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ORIGINAL ARTICLE

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Dutch Caucasian population data on the loci LDLR, GYPA, HBGG, D7S8, and GC

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Abstract To introduce the loci LDLR, GYPA, HBGG, D7S8, and GC (PM loci) in Dutch forensic identity testing, allele and genotype frequencies were determined in a Dutch Caucasian population sample, which had previously been typed for the HLADQA1 locus [12]. All 6 loci met Hardy-Weinberg equilibrium expectations, and there is little evidence for association between pairs of loci. The combined power of discrimination for all 6 loci is 0.9997. The allele frequencies of the PM loci were similar to 2 other Caucasian populations [3, 10], and differed from 3 non-Caucasian populations [3].

Key words PM loci (LDLR, GYPA, HBGG, D7S8 and GC) · HLADQA1 · Polymerase chain reaction (PCR) · Multiplex PCR · Allele frequencies · Genotype frequencies · Population genetics · Forensic DNA typing

Introduction

The polymarker (PM) loci LDLR, GYPA, HBGG, D7S8, and GC (Table 1) were studied for usage in Dutch forensic identity testing in combination with the locus HLADQA1. We report the genotype and allele frequencies, and the test results for Hardy Weinberg (HWE) and linkage equilibrium (LE). Furthermore, we computed the power of discrimination, and compared the allele frequencies of the PM loci with 2 other studies [3, 10].

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Materials and methods

We typed 155 unrelated Dutch Caucasian donors (employees and students of the Dutch Forensic Science Laboratory), using the Amplitype PM PCR Amplification and Typing Kit (Roche Molecular Systems, Alameda, Cal.) and following the manufacturer's instructions [1]. The donors had previously been typed for the HLADQA1 locus [12].

The powers of discrimination of the loci were calculated as $1 - \Sigma (P_i)^2$, where P_i represents the frequency of genotype i [5]. The exact test [6] was used to investigate deviations from HWE. The exact significance level of this test for HLADQA1 was estimated using a Monte Carlo method [6]. A test for pairs of loci [13] was used to investigate deviations from LE. Population homogeneity was tested pairwise with the adjusted chi-squared test for homogeneity [14], using a computer program kindly provided by G. Carmody (Carleton University, Ottawa).

Results and discussion

Our data did not deviate from HWE (Table 2). Furthermore, we found little evidence for correlation between the alleles at any of the pairs of loci (Table 3). One of the 15 locus pairs showed deviation from LE (HLADQA1 and GYPA). However, the combined findings are consistent with LE (improved Bonferroni method [9], overall significance level 0.05). To investigate the practical conse-

Table 1 Characteristics of the PM loci and the HLADQA1 locus

Locus [ref]	LDLR [17]	GYPA* [15]	HBGG [16]	D7S8 [11]	GC** [18]	HLADQA1 [7]
Chromosome	19p13.1–13.3	4q28–31	11p15.5	7q22–31.1	4q11–13	6p21.3
PCR-product (bp)	214	190	172	151	138	242/239***

* Alleles A and B are equivalent to alleles M and N of the blood-group MN-system resp.
** Alleles A, B and C are equivalent to alleles 2, 1F and 1S of the GC-protein system resp.
*** A 242 bp fragment is amplified for alleles 1.1, 1.2, 1.3, and 3, and a 239 bp fragment for alleles 2 and 4

Table 2 Estimates of allele and genotype frequencies, heterozygosity, discrimination power, and the *P*-values of the exact test for HWE (155 individuals)

	LDLR	GYPA	HBGG	D7S8	GC	HLADQA1
AA	0.187	0.297	0.342	0.387	0.058	
AB	0.477	0.44	0.458	0.465	0.077	
BB	0.335	0.26	0.2	0.148	0.303	
AC			0		0.019	
BC			0		0.161	
CC			0		0.381	
A	0.426	0.516	0.571	0.619	0.248	
B	0.574	0.484	0.429	0.381	0.139	
C			0		0.613	
Het	0.477	0.439	0.458	0.465	0.542	
DP	0.625	0.651	0.633	0.612	0.727	0.943 [12]
p(HWE)	0.75	0.15	0.42	0.87	0.95	0.87*

Combined power of discrimination: 0.9997

*: *P*-value estimated using a Monte Carlo method [6] (10,000 simulations)

Table 3 *P*-values* for linkage equilibrium test [13] between 2 loci Dutch Caucasian population sample (155 individuals)

