandem repeat (STR) loci) or by differences in the sequence (i.e., sequence specific allele systems). Simultaneous amplification and typing of a number of loci have enhanced the capabilities of the forensic laboratory.

This current study investigated Italian population data for the STR loci HumvWA31, HumF13B, HumTH01, HumTPOX and HumCSF1PO (1–5) and the PolyMarker (PM) loci LDLR, GYPA, HBGG, D7S8 and Gc (6). The data can be useful for human identification testing cases.

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## Materials and Methods

### *Sample Preparation*

Whole blood samples from unrelated Italians were drawn in EDTA vacutainer tubes. Approximately 150 to 200 μL blood samples were placed onto cotton cloth and allowed to air dry, and only a portion (2 mm by 2 mm) was used for extraction. The DNA was extracted organically and quantified using the slot-blot procedure described by Waye et al. (7).

### *PCR Amplification*

Amplification by PCR of the STR loci was performed using the Geneprint™ STR Systems Kit (Promega, Corp., Madison, WI) according to the manufacturer's recommendations. The PM loci were analyzed using the Amplitype PM Typing Kit (Perkin Elmer, Foster City, CA). The PCRs were carried out with slight modification to the user manual guide by mixing 25 μL final volume containing 10 to 20 ng of template DNA and 1.25 units of Taq DNA polymerase. The PCR was performed in a Perkin Elmer 2400 Thermal Cycler after a 2 min hot start denaturation.

### *STR Typing*

The amplified DNA samples and allelic ladders were prepared by mixing 2.5 μL of sample and 2.5 μL of STR 2X loading dye (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol FF), heating the mixture at 95°C for two min, and then snap cooling on ice.

The STR products were separated on the SA-32 vertical gel electrophoresis apparatus (BRL, Bethesda, MD) using a 4% polyacrylamide gel (4% T, 5% C, 31 cm long and 0.4 mm thick) containing 7M urea and 0.5X Tris-Borate-EDTA buffer. After polymerization and prior to sample loading, the PAG was subjected to electrophoresis for 60 min at 40 W. Subsequently, the samples were subjected to electrophoresis for approximately 80 min at 40 W. The runs were stopped approximately 10′ to 15′ after the xylene cyanol migrated out of the anodal end of the gel. The separated STR fragments then were visualized by silver staining (9,10). Allele designation was determined by comparison with allelic ladders that were run in adjacent lanes.



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*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 heterogosity were computed as described by Edwards et al. (11). Possible divergence from Hardy-Weinberg expectations (HWE) was tested by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (12–15), the likelihood ratio test (11,13,16), and the exact test (17), based on 2000 shuffling experiments. An interclass correlation criterion (18) for two locus associations was used for detecting disequilibrium between loci.

The power of discrimination $P_D$ (the probability that two individuals chosen at random from a given population have different phenotypes) was calculated using the Fisher's formula:

$$P_D = 1 - \sum_{i=1}^{n} p_i^2$$

where $p_i$ = expected phenotype frequencies.

## Results and Discussion

The distribution of allele frequencies and observed genotype counts at the ten PCR-based loci in our Italian sample population are shown in Tables 1–6. All observed STR alleles were based on a tetranucleotide repeat motif, except for the commonly occurring TH01 9.3 allele. The observed heterozygosities for the STR loci range from 64.4% for the TPOX locus to 82.8% for the vWA locus. The heterozygosities values for the PM loci were slightly lower than the STR loci, ranging from 45.9% for the GYPA locus to 54.8% for the Gc locus.

Nine of ten loci meet HWE (Table 7). The only locus to depart from HWE was the Gc locus ($p = 0.031$, based on the exact test). This departure from HWE is significant, but not highly significant, and the Gc allele frequencies are not substantially different from other population data. There was no detectable departure from the

