Int J Leg Med (1993) 106:111–114

International Journal of
Legal Medicine

Original articles

Allele frequency distribution of the D1S80 (pMCT118) locus polymorphism in the Japanese population by the polymerase chain reaction

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Received May 5, 1993 / Received in revised form July 27, 1993

Summary. Population studies among Japanese were carried out at the D1S80 locus by the polymerase chain reaction and subsequent analysis in agarose gel electrophoresis. A total of 58 genotypes and 25 alleles ranging from 16 to 45 repeat units were observed in a population group of 121 unrelated individuals. The alleles with 18, 24 and 30 repeat units were found to be most common. Some large alleles with more than 42 repeat units were first observed in this study. Statistical tests for Hardy-Weinberg equilibrium showed that no significant deviations could be found in this Japanese population sample. The values of the mean exclusion chance and the discriminating power (DP) were calculated to be 0.76 and 0.91, respectively. The observed heterozygosity was 0.91.

Key words: Population genetics – PCR-VNTR – D1S80 – Japanese

Zusammenfassung. Eine populationsgenetische Untersuchung am Locus D1S80 wurde mit Hilfe der Polymerase-Kettenreaktion und anschließender Untersuchung durch Agarose-Gelelektrophorese durchgeführt. Insgesamt wurden 58 Genotypen und 25 Allele (Repeat-Anzahl zwischen 16 and 45) in der 121 nicht-verwandte Personen umfassenden Populationsstichprobe gefunden. Die Allele mit 18, 24 und 30 Repeats waren die häufigsten. Einige große Allele mit mehr als 42 Repeats wurden in dieser Untersuchung erstmalig beobachtet. Die statistische Prüfung des Hardy-Weinberg-Gleichgewichts ergab keine signifikanten Abweichungen in dieser japanischen Populationsstichprobe. Die Werte der mittleren Ausschlußchance und des Diskriminationsindex wurden mit 0,76 und 0,91 errechnet. Die beobachtete Heterozygotierate war 0,91.

Schlüsselwörter: Populationsgenetische Untersuchung – PCR-VNTR – D1S80 – Japaner

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Introduction

Analysis of variable number of tandem repeats (VNTR) loci is widely used for individualization at the DNA level in forensic testing [1]. Amplifiable fragment length polymorphisms (AMPFLP's) have been investigated in several VNTR loci since the polymerase chain reaction (PCR) was first applied. AMPFLP systems such as D1S80 [2–8], 2p24–p23 (ApoB) [4, 9–11], D17S30 (pYNZ22) [4, 12, 13] and 12q13.1 (COL2A1) [4, 14, 15] can be useful tools for forensic studies because of their high sensitivity.

The VNTR locus D1S80 was first isolated and identified by Nakamura et al. [2], and successfully amplified by the PCR [3]. Genetic evidence concerning the D1S80 locus has already been reported for German Caucasian [4, 5], Dutch Caucasians [6]. American Caucasians [7], Black Americans [8], hispanic Americans [8], and a mixed population from Japanese and Caucasians [3]. These studies show that the VNTR D1S80 locus has many alleles and genotypes. The AMPFLP system at the D1S80 locus may be informative for the Japanese population, but at present no informative allelic data are available.

In the present study, the distribution of D1S80 alleles in the Japanese population was examined and compared with the allelic data from other ethnic groups.

Materials and methods

Preparation of DNA. Blood samples were obtained from healthy unrelated individuals. Genomic DNA was isolated from cells by phenol extraction after sodium dodecyl sulfate and proteinase K treatment [16].

Amplification. Amplification was carried out in a Thermal sequence TSR-300 (Iwaki Glass Co., Chiba, Japan). After an initial denaturation step at 94°C for 3 min, reactions were carried out for 30 cycles at an annealing temperature of 65°C for 1 min an extension temperature of 72°C for 2 min and a heat denaturation

