Int J Legal Med (2003) 117 : 34–38
DOI 10.1007/s00414-002-0319-6

ORIGINAL ARTICLE

**Rieko Uchihi · Toshimichi Yamamoto · Kanako Usuda
Takashi Yoshimoto · Miwa Tanaka · Seisaku Tokunaga
Rina Kurihara · Katsushi Tokunaga ·
Yoshinao Katsumata**

Haplotype analysis with 14 Y-STR loci using 2 multiplex amplification and typing systems in 2 regional populations in Japan

Received: 27 December 2001 / Accepted: 17 May 2002 / Published online: 10 January 2003

Abstract In this study 14 Y-STR loci (DYS393, DYS19, DYS391, DYS437, DYS435, DYS439, DYS389II, DYS438, DYS436, DYS390, Y-GATA-H4, DYS385, Y-GATA-A7.1 and DYS392) were analysed in 207 Japanese males from Honshu (main island of Japan, Nagoya City) and 87 Japanese males from Okinawa (southernmost islands of Japan) using two multiplex PCR typing systems, a novel 10-plex amplification system and a new commercially available 6-plex typing kit which had two loci in common. The allele frequency distributions were similar at almost all of the 14 loci. Of the haplotypes observed, 244 were unique in both Japanese populations and 17 haplotypes were observed more than once but the 2 populations shared only 7 haplotypes. The haplotype diversities for the 14 loci were 0.9987 and 0.9976 in Honshu and Okinawa Japanese, respectively. The haplotype analysis at 14 Y-STR loci would be useful for personal identification in forensic fields and for population genetics because of the high divergence of these haplotypes.
Electronic supplementary material to this paper (Table S1) can be obtained by using the Springer Link server located at http://dx.doi.org/10.1007/s00414-002-0319-6

Keywords Y chromosome · STRs · Y-haplotypes · Japanese population · Okinawa

Electronic supplementary material to this paper (Table S1) can be obtained by using the Springer Link server located at http://dx.doi.org/10.1007/s00414-002-0319-6

R. Uchihi (✉) · T. Yamamoto · K. Usuda · T. Yoshimoto
M. Tanaka · S. Tokunaga · R. Kurihara · Y. Katsumata
Department of Legal Medicine and Bioethics,
Graduate School of Medicine, Nagoya University,
Nagoya 466-8550, Japan
e-mail: rukko@med.nagoya-u.ac.jp,
Fax: +81-52-744-2121

K. Tokunaga
Department of Human Genetics,
Graduate School of Medicine, The University of Tokyo,
Tokyo 113-0033, Japan

Introduction

STRs (microsatellites) are widely distributed throughout the whole human genome and it was reported that one STR exists at every 2 kb in the human genome (International Human Genome Sequencing Consortium 2001). Numerous Y-STRs (STRs on the Y chromosome) have been discovered and some have been used for forensic purposes (Gill et al. 2001), and population studies (de Knijff et al. 1997; Bosch et al. 2000; Hidding and Schmitt 2000) to evaluate diversity of the haplotypes, but multiplex methods are needed to analyse them effectively.

A novel multiplex amplification system for 10 Y-STR loci was presented at the 11th International Symposium on Human Identification in Biloxi in 2000 by Christian M. Ruitberg and John M. Butler. This system contains the five new Y-STR loci (DYS435, DYS436, DYS437, DYS438, and DYS439) selected from sequence databases by Ayub et al. (2000) and the two loci (Y-GATA-A7.1 and Y-GATA-H4) developed by White et al. (1999) in addition to the three well-known loci, DYS19, DYS391, and DYS392. These 7 new loci are all tetrameric Y-STRs with the exception of the trimeric DYS436 and the pentameric DYS438. Some researchers have analysed these loci and reported high diversities (Grignani et al. 2000; González-Neira et al. 2001; Hou et al. 2001; Mohyuddin et al. 2001).

Recently, a new multiplex amplification and typing kit for six Y-STRs (Y-PLEX 6 kit, Reliagene) was released commercially in the U.S.A. It is very convenient to use this kit, which includes an allelic ladder marker, in order to obtain the haplotypes for Y-STRs effectively and accurately. Each of the six loci (DYS393, DYS19, DYS391, DYS389II, DYS390, and DYS385) in the kit has been well investigated (de Knijff et al. 1997).

We applied both systems (for a total of 14 loci) to analyse the haplotypes in 2 regional populations in Japan. We calculated the allele or haplotype frequencies and compared them in both populations. We also examined female samples to confirm Y-chromosome specificity at each locus in both populations.