TABLE 1—*Distribution of allele frequencies and observed genotype counts of vWA locus in 204 unrelated Italians.*

| Genotype counts | | | | | | | | | | | | | Allele observed | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <11 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | | Num. | Percent |
| <11 | - | | | | | | | | | | | | 13 | 1 | 0.245 |
| 11 | - | - | | | | | | | | | | | 14 | 34 | 8.333 |
| 12 | - | - | - | | | | | | | | | | 15 | 49 | 12.010 |
| 13 | - | - | - | - | | | | | | | | | 16 | 97 | 23.775 |
| 14 | - | - | - | 1 | 2 | | | | | | | | 17 | 98 | 24.020 |
| 15 | - | - | - | - | 4 | 2 | | | | | | | 18 | 83 | 20.343 |
| 16 | - | - | - | - | 9 | 10 | 13 | | | | | | 19 | 38 | 9.314 |
| 17 | - | - | - | - | 10 | 17 | 24 | 8 | | | | | 20 | 8 | 1.961 |
| 18 | - | - | - | - | 4 | 11 | 20 | 18 | 8 | | | | | | |
| 19 | - | - | - | - | 2 | 3 | 8 | 10 | 12 | 1 | | | | | |
| 20 | - | - | - | - | - | - | - | 3 | 2 | 1 | 1 | | | | |
| 21 | - | - | - | - | - | - | - | - | - | - | - | - | | | |

TABLE 2—*Distribution of allele frequencies and observed genotype counts of F13B locus in 200 unrelated Italians.*

| Genotype counts | | | | | | | Allele observed | | |
|---|---|---|---|---|---|---|---|---|---|
| | 6 | 7 | 8 | 9 | 10 | 11 | | Num. | Percent |
| 6 | 3 | | | | | | 6 | 40 | 10.000 |
| 7 | - | 1 | | | | | 7 | 7 | 1.750 |
| 8 | 9 | 1 | 14 | | | | 8 | 105 | 26.250 |
| 9 | 9 | 3 | 28 | 6 | | | 9 | 98 | 24.500 |
| 10 | 16 | 1 | 38 | 46 | 24 | | 10 | 149 | 37.250 |
| 11 | - | - | 1 | - | - | - | 11 | 1 | 0.250 |

TABLE 3—*Distribution of allele frequencies and observed genotype counts of TH01 locus in 205 unrelated Italians.*

| Genotype counts | | | | | | | | | | | Allele observed | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <5 | 5 | 6 | 7 | 8 | 8.3 | 9 | 9.3 | 10 | 11 | | Num. | Percent |
| <5 | - | | | | | | | | | | 6 | 120 | 29.268 |
| 5 | - | - | | | | | | | | | 7 | 53 | 12.927 |
| 6 | - | - | 20 | | | | | | | | 8 | 45 | 10.976 |
| 7 | - | - | 15 | 2 | | | | | | | 8.3 | 0 | 0.000 |
| 8 | - | - | 12 | 4 | 5 | | | | | | 9 | 80 | 19.512 |
| 8.3 | - | - | - | - | - | - | | | | | 9.3 | 106 | 25.854 |
| 9 | - | - | 24 | 11 | 6 | - | 10 | | | | 10 | 5 | 1.220 |
| 9.3 | - | - | 27 | 19 | 13 | - | 18 | 14 | | | 11 | 1 | 0.244 |
| 10 | - | - | 2 | - | - | - | 1 | - | 1 | | | | |
| 11 | - | - | - | - | - | - | - | 1 | - | - | | | |

TABLE 4—*Distribution of allele frequencies and observed genotype counts of TPOX locus in 202 unrelated Italians.*

| Genotype counts | | | | | | | | | | Allele observed | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <6 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | | Num. | Percent |
| <6 | - | | | | | | | | | 6 | 2 | 0.495 |
| 6 | - | - | | | | | | | | 7 | 2 | 0.495 |
| 7 | - | - | - | | | | | | | 8 | 215 | 53.218 |
| 8 | - | 1 | 2 | 58 | | | | | | 9 | 55 | 13.614 |
| 9 | - | - | - | 25 | 4 | | | | | 10 | 25 | 6.188 |
| 10 | - | 1 | - | 14 | 3 | 1 | | | | 11 | 95 | 23.515 |
| 11 | - | - | - | 50 | 19 | 5 | 9 | | | 12 | 10 | 2.475 |
| 12 | - | - | - | 7 | - | - | 3 | - | | | | |
| 13 | - | - | - | - | - | - | - | - | - | | | |

