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A POPULATION STUDY OF 5 PCR GENETIC MARKERS, LDLR, GYPA, HBGG, D7S8 AND Gc, IN ITALY

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The Amplitype® PM PCR Amplification and Typing Kit (Perkin Elmer Corporation, Norwalk, CT) permits multiplex PCR amplification of six loci:
1) low density lipoprotein receptor (LDLR) (Yamamoto et al. 1984), on chromosome 19, PCR product of 214 bp, 2 alleles (A and B);
2) glycophorin A (GYPA) (Siebert and Fukuda 1987), on chromosome 4, PCR product of 190 bp, 2 alleles (A and B) and other variants in African-American populations not distinguishiable using the above kit;
3) haemoglobin G-gammaglobin (HBGG) (Slightom et al. 1980), on chromosome 11, PCR product of 172 bp, 3 alleles (A, B and C);
4) D7S8 (Horn et al. 1990), on chromosome 7, PCR product of 151 bp, 2 alleles (A and B);
5) group-specific component (Gc) (Yang et al. 1985), on chromosome 4, PCR product of 138 bp, 3 alleles (A, B and C);
6) HLA-DQA1 (previously named HLA-DQa) (Gyllestein and Erlich 1988) on chromosome 6, PCR product of 239/242 bp, 6 alleles.

The amplification products of the first five loci can subsequently be typed simultaneously on the same nylon strip using a reverse dot blot method (Saiki et al. 1989), whereas the HLA-DQA1 PCR product hybridizes with the S specific probe which acts as control (Fig. 1b).

Validation studies on the suitability and forensic efficiency of this system were recently reported (Budowle et al. 1995; Fildes and Reynolds 1995) together with allele frequencies from several populations (Hochmeister et al. 1994; Budowle et al. 1995; Hausmann et al. 1995). However, further studies on allele frequencies from populations of various countries are desirable, to improve knowledge of the genetic profiles of these loci and to create a wide data-base. Since such information has not yet been reported for Italians, we investigated a suitable sample population with the aims of: 1) analysing the polymorphism of these 5 loci; 2) establishing a database of allele frequencies, in view of its application in forensic investigations, and 3) examining the performance of amplitype kit and problems arising from its use.

MATERIALS AND METHODS

DNA was extracted from samples of fresh peripheral blood from 98 healthy unrelated donors living in Northern (Parma= n. 46) and Central Italy (Ancona= n. 52), following the method suggested by Budowle and Baechtel (1990). In addition, 5 mother-child pairs were examined in the same conditions using the AmpliType® PM PCR Amplification and Typing Kit (supplied by Cetus Corporation). Amplification was carried out in a thermal cycler Gene Amp PCR System 2400 (Perkin Elmer) in the conditions suggested by the manufacturer, using quantities of DNA ranging from 10 to 50 ng. The PCR product was checked before hybridization by electrophoresis on a silver stained polyacrylamide gel (Fig. 1a). The frequency of alleles for each locus was calculated from the number of genotypes. The Hardy-Weinberg law was verified by the chi-square test between observed and expected genotype frequencies. The power of discrimination (PD) was calculated using Fischer's (1951) equation. A computer program kindly supplied by G. Carmody (Carleton University, Ottawa, Canada) was used to test for homogeneity between various population samples.

RESULTS AND DISCUSSION

The distributions of observed phenotypes and allele frequencies for the five PM genetic markers are shown in Tables 1 and 2. All five loci were polymorphic in our sample. The chi-square test did not detect any deviation from the Hardy-Weinberg expectations for the five loci. The combined PD was 0.994.The distribution of PM allele frequencies were found to be similar to those described for Caucasians (Hochmeister et al. 1994, Budowle at al. 1995, Hausmann et al. 1995) for all five loci (Table 3).

In our experience, care must be taken when interpreting the typing of the Gc locus. In one case of mother-child pair typing there was an apparent exclusion (mother A, child C), due to signal imbalance for the Gc B dot, which appeared less intense than the control. This problem was solved by adding EDTA after amplified product denaturation, to prevent primer extension which may mask the Gc B probe binding site on the strip (Reynolds, pers. com.).

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Fig. 1. Polyacrylamide gel electrophoresis of PCR amplified product (a) and probe strip typing (b) of PM loci

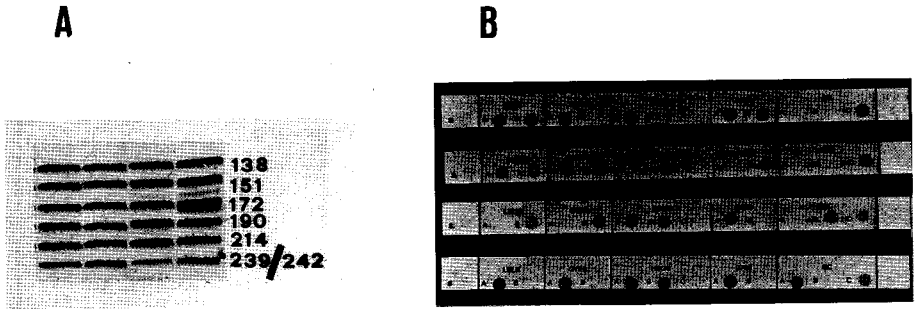

Table 1. Observed PM loci genotype frequencies in a sample of 98 Italians

Genotype	LDLR	GYPA	HBGG	D7S8	Gc
AA	0.133	0.378	0.245	0.286	0.051
AB	0.561	0.459	0.367	0.541	0.071
BB	0.306	0.163	0.357	0.173	0.031
AC	NG[a]	NG	0.010	NG	0.275
BC	NG	NG	0.021	NG	0.143
CC	NG	NG	0.000	NG	0.429

[a] NG, no genotype, there is no allele C.

Table 2. PM loci allele frequencies in a sample of 98 Italians

Allele	LDLR	GYPA	HBGG	D7S8	Gc
A	0.413	0.607	0.434	0.556	0.224
B	0.587	0.393	0.551	0.444	0.138
C	NA[a]	NA	0.015	NA	0.638

[a] NA, there is no allele C in AmpliType® PM kit

χ test	2.4240	0.1373	5.2799	0.8925	1.6023
Prob.	0.10<P<0.25	0.50<P<0.75	0.10<P<0.25	0.25<P<0.50	0.50<P<0.75
d.f.	n = 1	n = 1	n = 3	n = 1	n = 3

Table 3. Results of test for heterogeneity between Italians, Americans, North Bavarians and Swiss

	LDLR	GYPA	HBGG	D7S8	Gc
G-stat.	8.9407	5.7412	24.1789	2.5166	18.1277
Prob.	0.1810±0.0122	0.4480±0.0157	0.0490 ±0.0068	0.8710±0.0106	0.3000 ±0.0145