Table 1 Allele frequencies at 13 Y-STRs in the Honshu- and Okinawa-Japanese populations

Locus	Alleles	Frequency		Locus	Alleles	Frequency	
		Honshu	Okinawa			Honshu	Okinawa
DYS393	11	0.024	0	DYS438	8	0.005	0
	12	0.222	0.230		9	0.014	0.058
	13	0.618	0.506		10	0.556	0.667
	14	0.126	0.218		11	0.106	0.046
	15	0.010	0.046		12	0.034	0
					13	0.266	0.230
DYS19	13	0.048	0.081		14	0.019	0
	14	0.058	0.058				
	15	0.488	0.299	DYS436	12	0.990	1.000
	16	0.217	0.287		13	0.010	0
	17	0.184	0.276				
	18	0.005	0	DYS390	22	0.213	0.207
					23	0.227	0.184
DYS391	9	0.039	0		24	0.227	0.195
	10	0.865	0.805		25	0.300	0.368
	11	0.092	0.184		26	0.029	0.046
	12	0.005	0.012		27	0.005	0
DYS437	13	0.005	0	Y-GATA-H4	9	0.014	0.012
	14	0.845	0.874		10	0.425	0.414
	15	0.150	0.115		11	0.512	0.517
	16	0	0.012		12	0.048	0.035
					13	0	0.012
DYS435	11	0.850	0.862		14	0	0.012
	12	0.150	0.115				
	13	0	0.023	Y-GATA-A7.1	9	0.092	0.103
					10	0.179	0.241
DYS439	10	0.034	0.023		11	0.662	0.632
	11	0.179	0.161		12	0.063	0.023
	12	0.565	0.517		13	0.005	0
	13	0.198	0.253				
	14	0.019	0.035	DYS392	10	0.005	0
	15	0.005	0.012		11	0.391	0.506
					12	0.053	0.035
DYS389II	27	0.029	0.081		13	0.449	0.391
	28	0.140	0.092		14	0.077	0.069
	29	0.295	0.276		15	0.014	0
	30	0.300	0.264		16	0.005	0
	31	0.198	0.253		17	0.005	0
	32	0.039	0.035				

n=207 in Honshu and 87 in Okinawa Japanese.

Materials and methods

DNA samples

Blood samples were collected with written informed consent from 227 healthy unrelated Japanese volunteers in Honshu (the main island of Japan), including 207 males and 20 females. DNA was extracted from blood samples as described previously (Tamaki et al. 1991). DNA samples from 87 unrelated Japanese males in Okinawa (the southernmost islands of Japan) were previously collected for studies on HLA alleles and haplotypes (Hatta et al. 1999).

PCR amplification

Two multiplex PCR amplification systems were used. A multiplex amplification and typing system for 10 STR loci (DYS19, DYS391, DYS437, DYS435, DYS439, DYS438, DYS436, Y-GATA-H4, Y-GATA-A7.1, and DYS392), which was devised by Christian M. Ruitberg and John M. Butler and presented at the 11th International Symposium on Human Identification in Biloxi in 2000, was used with minor modifications by increasing the number of PCR cycles from 25 to 30 and the annealing temperature from 55°C to 59°C, by increasing a primer concentration 2.5 times for DYS19, and by changing the labelled dye set from Set C (FAM, TET and HEX) to Set D (FAM, HEX and NED).

We also used a commercially available multiplex amplification and typing kit (Y-PLEX 6, Reliagene Technologies, La.) for six STR loci (DYS393, DYS19 and DYS389II labeled with FAM, and

Table 2 Haplotype frequencies at the DYS385 in Honshu- and Okinawa-Japanese

Haplotype	Frequency		Haplotype	Frequency	
	Honshu	Okinawa		Honshu	Okinawa
9–15	0.005	0.023	13–14	0.010	0.058
9–18	0.005	0	13–15	0.029	0.046
10–10	0.015	0	13–16	0.039	0.023
10–14	0.005	0	13–17	0.097	0.126
10–15	0.005	0	13–18	0.048	0.046
10–17	0.010	0.012	13–19	0.005	0
10–18	0.077	0.023	13–20	0.010	0.023
10–19	0.077	0.092	13–23	0.005	0
10–20	0.101	0.069	13–24	0.015	0
10–21	0.015	0.035	14–14	0.010	0.012
10–23	0.005	0	14–15	0	0.012
11–12	0.024	0.023	14–16	0.029	0.012
11–13	0.005	0	14–17	0.073	0.035
11–18	0.005	0	14–18	0.024	0.058
11–19	0.015	0.023	14–19	0.010	0.012
11–21	0.005	0.046	14–20	0.005	0.046
12–12	0.015	0	14–21	0.005	0
12–13	0.010	0.012	14–22	0.005	0
12–14	0.015	0	15–16	0	0.012
12–15	0	0.012	15–17	0.010	0
12–16	0.015	0.023	15–18	0.005	0
12–17	0.034	0.012	15–20	0.010	0
12–18	0.024	0.023	16–16	0.005	0
12–19	0.029	0.012	16–17	0.005	0
12–20	0.010	0.012	16–18	0	0.012
12–21	0.015	0.012	16–20	0.005	0
13–13	0.005	0.012	17–17	0.005	0

DYS390, DYS391 and DYS385 labeled with TAMRA) including the allelic ladder marker. These six loci were amplified according to the instruction manual with the minor modification of decreasing the total PCR volume.