TABLE 5—*Distribution of allele frequencies and observed genotype counts of CSF1PO locus in 202 unrelated Italians.*

| Genotype counts | | | | | | | | | | | Allele observed | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <7 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | Num. | Percent |
| <7 | - | | | | | | | | | | 7 | 2 | 0.495 |
| 7 | - | - | | | | | | | | | 8 | 3 | 0.743 |
| 8 | - | - | - | | | | | | | | 9 | 20 | 4.950 |
| 9 | - | - | - | 1 | | | | | | | 10 | 94 | 23.267 |
| 10 | - | 2 | 1 | 6 | 8 | | | | | | 11 | 128 | 31.683 |
| 11 | - | - | 1 | 5 | 29 | 24 | | | | | 12 | 129 | 31.931 |
| 12 | - | - | - | 5 | 31 | 38 | 23 | | | | 13 | 22 | 5.446 |
| 13 | - | - | - | 2 | 7 | 7 | 6 | - | | | 14 | 6 | 1.485 |
| 14 | - | - | 1 | - | 2 | - | 3 | - | - | | | | |
| 15 | - | - | - | - | - | - | - | - | - | - | | | |

TABLE 6—*Distribution of allele frequencies and observed genotype counts of PM loci in 15 unrelated Italians.*

| • **LDLR**: | *Genotype counts* | | *Allele Observed* | | |
|---|---|---|---|---|---|
| | A | B | | Num. | Percent |
| A | 31 | | A | 146 | 46.497 |
| B | 84 | 42 | B | 168 | 53.503 |

| • **GYPA:** | *Genotype counts* | | *Allele Observed* | | |
|---|---|---|---|---|---|
| | A | B | | Num. | Percent |
| A | 48 | | A | 168 | 53.503 |
| B | 72 | 37 | B | 146 | 46.497 |

| • **HBGG**: | *Genotype counts* | | | *Allele Observed* | | |
|---|---|---|---|---|---|---|
| | A | B | C | | Num. | Percent |
| A | 28 | | | A | 138 | 43.949 |
| B | 78 | 46 | | B | 171 | 54.459 |
| C | 4 | 1 | | C | 5 | 1.592 |

| • **D7S8**: | *Genotype counts* | | *Allele Observed* | | |
|---|---|---|---|---|---|
| | A | B | | Num. | Percent |
| A | 60 | | A | 200 | 63.694 |
| B | 80 | 17 | B | 114 | 36.306 |

| • **GC**: | *Genotype counts* | | | *Allele Observed* | | |
|---|---|---|---|---|---|---|
| | A | B | C | | Num. | Percent |
| A | 11 | | | A | 78 | 24.841 |
| B | 20 | 1 | | B | 52 | 16.561 |
| C | 36 | 30 | 59 | C | 184 | 58.599 |

TABLE 7—*Summary of Hardy-Weinberg tests.*

| | |
|---|---|
| **1 LDLR** | |
| Observed Homozygosity | 46.5% |
| Expected Homozygosity (unbiased) | 50.1% |
| Homozygosity Test* | 0.368 |
| Likelihood Test* | 0.408 |
| Exact Test* | 0.408 |
| **2 GYPA** | |
| Observed Homozygosity | 54.1% |
| Expected Homozygosity (unbiased) | 50.1% |
| Homozygosity Test* | 0.310 |
| Likelihood Test* | 0.340 |
| Exact Test* | 0.340 |
| **3 HBGG** | |
| Observed Homozygosity | 47.1% |
| Expected Homozygosity (unbiased) | 48.8% |
| Homozygosity Test* | 0.670 |
| Likelihood Test* | 0.287 |
| Exact Test* | 0.280 |
| **4 D7S8** | |
| Observed Homozygosity | 49.0% |
| Expected Homozygosity (unbiased) | 53.6% |
| Homozygosity Test* | 0.252 |
| Likelihood Test* | 0.238 |
| Exact Test* | 0.238 |
| **5 Gc** | |
| Observed Homozygosity | 45.2% |
| Expected Homozygosity (unbiased) | 43.1% |
| Homozygosity Test* | 0.586 |
| Likelihood Test* | 0.029 |
| Exact Test* | 0.031 |
| **6 vWA** | |
| Observed Homozygosity | 17.2% |
| Expected Homozygosity (unbiased) | 18.4% |
| Homozygosity Test* | 0.646 |
| Likelihood Test* | 0.424 |
| Exact Test* | 0.437 |
| **7 F13B** | |
| Observed Homozygosity | 24.0% |
| Expected Homozygosity (unbiased) | 27.6% |
| Homozygosity Test* | 0.252 |
| Likelihood Test* | 0.144 |
| Exact Test* | 0.089 |
| **8 THO1** | |
| Observed Homozygosity | 25.4% |
| Expected Homozygosity (unbiased) | 21.8% |
| Homozygosity Test* | 0.211 |
| Likelihood Test* | 0.279 |
| Exact Test* | 0.270 |
| **9 TPOX** | |
| Observed Homozygosity | 35.6% |
| Expected Homozygosity (unbiased) | 36.0% |
| Homozygosity Test* | 0.917 |
| Likelihood Test* | 0.547 |
| Exact Test* | 0.699 |
| **10 CSF1P0** | |
| Observed Homozygosity | 27.7% |
| Expected Homozygosity (unbiased) | 26.0% |
| Homozygosity Test* | 0.585 |
| Likelihood Test* | 0.386 |
| Exact Test* | 0.419 |

