BRIEF COMMUNICATION

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PM and D1S80 Loci Gene Frequencies in the Zaragoza Population of Northern Spain

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ABSTRACT: LDLR, GYPA, HBGG, D7S8, GC (PM loci) and D1S80 are widely used in forensic casework analyses and population data are required to estimate the frequency of a DNA profile. This paper presents the results of a survey aimed at investigating the allele and genotype frequency distribution of these loci in an important Spanish population (Zaragoza, North Spain). Statistical analysis to determine whether allele frequencies were in Hardy-Weinberg equilibrium was carried out as well as to obtain some parameters of medicolegal interest. There was no evidence of association between the alleles of the loci. The Zaragoza sample does not differ substantially from other Caucasian populations.

KEYWORDS: forensic science, DNA typing, genetic markers, polymerase chain reaction, LDLR, GYPA, HBGG, D7S8, GC, D1S80, population genetics, Spain

D1S80 (pMCT118) is a variable number of a tandem repeat (VNTR) locus with a repeat size of 16 base pairs (1). Because of its robust discrimination power and ease in typing (2–4), this system has been applied in forensic laboratories worldwide. Multiplex amplification and typing of low density lipoprotein receptor (LDLR) (5), glycophorin A (GYPA) (6), hemoglobin G gammaglobin (HBGG) (7), D7S8 (8) and group specific component (GC) (9) loci have also proved to be very useful for forensic identification (10,11–18).

We report here the allele and genotype frequencies for the LDLR, GYPA, HBGG, D7S8, GC and D1S80 loci in a sample of population from Zaragoza (North Spain).

Material and Methods

Blood samples from 201 healthy unrelated individuals from Zaragoza were collected. DNA was extracted with Chelex™ 100 using the method described by Walsh et al. (19).

The LDLR, GYPA, HBGG, D7S8 and GC loci were simultaneously amplified and typed using the AmpliType^{R} PM PCR Amplification Typing Kit (Perkin Elmer Corp., Norwalk, CT). Amplification was carried out in a Perkin-Elmer DNA Thermal Cycler 480. The conditions were those recommended by the manufacturer (Roche Molecular System, Branchburg, NJ). The D1S80 locus was typed using the method described by Lareu et al. (20). Statistical analyses were performed as described previously (see Refs 21 and 22 for details).

Results and Discussion

The distributions of observed genotype and allele frequencies for the six loci are summarized in Tables 1 and 2. Table 3 shows a summary of Hardy-Weinberg equilibrium tests.

There was no evidence of association detected between the alleles of the loci (Table 4), thus multiplication of the individual locus profile frequencies can be carried out to estimate multiple locus profile frequencies. However, two loci HBGG and D7S8 loci did depart from Hardy-Weinberg equilibrium. There was an excess of observed homozygotes at both loci. This observation must be due to population substructure, sampling and/or technical limitations that failed to detect allele(s). A review of the data does not support technical limitations as a cause for the excess homozygosity (10–13,15–18). It is interesting that these results (i.e., excess of observed homozygotes) were observed for population samples from areas near Zaragoza (22). Zaragoza has had some migrations from those areas, particularly from isolated Pyrenean valleys free of Arabic invasions of the lberian Peninsula. Thus, there may be some population substructures to consider. Regardless, to avoid assumptions of independence when estimating profile frequencies, the observed genotype frequencies could be used for the loci HBGG and D7S8. However, multiple locus profile frequencies for the six PCR-based loci would not be substantially different if the observed or expected genotype frequencies were used for these loci (data not shown).

The Zaragoza data were compared with other sample populations (3,10–19) using a RxC contingency table χ^2 test for homogeneity. The Zaragoza population does not differ significantly from other Caucasians, but significant differences were observed with SW Hispanics, Afro-Americans, Arabs, Koreans and Japanese (Table 5). As indicated in Table 6, the combined chance of exclusion (CE) is 88.72% and the combined discrimination power (PD)

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TABLE 1—*Observed allele frequency distribution for LDLR, GYPA, D7S8, HBGG and GC in 201 unrelated individuals from Zaragoza.*

System	Genotype Counts n = 201	Observed alleles n = 402 Number	Percent
LDLR	AA = 40	A 178	44.279
	AB = 98	B 224	55.721
	BB = 63	...	...
GYPA	AA = 50	A 204	50.746
	AB = 104	B 198	49.254
	BB = 47		
HBGG	AA = 61	A 205	50.995
	AB = 82	B 196	48.756
	AC = 1	C 1	0.249
	BB = 57	...	...
	BC = 0	...	...
D7S8	AA = 62	A 208	51.741
	AB = 84	B 194	48.259
	BB = 55	...	...
GC	AA = 25	A 132	32.836
	AB = 10	B 51	12.687
	AC = 72	C 219	54.478
	BB = 4	...	...
	BC = 33	...	...
	CC = 57	...	...

