

SHORT COMMUNICATION

S. C. Halos · E. S. Fortuno III · A. C. M. Ferreon
J. Y. Chu · J. Miranda · S. Harada · M. Benecke

Allele frequency distributions of the polymorphic STR loci HUMVWA, HUMFES, HUMF13A01 and the VNTR D1S80 in a Filipino population from Metro Manila

Received: 7 July 1997 / Received in revised form: 18 September 1997

Abstract Allele frequency distributions at the short tandem repeat (STR) loci HUMVWA, HUMFES, HUMF13A01 and of the variable number of tandem repeat (VNTR) locus D1S80 were determined in a Filipino population from Metro Manila (103 individuals) by use of the polymerase chain reaction (PCR) followed by polyacrylamide gel electrophoresis (PAGE). The exact test demonstrated that all four loci had no deviations from Hardy-Weinberg equilibrium (HWE) with the only reservation that the exact test p-value for F13A01 is weak. The discriminating power is 0.82 for D1S80, and the expected exclusion chance is 0.85 for F13A01, 0.83 for FES, and 0.93 for VWA. The observed heterozygosity rates are 0.63 for D1S80, 0.66 for F13A01, 0.67 for FES, and 0.80 for VWA. The exact test for independence between all loci gave a p-value of 0.0195. This is the first time that Filipino population data of DNA loci of forensic importance are reported.

Key words Population study · HUMFES · HUMVWA · HUMF13A01 · D1S80 · Philippines

S. C. Halos (✉) · A. C. M. Ferreon · J. Miranda · M. Benecke
Molecular Biology & Biotechnology Program, Albert Hall,
University of the Philippines, Diliman 1101 Quezon City,
Metro Manila, Philippines

S. C. Halos · E. S. Fortuno III · J. Y. Chu
DNA Analysis Laboratory, Natural Sciences Research Institute,
University of the Philippines, Diliman 1101 Quezon City,
Metro Manila, Philippines

S. Harada
Department of Forensic Medicine, Tsukuba University,
Tsukuba, Japan

M. Benecke
Institute for Legal Medicine, University of Cologne,
Melatengürtel 60-62, D-50823 Köln, Germany
e-mail: benecke@uni-koeln.de

Introduction

This population study was performed as an initial approach towards introduction of medico-legal DNA typing in the Philippines. Currently, a network of existing DNA typing laboratories inside the University of the Philippines is being set up and will be extended to the Philippine National Police (PNP) and the National Bureau of Investigations (NBI) in the near future [1].

Materials and methods

DNA from 103 unrelated individuals from Metro Manila was extracted from whole blood by isopropanol-fractionation [2]. Amplification of the D1S80 locus was performed with original primers [3] and with the AmpliFLP D1S80 Amplification Kit (Perkin Elmer) and STRs were amplified with the multiplex GenePrint Kit (Promega). PCR was performed on an AmpliTron I thermocycler (Thermolyne/Barnstead). The PCR products were separated by vertical polyacrylamide gels (43 cm for STRs, and 24 cm for D1S80), and visualization was carried out by silver staining (STRs: conventional protocol, D1S80: PlusOne DNA Silver Staining Kit, Pharmacia Biotech). Statistical analysis of STRs was performed with the DNA View software package and the exact test [4, 5] and statistics for D1S80 additionally followed the approach of Budowle et al. [6] and Sugiyama et al. [7]. To perform a χ^2 -test for determination of Hardy-Weinberg equilibrium of D1S80, alleles were categorized into four allele groups to overcome the small population sample [8].

Results and discussion

For D1S80 16 different alleles were found (Table 1). The order of the most common alleles in our sample was the same as in a general Asian sample [6] and a Japanese sample [7]. The frequency of allele 31 in our sample is the highest observed so far among Asian population groups [6, 7, 9]. By χ^2 analysis and the exact test, no significant deviations between expected and observed values were observed. Compared to other Asian populations [6, 7, 9, 10] the Filipino sample shows the lowest observed heterozygosity (0.63) which might possibly be due to the geo-

Table 1 Distribution of observed D1S80 alleles in a Filipino population from Metro Manila

Allele (repeat no.)	Frequency in (%)
18	16.0
21	3.4
22	2.4
23	0.5
24	31.6
25	3.9
27	8.3
28	1.5
29	2.4
30	18.0
31	10.7
32	1.0
40	0.5
> 41	0.4

Table 2 Statistical values (%) for FES, F13A01, VWA and D1S80 in a Filipino population from Metro Manila ($n = 103$ individuals).

	FES	VWA	F13A01	D1S80
Heterozygosity (observed)	67.1	80.4	66.1	62.7
Expected exclusion chance in stain cases	83.0	93.4	84.7	81.6
Expected exclusion chance in paternity cases	40.1	61.5	43.9	39.5
Exact test p -value	0.208	0.390	0.067	0.616

Table 3 Allele frequencies (in %) of FES, F13A01 and VWA in a Filipino population from Metro Manila ($n = 103$ individuals)

Allele	FES	F13A01	VWA
3.2		22.8	
4		10.2	
5		8.7	
6		51.5	
7	0.5	2.4	
8		2.4	
9		1.5	
10	2.9		
11	43.2		
12	30.6		
13	21.8		
14	1.0	1.0	18.0
15		1.0	9.2
16		1.0	14.6
17			28.6
18			21.4
19			6.8
20			1.5

graphic isolation (island complex) and only very distinct waves of immigration, e.g. by the Spanish. However, the discriminating power is 0.95 (calculated after [7]) and the exclusion chance in stain cases is 0.82 (DNA View) (Table 2).