*These values are probability values.

TABLE 8—*Linkage disequilibrium tests.*

| Loci | p-value |
|---|---|
| 1 LDLR / 2 GYPA | 0.481 |
| 1 LDLR / 3 HBGG | 0.815 |
| 1 LDLR / 4 D7S8 | 0.788 |
| 1 LDLR / 5 Gc | 0.149 |
| 1 LDLR / 6 vWA | 0.840 |
| 1 LDLR / 7 F13B | 0.154 |
| 1 LDLR / 8 THO1 | 0.280 |
| 1 LDLR / 9 TPOX | 0.692 |
| 1 LDLR / 10 CSF1PO | 0.463 |
| 2 GYPA / 3 HBGG | 0.824 |
| 2 GYPA / 4 D7S8 | 0.506 |
| 2 GYPA / 5 Gc | 0.843 |
| 2 GYPA / 6 vWA | 0.249 |
| 2 GYPA / 7 F13B | 0.896 |
| 2 GYPA / 8 THO1 | 0.715 |
| 2 GYPA / 9 TPOX | 0.775 |
| 2 GYPA / 10 CSF1PO | 0.287 |
| 3 HBGG / 4 D7S8 | 0.719 |
| 3 HBGG / 5 Gc | 0.931 |
| 3 HBGG / 6 vWA | 0.573 |
| 3 HBGG / 7 F13B | 0.313 |
| 3 HBGG / 8 THO1 | 0.187 |
| 3 HBGG / 9 TPOX | 0.640 |
| 3 HBGG / 10 CSF1PO | 0.355 |
| 4 D7S8 / 5 Gc | 0.126 |
| 4 D7S8 / 6 vWA | 0.274 |
| 4 D7S8 / 7 F13B | 0.887 |
| 4 D7S8 / 8 THO1 | 0.337 |
| 4 D7S8 / 9 TPOX | 0.512 |
| 4 D7S8 / 10 CSF1PO | 0.088 |
| 5 Gc / 6 vWA | 0.501 |
| 5 Gc / 7 F13B | 0.612 |
| 5 Gc / 8 THO1 | 0.514 |
| 5 Gc / 9 TPOX | 0.058 |
| 5 Gc / 10 CSF1PO | 0.966 |
| 6 vWA / 7 F13B | 0.253 |
| 6 vWA / 8 THO1 | 0.260 |
| 6 vWA / 9 TPOX | 0.942 |
| 6 vWA / 10 CSF1PO | 0.544 |
| 7 F13B / 8 THO1 | 0.149 |
| 7 F13B / 9 TPOX | 0.336 |
| 7 F13B / 10 CSF1PO | 0.575 |
| 8 THO1 / 9 TPOX | 0.175 |
| 8 THO1 / 10 CSF1PO | 0.863 |
| 9 TPOX / 10 CSF1PO | 0.283 |

expectation of independence between the alleles of any pairwise locus analysis (Table 8). The combined power of discrimination (PD) for the five STR loci is 0.9999862 and then for the PM loci is 0.99503. The PD for all ten loci is 0.9999993.

The Italian population allele frequency data for these ten PCR-based loci do not differ substantially from other Caucasian data for the same loci (data not shown).

In conclusion, the use of multiplex systems provides a number of genetic markers that enable a high degree of discrimination for forensic analyses, paternity testing, and linkage studies. The multiplex analysis is attractive for routine analyses because less sample is used compared with single locus typing and less sample manipulations are required, which is an ancillary benefit of reducing the risk for contamination within the laboratory. Moreover, the small allele size of the loci (generally less than 350 bp) enables analysis of many degraded DNA samples. Finally, population data are available such that routine interpretation of DNA profiles can be made.

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