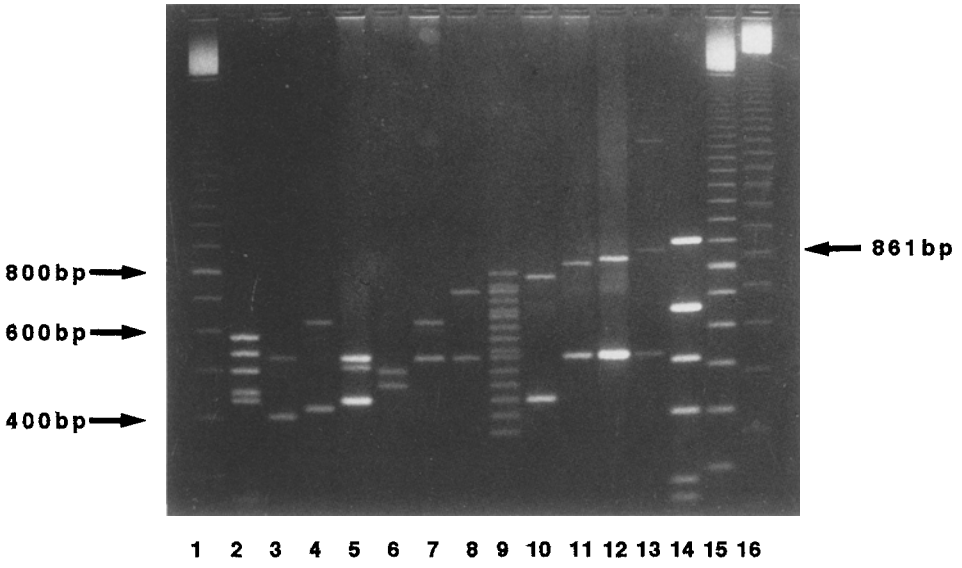

Fig. 1. Agarose gel electrophoresis of PCR amplification of locus D1S80 and DNA markers. Lane 1 and 15, 100 Base-Pair Ladder (Pharmacia-LKB); lane 2, *Hae*III digest of pBR322; lane 3–8 and 10–13, D1S80 alleles: lane 3, 16–24; lane 4, 17–30; lane 5, 18–24; lane 6, 20–22; lane 7, 24–30; lane 8, 24–36; lane 10, 18–39: lane 11, 24–42; lane 12, 24–43; lane 13, 24–45; lane 9, standard D1S80 Allelic Ladder (Cetus Co.); lane 14, *Alu*I digest of pBR322; lane 16, 123 bp DNA Ladder (Life Technologies Inc)

temperature of 94°C for 1 min, followed by a final extension step at 72°C for 5 min. Primer sequences [3]:

5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′
5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′

Each reaction mixture contained 10–300 ng DNA, 10 m*M* Tris-HCl (pH 9.0 at 25°C), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.1% Triton X-100, 0.5 μ*M* each primer, 200 μ*M* dNTPs and 2.5 U Taq DNA polymerase (Promega, Madison, USA). The total volume was 50 μl, and 1 drop of mineral oil was added. Amplification was also carried out using the D1S80 forensic amplification reagent set (Cetus, USA).

Electrophoresis. Amplified products were subjected to electrophoresis in ethidium bromide-stained 6% polyacrylamide gels (1 mm thick, 30 cm or 11 cm long) in 0.09 *M* Tris-borate (pH 8.0) or 0.025 *M* Tris-glycine (pH 8.8) buffer and 2% agarose gels (4 mm thick, 20 cm long) in 0.09 *M* Tris-borate (pH 8.0) buffer. The electrophoresis was carried out at 200 V (for polyacrylamide gels), or 25 V (for agarose gels). After electrophoresis, the gel was photographed by ultraviolet trans-illumination.

Size determination. The fragment sizes were determined by co-migration of a 123 bp ladder, 100 bp ladder and plasmid DNAs (pBR322 and ΦX174) digested with the various restriction enzymes (*Hae*III, *Fok*I, *Hin*fI, *Alu*I and *Mbo*II). The migration of D1S80 alleles and markers were plotted on a semilogarithmic scale vs. distance. In addition, the D1S80 allelic ladder (15 alleles) included in the D1S80 forensic amplification reagent set was also subjected to electrophoresis for allelic determination of each sample (Fig. 1). The allelic ladder is a sizing marker consisting of 15 D1S80 alleles ranging 14 to 40 repeat units (Cetus, USA).

Chemicals. For agarose gel electrophoresis, Nusieve 3:1 (FMC BioProducts, Rockland, ME, USA) was used. The D1S80 forensic amplification reagent set was purchased from Cetus Co. (Emeryville, CA, USA). The 100 Base-Pair Ladder was purchased from Pharmacia-LKB (Uppsala, Sweden) and the 123 bp DNA ladder was from Life Technologies, Inc. (Gaitherburg, MD, USA). Other molecular markers were made in our laboratory by digestion of plasmid DNAs with several restriction enzymes. The plasmid DNAs, pBR322 and ΦX174 (Kyoto, Japan) and the restriction enzymes, *Hae*III, *Fok*I, *Hin*fI, *Alu*I and *Mbo*II were purchased from Takara (Kyoto, Japan).