TABLE 2—*Observed allele frequency distribution for D1S80 in 166 unrelated individuals from Zaragoza.*

Allele	Observed Number	Observed Percent
14	1	0.301
17	2	0.602
18	77	23.193
19	3	0.904
20	6	1.807
21	21	6.325
22	10	3.012
23	2	0.602
24	119	35.843
25	16	4.819
26	4	1.205
27	6	1.807
28	12	3.614
29	27	8.133
30	3	0.904
31	19	5.723
32	0	0.000
33	0	0.000
34	1	0.301
35	0	0.000
36	2	0.602
37	0	0.000
38	1	0.301
Total	332	100,000

TABLE 3—*Summary of HWE tests.*

	LDLR	GYPA	HBGG	D7S8	GC	D1S80
Observed homozygosity	51.2%	48.3%	58.7%	58.2%	42.8%	22.9%
Expected homozygosity	50.5%	49.9%	49.7%	49.9%	41.9%	19.9%
Homozygosity test*	0.840	0.644	0.010†	0.019†	0.805	0.341
Likelihood test*	0.898	0.693	0.014†	0.024†	0.186	0.093
Exact test*	0.898	0.693	0.011†	0.024†	0.169	0.138

* These values are probability values.
† Statistically significant.

TABLE 4—*Summary of linkage analyses.*

Loci	*p*-Value
1 LDLR/2 GYPA	0.656
1 LDLR/3 HBGG	0.797
1 LDLR/4 D7S8	0.485
1 LDLR/5 Gc	1.000
1 LDLR/6 D1S80	0.834
2 GYPA/3 HBGG	0.740
2 GYPA/4 D7S8	0.444
2 GYPA/5 Gc	0.526
2 GYPA/6 D1S80	0.660
3 HBGG/4 D7S8	0.900
3 HBGG/5 Gc	0.502
3 HBGG/6 D1S80	0.906
4 D7S8/5 Gc	0.877
4 D7S8/6 D1S80	0.733
5 GC/6 D1S80	0.888

TABLE 5—*Comparison of different populations for LDLR, GYPA, HBGG, D7S8, GC and D1S80 loci.*

Populations	LDLR	GYPA	HBGG	D7S8	GC	D1S80
Zaragoza/USA Afro-American (3) (10)	0.0001	0.5693	0.0002	0.0472	0.0001	0.0024
Zaragoza/USA Cauc. (3) (10)	0.9391	0.1203	0.6469	0.0449	0.1933	0.3544
Zaragoza/USA S-W Hispanic (3) (10)	0.0159	0.0018	0.0007	0.0004	0.1061	0.1272
Zaragoza/Korean (12)	0.0001	0.0001	0.0001	0.9299	0.0001	0.0001
Zaragoza/Arab (13)	0.7754	0.0242	0.0215	0.0058	0.0012	0.1177
Zaragoza/Portugal S (14)	0.2292	0.6069	0.4185	0.3618	0.7000	. . .
Zaragoza/Basque Country (15)	0.9391	0.8954	0.7416	0.2439	0.7125	0.4192
Zaragoza/Portugal N (16)	0.5911	0.9113	0.4230	0.1328	0.6531	. . .
Zaragoza/Japanese (18)	0.0001	0.0745	0.0001	0.0635	0.0001	. . .

TABLE 6—*Statistical parameters of medicolegal interest.*

Loci	H %	PD %	CE %
LDLR	49.47	63	18.58
GYPA	50.12	62.37	18.74
HBGG	50.35	66.10	19.06
D7S8	50.06	65.55	18.73
Gc	58.07	74.60	29.95
D1S80	81.10	93.85	63.00
Total	98.90	99.96	88.72

H = allelic diversity; PD = power of discrimination; CE = chance of exclusion.

is 99.96%. This data are comparable to those for other Spanish population samples (21,22).