	LDLR	HBGG	GYPA	D7S8	GC
HBGG	0.83				
GYPA	0.73	0.78			
D7S8	0.80	0.26	0.89		
GC	0.08	0.35	0.36	0.39	
HLADQA1	0.58	0.60	0.004**	0.58	0.26

* *P*-values estimated by a bootstrap method using 1000 bootstrap resamples

** To increase precision, this low p-value was estimated with 10,000 bootstrap resamples. The rejection level at an overall significance level of 0.05 is 0.003 (Improved Bonferroni [9])

Fig. 1 Polymarker allele frequencies in 6 different population samples. 1 = Dutch Caucasians (N = 155); 2 = US Caucasians (N = 148); 3 = Swiss Caucasians (N = 100); 4 = US African Americans (N = 145); 5 = US South East Hispanics (N = 94); 6 = US South West Hispanics (N = 96); N = number of individuals typed; Bars represent point estimate frequency (%) data

quences of a possible correlation between the HLADQA1 and the GYPA locus, we compared the observed HLADQA1/GYPA profile frequencies and the estimated frequencies assuming LE and HWE. Since the maximum ratio (observed/estimated) was only 2.3, we conclude that correlation would have a negligible effect on the magnitude of the complete profile frequency estimate.

Although the power of discrimination of a single comparison of a pair of profiles is high (0.9997, Table 2), it should be emphasized that the probability of a match when a person is compared to a large database is much higher [2]. To illustrate this point, we pairwise compared the 155 individuals in our database. Of the 11,935 pairs, 5 pairs (10 different individuals) matched at all 6 loci, and 106 pairs matched at 5 loci (104 different individuals). Hence, the probability of a match when a sample of unknown origin is compared with all individuals in our database can be estimated as 10/155 (= 0.06), and the probability of a match at 5 of the 6 loci as 104/155 (= 0.68).

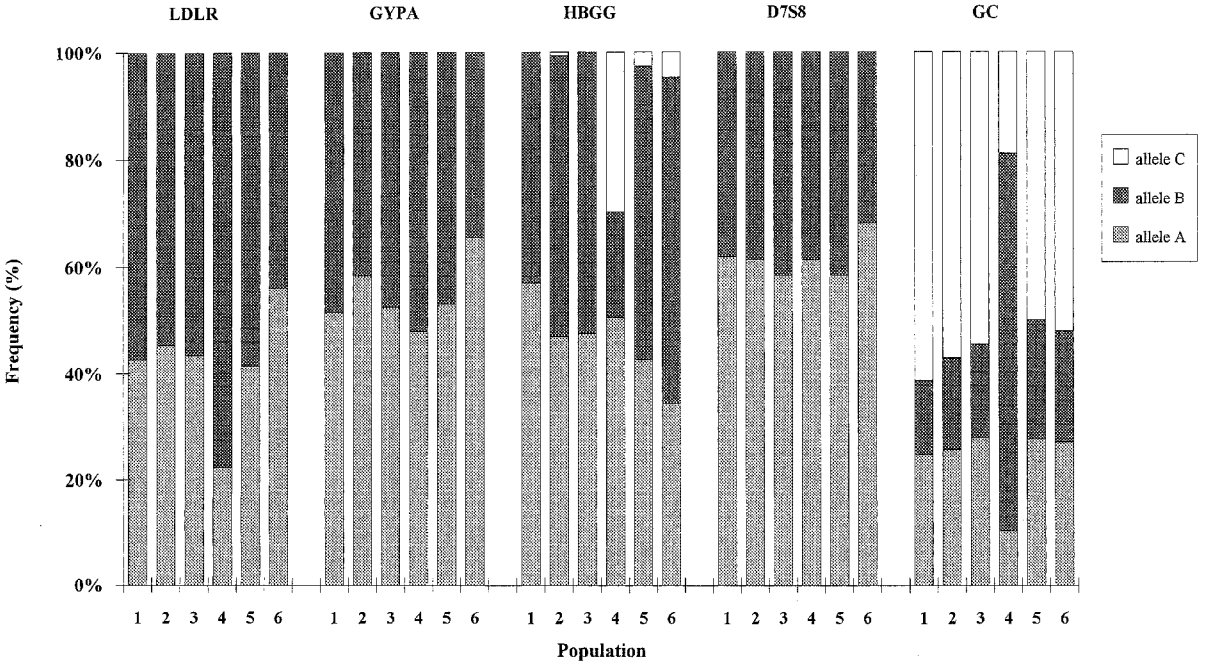

Table 4 P-values for pairwise comparisons of Dutch Caucasian allele frequencies (n = 155) with 2 Caucasian population samples, an African American and 2 American Hispanic population samples