Typing

The PCR products were mixed with GeneScan-500 (ROX) size standard (Applied Biosystems) and separated by capillary electrophoresis with the Genetic Analyzer 310 (PE Applied Biosystems, Foster City, Calif.). The typing of those products at 10 Y-STR loci was performed by comparing to an allelic ladder marker which we constructed after confirming the sequences. The PCR products for the six Y-STR loci were typed automatically using the Genotyper software (PE Applied Biosystems, Calif.).

Statistical analysis

We calculated allele frequencies at each locus in the two regional Japanese populations, and compared them using the software Genepop 3.1b (Raymond and Rousset 1995). The gene or haplotype diversity (h) was calculated as $h = n\left(\sum x_i^2\right) / (n-1)$, where n is the number of individuals and x_i is the frequency of each allele or haplotype (Nei 1987). The discrimination capacity was calculated as the percentage proportion of unique haplotypes (Gené et al. 1999).

Results and discussion

We performed the typing of Y-STR loci using the two multiplex (10-plex and 6-plex) amplification and typing

Table 3 Gene (haplotype) diversity values and the P-values for difference between Honshu- and Okinawa-Japanese at each Y-STR loci

Locus	Gene diversity		*P*-value
	Honshu	Okinawa	
DYS393	0.5545	0.6490	0.0271*
DYS19	0.6785	0.7509	0.0470*
DYS391	0.2435	0.3224	0.0239*
DYS437	0.2641	0.2261	0.3978
DYS435	0.2559	0.2459	0.0924
DYS439	0.6108	0.6482	0.7089
DYS389II	0.7660	0.7829	0.3176
DYS438	0.6107	0.5031	0.0436*
DYS436	0.0192	0.0000	1.0000
DYS390	0.7645	0.7565	0.7669
Y-GATA-H4	0.5572	0.5662	0.4668
DYS385	0.9558[a]	0.9583[a]	0.3094
Y-GATA-A7.1	0.5201	0.5370	0.4657
DYS392	0.6390	0.5924	0.7378
Haplotype diversity	0.9987	0.9976	

*significantly different ($P<0.05$) between Honshu and Okinawa Japanese
[a]haplotype diversity

Table 4 The comparisons of haplotype information parameters between the two multiplex systems and a combined system at Y-STR loci

	Combined (14 loci)		6-Plex		10-Plex	
	Honshu	Okinawa	Honshu	Okinawa	Honshu	Okinawa
Different haplotypes	189	79	161	72	146	66
Discrimination capacity	0.8599	0.8276	0.6860	0.7126	0.5894	0.6092
Haplotype diversity	0.9987	0.9976	0.9943	0.9931	0.9890	0.9915

systems, which shared 2 loci (DYS19 and DYS391), and therefore a total of 14 loci could be typed. The allele frequencies for 13 Y-STR loci excluding DYS385 and the haplotype frequencies for DYS385 were calculated from 207 Honshu and 87 Okinawa Japanese males, and were compared between both populations (Tables 1 and 2). The patterns of allele frequency distributions between them were almost identical for DYS439, DYS436, and DYS390. In terms of statistical comparative analysis by the GENEPOP software, the distributions at DYS393, DYS19, DYS391, and DYS438 were significantly different ($p<0.05$), but the values were close to 0.05 (0.027, 0.047, 0.024, and 0.044, respectively, Table 3).

All haplotypes for the 14 Y-STR loci observed in the present study are listed in Table S1 which is available as ESM. A sample of 294 males presented 261 different haplotypes. In Honshu-Japanese, 189 different haplotypes were observed and 79 in Okinawa-Japanese. Only 7 shared haplotypes were observed between the populations, and only a single one (haplotype 195) occurred more than once in both populations. Of the haplotypes, 11 were observed more than once in Honshu-Japanese and 7 in Okinawa-Japanese. The most frequent haplotype was observed 5 times in Honshu and 3 times in Okinawa. The haplotype diversities for the 14 loci were estimated as 0.9987 and 0.9976 in Honshu- and Okinawa-Japanese, respectively.

We compared the peak heights observed in PCR products from females to those from males at each of the 14 Y-STR loci. In the 10-plex system, low peaks appeared in female samples at a few loci, but the highest ones at DYS436 were 10–20 times lower than in males. No peaks were observed in females at any of the six loci in the Y-PLEX 6 kit.

