TECHNICAL NOTE

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LDLR, GYPA, HBGG, D7S8 and GC Allele and Genotype Frequencies in the Northwest Italian Population

REFERENCE: Gino S, Robino C, Torre C, Iorio M, Peruccio D. LDLR, GYPA, HBGG, D7S8 and GC allele and genotype frequencies in the Northwest Italian population. J Forensic Sci 1999;44(1): 171–174.

ABSTRACT: Allele and genotype frequencies for five PCR-based DNA markers (LDLR, GYPA, HBGG, D7S8 and GC) were determined in 100 unrelated individuals from Piedmont (Northwest Italy). All five Ioci met Hardy-Weinberg expectations in the sampled population. The combined PD and CE were, respectively, 0.995 and 0.697. Frequencies obtained were compared with other previously published data on Caucasian populations with no significant differences. The genetic data from this study, in addition to those already collected by other groups, contribute to the expansion of the Italian DNA database suitable for forensic casework and paternity testing.

KEYWORDS: forensic science, population genetics, Italy, DNA typing, polymorphism, polymerase chain reaction, LDLR, GYPA, HBGG, D7S8, GC

In the few years since the first applications of molecular biology techniques to analysis of forensic evidence, the study of DNA polymorphisms has become routine in laboratories conducting identity and paternity testing. Today, PCR-based typing kits, designed to simultaneously amplify different loci, expedite testing procedures by providing sufficient discrimination from a single test, thus overcoming the common problem of poor quality and a limited quantity of DNA available in forensic casework.

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Received 20 April 1998; and in revised form 22 June 1998; accepted 29 June 1998.

To estimate the frequency of a genetic profile, however, it is necessary to create reliable databases by collecting allele and genotype frequencies of these DNA markers from large samples of reference populations. In this study, the ''AmpliType® PM PCR Amplification and Typing Kit'' (1,2) was used to analyze a sample population from Piedmont, a Northwest region of Italy that, since the 1950s, has received a strong migration flow from Northeastern and Southern Italy. Five loci (PM loci) were simultaneously amplified: low density lipoprotein receptor (LDLR) (3), glycophorin A (GYPA) (4), hemoglobin G gammaglobin (HBGG) (5), D7S8 (6) and group-specific component (GC) (7). The allele and genotype frequencies obtained were compared with data reported from seven Caucasian populations (8–14).

Material and Methods

Whole blood samples were obtained from 100 unrelated individuals from the region of Piedmont (Northwest Italy). DNA was extracted either by 5% Chelex 100 (Bio-Rad Laboratories, Hercules, CA) following the procedure suggested by Walsh (15) or by ''QIAamp Blood and Tissue Kit'' (QIAGEN GmbH, Hilden, Germany). DNA was quantified by spectrophotometry.

About 2 ng of DNA were used for PCR. LDLR, GYPA, HBGG, D7S8 and GC loci were simultaneously amplified by ''AmpliType® PM PCR Amplification and Typing Kit'' (Perkin-Elmer Corporation, Norwalk, CT). Amplification was carried out in a ''GeneAmp PCR System 9700'' thermocycler (Perkin-Elmer). The presence and size of PCR products were verified by electrophoresis through agarose gel (3% NuSieve®, FMC BioProducts, Rockland, ME) in 1X TAE buffer containing 0.5 μg/mL ethidium bromide. Typing of the five loci was performed by reverse dot blot with ASO (Allele Specific Oligonucleotides) probes, using the ''AmpliType® PM PCR Amplification and Typing Kit'' according to the manufacturer's recommended protocol.

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Allele frequencies of the five loci were calculated by direct gene counting. Each locus was tested for Hardy-Weinberg equilibrium by the chi-square test (χ^2). Independence among the PM loci was verified by the test for nonrandom association of alleles, (s_k^2) test, described by Brown (16), which examines whether or not the observed variance (s_k^2) of the number of heterozygous loci exceeds its confidence interval, under the assumption of independence. Power of discrimination (PD), chance of exclusion (CE) and heterozygosity value (h) were calculated using the formulas described by Fisher (17), Ohno (18), and Nei and Roychoudhury (19), respectively. Comparison of population data was carried out with a two-way RxC contingency table using a conventional chi-square test.