*: [3]
**: [10]

Population Origin Nr individuals	Caucasian* US (n = 148)	Caucasian** Switzerland (n = 100)	African* US (n = 145)	SE Hispanic* US (n = 94)	SW Hispanic* US (n = 96)
LDLR	0.5	0.84	< 0.001	0.81	0.003
GYPA	0.09	0.84	0.37	0.73	0.0021
HBGG	0.018	0.053	< 0.001	< 0.001	< 0.001
D7S8	0.91	0.44	0.89	0.45	0.15
GC	0.45	0.29	< 0.001	0.016	0.06

Figure 1 shows the allele frequency distributions of the PM loci for 3 Caucasian population samples [3, 10] and 3 non-Caucasian samples [3]. Whereas the US and Swiss Caucasian samples appear similar to the Dutch (except for the US sample at the HBGG locus, p = 0.018), many differences were found between the Dutch sample and the non-Caucasian population samples (Table 4). The most striking difference is observed for the C allele of the HBGG locus, which is absent in the Swiss and Dutch Caucasian samples and occurs with a frequency of 0.30 in the African American population.

In conclusion, the Dutch data are consistent with HWE and LE, and comparable with other Caucasian population genetic studies. Combined with extensive validation studies [3, 4, 8], this study contributes to the acceptance of PM typing in forensic identity testing in the Netherlands.

References

1. Amplitype PM User Guide – Version 2 (1994) Roche Molecular Systems, Inc. Emmeryville CA, USA
2. Balding DJ, Donnelly P (1994) How convincing is DNA evidence ? Nature 368: 285–286
3. Budowle B, Lindsey JA, DeCou JA, Koons BW, Giusti AM, Comey CT (1995) 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 40: 45–54
4. Fildes N, Reynolds R (1995) Consistency and reproducibility of Amplitype PM Results between seven laboratories. J Forensic Sci (in press)
5. Fisher RA (1951) Standard calculations for evaluating a blood group system. Heredity 5: 95–102
6. Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361–372
7. Helmuth R, Fildes N, Blake E, Luce MC, Chimera J, Madej R, Gorodezky C, Stoneking M, Schmill N, Klitz W, Higuchi R, Erlich HA (1990) HLA DQα allele and genotype frequencies in various human populations, determined by using enzymatic amplification and oligonucleotide probes. Am J Hum Genet 47: 515–523
8. Herrin G Jr, Fildes N, Reynolds R (1994) Evaluation of the Amplitype® PM DNA test system on forensic case samples. J Forensic Sci 39: 1247–1253
9. Hochberg Y (1988) A sharper Bonferroni procedure for multiple tests of significance. Biometrika 75: 800–802
10. Hochmeister MN, Budowle B, Borer UV, Dirnhofer R (1994) Swiss population data on the loci HLA-DQα, LDLR, GYPA, HBGG, D7S8, Gc and D1S80. Forensic Sci Int 67: 175–184
11. Horn GT, Richards B, Merill JJ, Klinger KW (1990) Characterization and rapid diagnostic analysis of DNA polymorphisms closely linked to the cystic fibrosis locus. Clin Chem 36: 1614–1619
12. Kloosterman AD, Budowle B, Riley EL (1993) Population data of the HLA DQ(locus in Dutch Caucasians. Comparison with seven other population genetic studies. Int J Legal Med 105: 233–238
13. Risch NJ, Devlin B (1992) On the probability of matching DNA fingerprints. Science 25: 717–720
14. Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA-polymorphisms and the problem of small samples. Mol Biol Evol 6: 539–545
15. Siebert PD, Fukuda M (1987) Molecular cloning of a human Glycophorin B cDNA: nucleotide sequence and genomic relationship to Glycophorin A. Proc Natl Acad Sci USA 84: 6735–6739
16. Slightom JL, Blechl AE, Smithies O (1980) Human fetal Gγ and Aγ-globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes. Cell 21: 627–638
17. Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW (1984) The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. Cell 39: 27–38
18. Yang F, Brune JL, Naylor SL, Apples RL, Naberhaus KH (1985) Human group specific component (Gc) is a member of the albumin family. Proc Natl Acad Sci USA 82: 7994–7998