Results and discussion

Identification of alleles

Because of its high resolution, polyacrylamide gel electrophoresis is useful to analyze low molecular weight PCR-amplified fragments. In this study, however, we faced a problem that molecular markers (e.g. plasmid DNA digested with the various enzymes, 123 bp and 100 bp ladder) did not migrate according to their molecular weights in the polyacrylamide gel electrophoresis (Fig. 2). However, this is not a limitation of agarose gel elec-

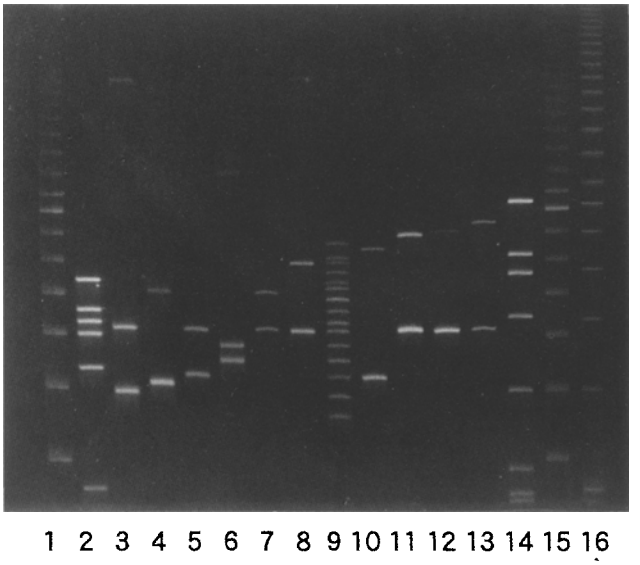

Fig. 2. Polyacrylamide gel electrophoresis of PCR amplification of locus D1S80 and DNA markers. Lane 1 and 15, 100 Base-Pair Ladder (Pharmacia-LKB); lane 2, *Hae*III digest of pBR322; lane 3–8 and 10–13, D1S80 alleles: lane 3, 16–24; lane 4, 17–30; lane 5, 18–24; lane 6, 20–22; lane 7, 24–30; lane 8, 24–36; lane 10, 18–39; lane 11, 24–42; lane 12, 24–43; lane 13, 24–45; lane 9, standard D1S80 Allelic Ladder (Cetus Co.); lane 14, *Alu*I digest of pBR322; lane 16, 123 bp DNA Ladder (Life Technologies Inc)

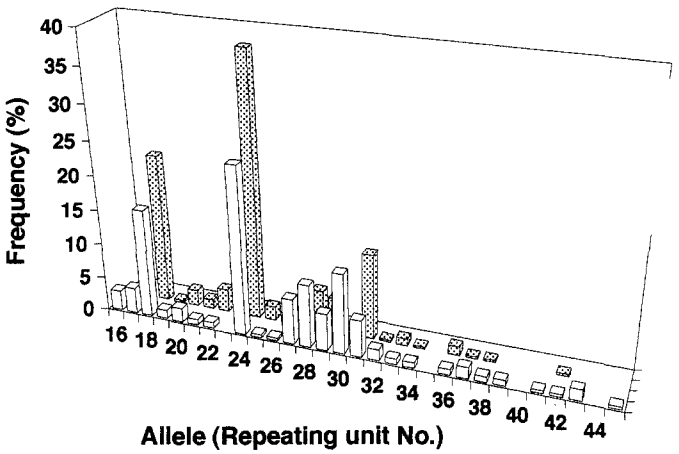

Fig. 3. Comparison of D1S80 allele frequencies in 121 unrelated Japanese (n = 242) with Caucasian data (n = 300) from Kloosterman et al. (1993) (n = number of chromosomes). □ n = 242 Japanese; ▨ n = 300 Dutch Caucasians

Table 1. Distribution of D1S80 alleles in Japanese

Allele (Repeat No.)	N	Frequency (%)	Allele (Repeat No.)	N	Frequency (%)
16	7	2.89	30	28	11.6
17	9	3.72	31	13	5.37
18	38	15.70	32	4	1.65
19	3	1.24	33	2	0.83
20	5	2.07	34	2	0.83
21	2	0.83	36	2	0.83
22	2	0.83	37	4	1.65
24	59	24.4	39	2	0.83
25	1	0.41	41	1	0.41
26	1	0.41	42	1	0.41
27	16	6.61	43	4	1.65
28	22	9.09	45	1	0.41
29	13	5.37	Total	242	100