The present allele distributions at the DYS19, 389II, 390, 391, 392, 393, and 385 loci in Honshu-Japanese were quite similar to those in other Honshu-Japanese populations reported previously by Honda et al. (2001) and Nonaka and Minaguchi (2001). On the other hand, the allele distributions of these seven loci in Koreans (Shin et al. 2001) were significantly different ($p<0.05$) from those in Honshu and Okinawa reported in the present study.

We compared the haplotype frequencies and diversities in the two multiplex systems (Table 4). The 6-plex typing system showed higher haplotype diversities than those of the 10-plex system in both populations mainly because of the extremely high diversity at DYS385.

The haplotype diversity values for the 14 loci (0.9987 and 0.9976 in Honshu and Okinawa, respectively) were higher than the value of 0.9824 for 7 loci in Swiss (Gehrig et al. 2000), 0.9907 for 11 loci in Italian (Grignani et al. 2000), or 0.92–0.99 for 16 loci in the regional Pakistani populations (Mohyuddin et al. 2001). Haplotype analysis at 14 loci by combining the 2 systems revealed extremely high diversity.

In the present study, we confirmed the Y-chromosome specificity and high diversity at the 14 Y-STR loci in Japanese using the 2 novel multiplex amplification and typing systems. Therefore, if the systems were evaluated in more detail, they would be useful in the forensic field and population genetics because of their high divergence even in closely related populations.

Acknowledgements This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Ayub Q, Mohyuddin A, Qamar R, Mazhar K, Zerjal T, Mehdi SQ, Tyler-Smith C (2000) Identification and characterisation of novel human Y-chromosomal microsatellites from sequence database information. Nucleic Acid Res 28:e8

Bosch E, Calafell F, Pérez-Lezaun A, et al. (2000) Y chromosome STR haplotypes in four populations from northwest Africa. Int J Legal Med 114:36–40

Gehrig C, Hochmeister M, Budowle B (2000) Swiss allele frequencies and haplotypes of 7 Y-specific STRs. J Forensic Sci 45:436–439

Gené M, Borrego N, Xifró A, Piqué E, Moreno P (1999) Haplotype frequencies of eight Y-chromosome STR loci in Barcelona (North-East Spain). Int J Legal Med 112:403–405

Gill P, Brenner C, Brinkmann B, et al. (2001) DNA Commission of the International Society of Forensic Genetics: recommendations on forensic analysis using Y-chromosome STRs. Int J Legal Med 114:305–309

González-Neira A, Elmoznino M, Lareu MV, Sánchez-Diz P, Gusmão L, Prinz M, Carracedo A (2001) Sequence structure of 12 novel Y chromosome microsatellites and PCR amplification strategies. Forensic Sci Int 122:19–26

Grignani P, Peloso G, Fattorini P, Previderè C (2000) Highly informative Y-chromosomal haplotypes by the addition of three new STRs DYS437, DYS438 and DYS439. Int J Legal Med 114:125–129

Hatta Y, Ohashi J, Imanishi T, et al. (1999) HLA genes and haplotypes in Ryukyuans suggest recent gene flow to the Okinawa Islands. Hum Biol 71:353–365

Hidding M, Schmitt C (2000) Haplotype frequencies and population data of nine Y-chromosomal STR polymorphisms in a German and a Chinese population. Forensic Sci Int 113:47–53

Honda K, Tun Z, Young D, Terao T (2001) Examination of Y-STR mutations in sex chromosomal abnormality in forensic cases. Forensic Sci Int 118:136–140

Hou YP, Zhang J, Li YB, Wu J, Zhang SZ, Prinz M (2001) Allele sequences of six new Y-STR loci and haplotypes in the Chinese Han population. Forensic Sci Int 118:147–152

International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921

Knijff P de, Kayser M, Caglià A, et al. (1997) Chromosome Y microsatellites: population genetic and evolutionary aspects. Int J Legal Med 110:134–140

Mohyuddin A, Ayub Q, Qamar R, Zerjal T, Helgason A, Mehdi SQ, Tyler-Smith C (2001) Y-chromosomal STR haplotypes in Pakistani populations. Forensic Sci Int 118:141–146

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nonaka I, Minaguchi K (2001) Allele frequencies and haplotypes of ten Y-specific STRs in the Japanese population. J Forensic Sci 46:179–182

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Shin DJ, Jin HJ, Kwak KD, et al. (2001) Y-chromosome multiplexes and their potential for the DNA profiling of Koreans. Int J Legal Med 115:109–117

Tamaki K, Yamamoto T, Uchihi R, et al. (1991) Frequency of HLA-DQA1 alleles in the Japanese population. Hum Hered 41:209–214

White PS, Tatum OL, Deaven LL, Longmire JL (1999) New, male-specific microsatellite markers from the human Y chromosome. Genomics 57:433–437