The Filipino STR allele frequencies of allele 10 in FES, allele 3.2 in F13A01, and allele 6 in F13A01 (Table 3) show a significant difference to the allele frequencies known from Caucasians and African-Americans but correspond to data from Hispanic-Americans and Asian populations [11–19]. No significant deviation in heterozygosity compared to other populations from all over the world was observed [11–19].

The data presented here are the basis for future forensic casework in the Philippines. Furthermore, we plan extensive studies on population genetics of other Philippine native populations.

Acknowledgements This study was supported by the University of the Philippines Molecular Biology & Biotechnology Program (MBB), the Quezon City Local Government, the Philippine Anti Crime Commission, Pharmacia Biotech, and by the Japanese Society for the Promotion of Science. Jeanett Edelmann (University Leipzig, Germany) performed parts of the statistics on the DNA View software.

References

1. Proceedings from the Symposium on DNA Profiling: Technical, legal and ethical policy issues. University of the Philippines, Diliman, Metro Manila (in press)
2. Wang L, Hirayasu K, Ishizawa M, Kobayashi Y (1994) Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated NaI and SDS. *Nucleic Acids Res* 22:1774–1775
3. Kasai K, Nakamura Y, White R (1990) Amplification of a variable number of tandem repeat (VNTR) locus (pMCT 118) by the polymerase chain reaction (PCR) and its application to forensic science. *J Forensic Sci* 35:1196–1200
4. Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361–372
5. Benecke M, Schmitt C, Staak M (1997) Forensische Nutzbarkeit und Populationsdaten der beiden short tandem repeat-Systeme D8S306 und PKLR. *Rechtsmedizin* 7:98–100
6. Budowle B, Baechtel FS, Smerick JB, Presley KW, Giusti AM, Parsons G, Alevy C, Chakraborty R (1995) D1S80 population data in African Americans, Caucasians, Southeastern Hispanics, Southwestern Hispanics, and Orientals. *J Forensic Sci* 40:38–44
7. Sugiyama E, Honda K, Katsuyama Y, Uchiyama S, Tsuchikane A, Ota M, Fukushima H (1993) Allele frequency distribution of the D1S80 (pMCT118) locus polymorphism in the Japanese population by the polymerase chain reaction. *Int J Legal Med* 106:111–114
8. Skowasch K, Wiegand P, Brinkmann B (1992) A new allelic ladder and an improved electrophoretic separation by the polymerase chain reaction. *Int J Legal Med* 105:165–168
9. Woo KM, Budowle B (1995) Korean population data on the PCR-based loci LDLR, GYPA, HBG, D7S8, Gc, HLA-DQA1, and D1S80. *J Forensic Sci* 40:645–648
10. Tie J, Oshida S, Chiba S, Tsukamoto S, Sebetan I (1995) Frequency of D1S80 and the HLA-DQ α alleles in a Chinese population. *Int J Legal Med* 108:170–171
11. Klitschar M, Kubat M, Ebersold A (1995) The distribution of D1S80 (pMCT118) alleles in an Austrian population sample – description of two new alleles. *Int J Legal Med* 107:225–226
12. Sepulchre MA, Wiegand P, Brinkmann B (1995) D1S80 (pMCT118): analysis of 3 ethnic subpopulations living in Brussels. *Int J Legal Med* 108:45–47

13. Nakajima T, Matsuki T, Ohkawara H, Nara M, Furukawa K, Kishi K (1996) Evaluation of 7 DNA markers (D1S80, HLADQ α , LDLR, GYPA, HBG, D7S8 and GC) in a Japanese population. *Int J Legal Med* 109:47–48
14. Kimpton CP, Gill P, Walton A, Urquhart A, Millican ES, Adams M (1993) Automated DNA profiling employing multiplex amplification of short tandem repeat loci. *PCR Methods Appl* 3:13–22
15. Schumm JW, Lins AM, Micka KA, Sprecher CJ, Rabbach DR, Bacher JW (1997) Automated fluorescent detection of STR multiplexes – development of the GenePrint PowerPlex and FFFL multiplexes for forensic and paternity applications. *Proceedings from the First European Symposium on Human Identification, Toulouse*. Promega, Madison, Wisconsin, pp. 90–99
16. Evett IW, Lambert JA, Buckleton JS, Weir BS (1996) Statistical analysis of a large file of data from STR profiles of British Caucasians to support forensic casework. *Int J Legal Med* 109: 173–177
17. Sjerps M, Geest N, Pieron C, Gajadhar M, Kloosterman A (1995) A Dutch population study of the STR loci HUMTHO1, HUMFES/FPS, HUMVWA31/1 and HUMF13A1, conducted for forensic purposes. *Int J Legal Med* 108:127–134
18. Hochmeister MN, Jung JM, Budowle B, Borer UV, Dirnhofer R (1994) Swiss population data on the three tetrameric short tandem repeat loci – VWA, HUMTHO1, and F13A1 – derived using multiplex PCR and laser fluorescence detection. *Int J Legal Med* 107:34–36
19. Martin P, Alonso A, Budowle B, Albarrán C, Garcia O, Sancho M (1995) Spanish population data on 7 tetrameric short tandem repeat loci. *Int J Legal Med* 108:145–149