Table 2. Distribution of D1S80 genotypes observed

Genotypes	N	%	Genotypes	N	%
16–16	1	0.83	20–30	1	0.83
16–22	1	0.83	21–31	2	1.65
16–24	1	0.83	24–24	4	3.31
16–30	1	0.83	24–26	1	0.83
16–31	1	0.83	24–27	6	4.96
16–37	1	0.83	24–28	5	4.13
17–24	4	3.31	24–29	7	5.79
17–28	4	3.31	24–30	5	4.13
17–30	1	0.83	24–31	2	1.65
18–18	1	0.83	24–32	1	0.83
18–19	1	0.83	24–36	1	0.83
18–24	10	9.71	24–37	1	0.83
18–25	1	0.83	24–42	1	0.83
18–27	3	2.48	24–43	3	2.48
18–28	2	1.65	24–45	1	0.83
18–29	2	1.65	27–27	1	0.83
18–30	8	6.61	27–28	1	0.83
18–31	1	0.83	27–30	4	3.88
18–32	2	1.65	28–28	3	2.48
18–33	1	0.83	28–29	1	0.83
18–34	1	0.83	28–30	2	1.65
18–36	1	0.83	28–41	1	0.83
18–37	1	0.83	29–30	2	1.65
18–39	2	1.65	30–31	3	2.48
19–20	1	0.83	30–33	1	0.83
19–24	1	0.83	31–31	1	0.83
20–22	1	0.83	31–32	1	0.83
20–24	1	0.83	31–43	1	0.83
20–29	1	0.83	34–37	1	0.83
			Total	121	100

trophoresis (Fig. 1). Molecular sizes of all the amplified fragments were estimated by calculating based on the calibration curves made using 7 molecular weight markers.

Abnormal electrophoretic behaviour in the polyacrylamide gels was discovered more than 10 years ago [17] and was found to be caused by DNA bending at special base sequences [18] involving homopolymeric dA/dT residues [19]. These findings reveal that it is impossible to estimate net molecular weights of DNA fragments which contain the special sequences using polyacrylamide gel electrophoresis. The migration problem observed in our study might be due to the bending of the some DNA markers which involve dA/dT residues. Since the sequence of the D1S80 locus does not include so many dA/dT residues [3], no disparity was found among the amplified fragments. Fifteen amplified D1S80 alleles ranging from 14 to 40 repeat units were identified by side-to-side comparison with the D1S80 allelic ladder. No contradiction was found between the amplified fragments at D1S80 locus and the commercial D1S80 allelic ladder, using polyacrylamide and agarose gel electrophoresis (Figs. 1, 2).

Population study

In this population study (n = 242), 25 different alleles (Fig. 3, Table 1) were distinguished. The allele 24 (P = 0.24) was most common; alleles 18 (P = 0.16) and 30 (P = 0.12) were the next most common. As shown in Fig. 1 (lane 11–13), the alleles containing 43 and 45 repeating units, which have not been previously observed, were found in this study. In total 58 different genotypes (Table 2), were found of which 18–24 (Fig. 1, lane 5) was most common (P = 0.10). The mean exclusion chance [20] was calculated to be 0.76. The discriminating power (DP was calculated as $1 - \Sigma (Pi)^2$ and Pi represents the frequency of each genotype) was 0.91 and the observed heterozygosity was 0.91.

Hardy-Weinberg equilibrium

Because of relatively small population samples, a reliable estimation of deviations from the Hardy-Weinberg equi-

Table 3. Check for Hardy-Weinberg equilibrium

Allele groups	4 allele model				3 allele model			
I	Alleles 16–23				Alleles 16–23			
II	Allele 24				Allele 24			
III	Alleles 25–30				Alleles 25–45			
IV	Alleles 31–45							
	Observed		Expected		Observed		Expected	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)
I–I	6	(4.95)	9.00	(7.43)	6	(4.95)	9.00	(7.43)
I–II	17	(14.0)	16.1	(13.3)	17	(14.0)	16.1	(13.3)
I–III	24	(19.8)	22.1	(18.3)	37	(30.6)	31.9	(26.4)
I–IV	13	(10.7)	9.82	(8.12)				
II–II	4	(3.31)	7.19	(5.94)	4	(3.31)	7.19	(5.94)
II–III	24	(19.8)	19.7	(16.3)	34	(28.1)	28.5	(23.6)
II–IV	10	(8.26)	8.78	(7.26)				
III–III	14	(11.6)	13.6	(11.2)	23	(19.0)	28.3	(23.4)
III–IV	5	(4.13)	12.1	(10.0)				
IV–IV	4	(3.31)	2.68	(2.21)				
Total	121	(100)	121	(100)	121	(100)	121	(100)
χ^2	9.542 ($df = 9$)				5.318 ($df = 5$)			
	$0.3 < P < 0.5$				$0.3 < P < 0.5$			