Results and Discussion

The distributions of the observed allele and genotype frequencies for LDLR, GYPA, HBGG, D7S8 and GC in the Northwest Italian population sample are shown in Table 1 and Table 2. The observed heterozygosity ranged from 40% (LDLR) to 60% (GC). The genotype frequencies did not deviate from Hardy-Weinberg equilibrium on the bases of a chi-square test (Table 2). No evidence of association between the PM loci was found using the s_k^2 criterion (Table 3). To evaluate the usefulness of these five DNA markers in identity and paternity testing, PD, CE and h were calculated (Table 4). Combined values for PD and CE were 0.995 and 0.697, respectively. These results are consistent with those reported from previous studies on PM loci (8,9,11,13,14).

The genotype distributions found in Northwest Italians and seven other Caucasian populations, shown in Table 5, were compared by a two-way RxC contingency table using a conventional chi-square test (Table 6). Only one statistically significant deviation was found at locus D7S8 between Northwest Italians and the general Italian population sample studied by Spinella et al. (9). This isolated difference may be due to the small sample sizes, but also to the different sampling strategies chosen to analyze the Italian population: strictly regional in this study, grid-like (with few samples collected from each region of Italy) in that by Spinella. However, one significant deviation out of 35 comparisons is no more than expected by chance.

In conclusion, the allele and genotype frequencies obtained in the Northwest Italian population sample can be used to improve the Italian database for the PM loci used in forensic casework.

TABLE 1—*Observed allele frequency distributions for PM loci in 100 unrelated Northwest Italians.*

Allele	LDLR	GYPA	HBGG	D7S8	GC
A	0.440	0.540	0.450	0.635	0.305
B	0.560	0.460	0.545	0.365	0.155
C	NA*	NA*	0.005	NA*	0.540

* There is no C allele for LDLR, GYPA and D7S8.

TABLE 2—*Observed frequency distributions of PM loci genotypes in a sample of 100 unrelated Northwest Italians.*

Genotype	LDLR	GYPA	HBGG	D7S8	GC
AA	24	30	22	36	7
AB	40	48	46	55	8
AC	NA*	NA*	0	NA*	39
BB	36	22	31	9	5
BC	NA*	NA*	1	NA*	13
CC	NA*	NA*	0	NA*	28
χ^2	3.546 $\nu = 1$ $0.10 > p > 0.05$	0.114 $\nu = 1$ $0.75 > p > 0.50$	1.238 $\nu = 3$ $0.75 > p > 0.50$	3.474 $\nu = 1$ $0.10 > p > 0.05$	5.607 $\nu = 3$ $0.25 > p > 0.10$

* There is no C allele for LDLR, GYPA and D7S8.

TABLE 3—*Test of nonrandom association between alleles* (s_k^2).

Loci	s_k^2	Confidence interval 95%
LDLR/GYPA	0.4531	0.5999
LDLR/HBGG	0.4731	0.6000
LDLR/D7S8	0.5275	0.5989
LDLR/GC	0.5384	0.5916
GYPA/HBGG	0.5291	0.6000
GYPA/D7S8	0.4475	0.5987
GYPA/GC	0.5364	0.5915
HBGG/D7S8	0.5196	0.5987
HBGG/GC	0.4584	0.5915
D7S8/GC	0.5379	0.5902

TABLE 4—*Statistical parameters of interest in forensic casework and paternity testing.*

System	PD*	CE*	h*
LDLR	0.621	0.186	0.495
GYPA	0.623	0.187	0.499
HBGG	0.630	0.193	0.503
D7S8	0.605	0.178	0.466
GC	0.760	0.313	0.596
Combined values	0.995	0.697	. . .

* PD = power of discrimination; CE = chance of exclusion; h = heterozygosity.