References

1. Kasai K, Nakamura Y, White R. Amplification of variable number of tandem repeat (VNTR) locus (pMCT118) by polymerase chain reaction (PCR) and its application to forensic science. J Forensic Sci 1990;35:1196–200.
2. Sugiyama E, Honda K, Katsuyama Y, Uchiyama S, Tsuchikane A, Ota M, et al. Allele frequency distribution of the D1S80 (pMCT118) locus polymorphism in the Japanese population by the polymerase chain reaction. Int J Leg Med 1993;106:111–4.
3. Budowle B, Baechtel FS, Smerick JB, Presley KW, Giusti AM, Parsons G, et al. D1S80 population data in African Americans, Caucasians, Southeastern Hispanics, Southwestern Hispanics, and Orientals. J Forensic Sci 1995;40:38–44.
4. Sepulchre MA, Wiegand P, Brinkmann B. D1S80 (pMCT118); analysis of 3 ethnic subpopulations living in Brussels. Int J Leg Med 1995;108:45–7.
5. Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, et al. The human LDL receptor: A cysteine-rich protein with multiple Alu sequences in its m-RNA. Cell 1984;39: 27–38.
6. Siebert PD, Fukuda M. Molecular cloning of human glycophorin B cDNA: nucleotide sequence and genomic relationship to glycophorin A. Proc Natl Acad Sci 1987;84:6735–9.
7. Slightom JL, Blechl AE, 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 1980;21: 627–38.
8. Horn GT, Richards B, Merrill JJ, Klinger KW. Characterization and rapid diagnostic analysis of DNA polymorphisms closely linked to the cystic fibrosis locus. Clin Chem 1990;36:1614–9.
9. Yang F, Brune JL, Naylor SL, Apples RL, Naberhaus KH. Human group-specific component (Gc) is a member of the albumin family. Proc Natl Acad Sci 1985;82:7994–8.
10. Budowle B, Lindsey JA, DeCou JA, Koons BW, Giusti AM, Comey CT. 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. J Forensic Sci 1995; 40(1):45–54.
11. Hausmann R, Hantschel M, Lötterle J. Frequencies of the 5 PCR-based genetic markers LDLR, GYPA, HBGG, D7S8, and GC in a North Bavarian population. Int J Leg Med 1995;107:227–8.
12. Woo KM, Budowle B. Korean population data on the PCR-based loci LDLR, GYPA, HBGG, D7S8, HLA-DQA1, and D1S80. J Forensic Sci 1995;40(4):645–8.
13. Hayes JM, Budowle B, Freund M. Arab population data on the PCR-based loci: HLA-DQA1, LDLR, GYPA, HBGG, D7S8, Gc, and D1S80. J Forensic Sci 1995;40(5):888–92.
14. Espinheira R, Ribeiro T, Geada H, Reys L. Polymarker and HLA DQA1 genetic markers in forensic casework. In: Mangin P, Ludes B, editors. Acta Medicinae Legalis. Berlin, Heidelberg, New York: Springer-Verlag, 1994;37–8.
15. García O, Martín P, Albarrán C, Alonso A. Allele frequencies of HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and Gc in the resident population of the Basque Country. In: Mangin P, Ludes B, editors. Acta Medicinae Legalis. Berlin, Heidelberg, New York: Springer-Verlag, 1994:40–3.
16. Pinheiro MF, Pontes ML, Pinto da Costa J. Use of the AmpliType PM coamplification system on forensic analysis. In: Mangin P, Ludes B, editors. Acta Medicinae Legalis. Berlin, Heidelberg, New York: Springer-Verlag, 1994:81–2.
17. Rodriguez Calvo MS, Bellas S, Souto L, Vide C, Valverde E, Carracedo A. Population data on the loci LDLR, GYPA, HBGG, D7S8 and GC in three Southwest European populations. J Forensic Sci 1996;41(2):291–6.
18. Nakajima T, Marsuki T, Ohwara H, Nara M, Furukawa K, Kishi K. Evaluation of 7 DNA markers (D1S80, HLA DQα, LDLR, GYPA, HBGG, D7S8 and GC) in a Japanese population. Int J Leg Med 1996;109:47–8.
19. Walsh PS, Metzger DA, Higucchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 1991;10:506–13.
20. Lareu MV, Muñoz I, Pestoni C, Rodriguez MS, Vide C, Carracedo A. The distribution of HLA DQA1 and D1S80 (pMCT118) alleles and genotypes in the population of Galicia and Central Portugal. Int J Leg Med 1993;106:124–8.
21. Martínez-Jarreta B. Abecia E, Bell B, Casalod Y, Castellano M, Hinojal R. Frequencies of the five PCR-based genetic markers LDLR, GYPA, D7S8, HBGG and GC in the population of Asturias (North Spain). Int J Leg Med 1997;110:41–3.
22. Bell B, Budowle B, Martínez-Jarreta B, Casalod Y, Abecia E, Castellano M. Distribution of types for six PCR-based loci: LDLR, GYPA, D7S8, HBGG and GC and HLA DQA1 in Central Pyrenees and Teruel (Spain). J Forensic Sci 1997;42(3):510–3.

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