librium is not possible using each separate allele (Fig. 3). Alleles were therefore categorized into allele groups [4], and calculations were carried out using these models. The χ^2 values show no significant deviation between expected and observed values (Table 3).

In Japanese, the D1S80 locus can be a more informative marker of individualization than in Caucasians.

References

1. Lewontin RC, Hartl DL (1991) Population genetics in forensic DNA typing. Science 254: 1745–1750
2. Nakamura Y, Carlson M, Krapcho K, White R (1988) Isolation and mapping of a polymorphic DNA sequence, pMCT118, on chromosome 1p (D1S80). Nucleic Acids Res 16: 9364
3. Kasai K, Nakamura Y, White R (1990) Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction and its application to forensic science. J Forensic Sci 35: 1196–1200
4. Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B (1992) Population genetics and forensic efficiency data of 4 AMPFLP's. Int J Leg Med 104: 329–333
5. Skowasch K, Wiegand P, Brinkmann B (1992) pMCT118 (D1S80): a new allelic ladder and an improved electrophoretic separation lead to the demonstration of 28 alleles. Int J Leg Med 105: 165–168
6. Kloosterman AD, Budowle B, Daselaar P (1993) PCR-amplification and detection of the human D1S80 VNTR locus. Int J Leg Med 105: 257–264
7. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC (1991) Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 48: 137–144
8. Eisenberg M, Maha G (1991) AMPFLP analysis in parentage testing. In: Proceedings from the Second International Symposium on Human Identification. Promega Corporation, Madison, USA, pp 129–154
9. Boerwinkle E, Xiong W, Fourest E, Chan L (1989) Rapid typing of tandem repeated hypervariable loci by the polymerase chain reaction: application to the apolipoprotein B3′ hypervariable region. Proc Natl Acad Sci USA 86: 212–216
10. Ludwig EH, Friedl W, McCarthy BJ (1989) High-resolution analysis of a hypervariable region in the human apolipoprotein B gene. Am J Hum Genet 45: 458–464
11. Vuorio AF, Sajantila A, Hämäläinen T, Syvänen A-C, Ehnholm E, Peltonen L (1990) Amplification of the hypervariable region close to the apolipoprotein B gene: application to forensic problems. Biochem Biophys Res Commun 170: 616–620
12. Wolff RK, Nakamura Y, White R (1988) Molecular characterization of a spontaneously generated new allele at a VNTR locus: no exchange of flanking DNA sequence. Genomics 3: 347–351
13. Horn GT, Richard B, Klinger KW (1989) Amplification of a highly polymorphic VNTR segment by the polymerase chain reaction. Nucleic Acids Res 17: 2140
14. Wu S, Seino S, Bell GI (1990) Human collagen type II, alpha 21, (COL2A1) gene: VNTR polymorphism detected by gene amplification. Nucleic Acids Res 18: 3102
15. Priestley L, Kumar D, Sykes B (1990) Amplification of the COL2A1 3′ variable region used for segregation analysis in a family with Stickler syndrome. Hum Genet 85: 525–526
16. Inoko H, Ando A, Ito M (1986) Southern hybridization analysis of DNA polymorphism in the HLA-D region. Hum Immunol 16: 304–313
17. Marini JC, Levene SD, Crothers DM, Englund PT (1982) Bent helical structure in kinetoplast DNA. Proc Natl Acad Sci USA 79: 7664–7668
18. Hagerman PJ (1985) Sequence dependence of the curvature of DNA: a test of the phasing hypothesis. Biochemistry 24: 7033–7037
19. Koo H-S, Wu H-M, Crothers DM (1986) DNA bending at adenine-thymine tracts. Nature 320: 501–506
20. Krüger J, Fuhrmann W, Lichte KH, Steffens C (1968) Zur Verwendung des Polymorphismus der sauren Eryhtrocytenphosphatase bei der Vaterschaftsbegutachtung. Dtsch Z Gerichtl Med 64: 127–146