TABLE 5—*PM genotype distribution in eight Caucasian populations.*

System	Genotype	NW Italian (n = 100)*	Italian (n = 374)†	Italian (n = 200)‡	Croatian (n = 199)§	Spanish (n = 132)‖	Swiss (n = 100)¶	N Bavarian (n = 150)**	US Caucasian (n = 200)††
LDLR	AA	0.240	0.158	0.210	0.156	0.189	0.210	0.153	0.205
	AB	0.400	0.513	0.525	0.508	0.500	0.450	0.447	0.485
	BB	0.360	0.329	0.265	0.336	0.311	0.340	0.400	0.310
GYPA	AA	0.300	0.318	0.290	0.287	0.258	0.240	0.353	0.280
	AB	0.480	0.457	0.510	0.547	0.500	0.570	0.467	0.500
	BB	0.220	0.225	0.200	0.166	0.242	0.190	0.180	0.220
HBGG	AA	0.220	0.233	0.200	0.302	0.220	0.240	0.220	0.295
	AB	0.460	0.503	0.465	0.457	0.515	0.470	0.547	0.485
	AC	0.000	0.016	0.010	0.000	0.000	0.000	0.020	0.000
	BB	0.310	0.240	0.315	0.241	0.258	0.290	0.200	0.200
	BC	0.010	0.008	0.005	0.000	0.007	0.000	0.013	0.015
	CC	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.005
D7S8	AA	0.360	0.364	0.310	0.432	0.303	0.330	0.327	0.395
	AB	0.550	0.476	0.450	0.443	0.545	0.510	0.547	0.430
	BB	0.090	0.160	0.240	0.125	0.152	0.160	0.126	0.175
GC	AA	0.070	0.080	0.080	0.060	0.076	0.070	0.080	0.095
	AB	0.080	0.091	0.120	0.105	0.060	0.130	0.093	0.065
	AC	0.390	0.329	0.325	0.336	0.341	0.290	0.327	0.295
	BB	0.050	0.027	0.040	0.005	0.023	0.000	0.013	0.045
	BC	0.130	0.158	0.125	0.139	0.205	0.220	0.187	0.200
	CC	0.280	0.315	0.310	0.355	0.295	0.290	0.300	0.300

* Present study.
† Tagliabracci et al. (8).
‡ Spinella et al. (9).
§ Keys et al. (10).
‖ Rodriguez-Calvo et al. (11).
¶ Hochmeister et al. (12).
** Hausmann et al. (13).
†† Perkin-Elmer (14).

TABLE 6—*Comparison of PM genotype distibution between Northwest Italy and seven Caucasian populations calculated by means of two-way RxC contingency tables.*

System	Italian*	Italian†	Croatian‡	Spanish§	Swiss‖	N Bavarian¶	US Caucasian**
LDLR	$\chi^2 = 5.35$ $\nu = 2$ P = 0.069	$\chi^2 = 4.46$ $\nu = 2$ P = 0.108	$\chi^2 = 4.30$ $\nu = 2$ P = 0.116	$\chi^2 = 2.35$ $\nu = 2$ P = 0.308	$\chi^2 = 0.55$ $\nu = 2$ P = 0.759	$\chi^2 = 2.95$ $\nu = 2$ P = 0.228	$\chi^2 = 1.92$ $\nu = 2$ P = 0.379
GYPA	$\chi^2 = 0.18$ $\nu = 2$ P = 0.914	$\chi^2 = 0.27$ $\nu = 2$ P = 0.873	$\chi^2 = 1.69$ $\nu = 2$ P = 0.430	$\chi^2 = 0.54$ $\nu = 2$ P = 0.763	$\chi^2 = 1.66$ $\nu = 2$ P = 0.437	$\chi^2 = 1.03$ $\nu = 2$ P = 0.599	$\chi^2 = 0.15$ $\nu = 2$ P = 0.929
HBGG	$\chi^2 = 3.47$ $\nu = 4$ P = 0.482	$\chi^2 = 1.89$ $\nu = 5$ P = 0.864	$\chi^2 = 4.80$ $\nu = 3$ P = 0.187	$\chi^2 = 0.95$ $\nu = 3$ P = 0.814	$\chi^2 = 1.16$ $\nu = 3$ P = 0.762	$\chi^2 = 5.91$ $\nu = 4$ P = 0.206	$\chi^2 = 4.06$ $\nu = 4$ P = 0.398
D7S8	$\chi^2 = 3.57$ $\nu = 2$ P = 0.168	$\chi^2 = 9.78$ $\nu = 2$ P = 0.008	$\chi^2 = 3.21$ $\nu = 2$ P = 0.201	$\chi^2 = 2.29$ $\nu = 2$ P = 0.318	$\chi^2 = 2.24$ $\nu = 2$ P = 0.326	$\chi^2 = 0.92$ $\nu = 2$ P = 0.632	$\chi^2 = 5.54$ $\nu = 2$ P = 0.063
GC	$\chi^2 = 3.15$ $\nu = 5$ P = 0.678	$\chi^2 = 2.27$ $\nu = 5$ P = 0.811	$\chi^2 = 8.88$ $\nu = 5$ P = 0.114	$\chi^2 = 3.82$ $\nu = 5$ P = 0.575	$\chi^2 = 9.99$ $\nu = 5$ P = 0.075	$\chi^2 = 5.02$ $\nu = 5$ P = 0.413	$\chi^2 = 4.51$ $\nu = 5$ P = 0.478

* Tagliabracci et al. (8).
† Spinella et al. (9).
‡ Keys et al. (10).
§ Rodriguez-Calvo et al. (11).
‖ Hochmeister et al. (12).
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