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**Rina Kurihara · Toshimichi Yamamoto · Rieko Uchihi
Shi-Lin Li · Takashi Yoshimoto · Hiroyuki Ohtaki
Kiyofumi Kamiyama · Yoshinao Katsumata**

Mutations in 14 Y-STR loci among Japanese father-son haplotypes

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Abstract In the present study 161 Japanese father/son haplotype transfers in 147 pedigrees were analyzed at 14 Y-STRs with two multiplex PCR-based typing systems. Five isolated single repeat mutations were identified at the DYS389I, DYS439, Y-GATA-H4, DYS389II and DYS391 loci, and a pedigree showing triple alleles at the DYS385 locus (a duplicate locus) without allelic discrepancy between the father and son was also observed. The overall mutation rate estimated across the 14 Y-STRs in the Japanese population was 0.22%/locus/meiosis (95% C.I. 0.09–0.51%). This rate was not significantly different ($p>0.05$) from those of autosomal STRs and Y-STRs in other populations, including German, Austrian, Polish and Norwegian populations. Furthermore, 138 haplotypes were identified in 147 pedigrees with a haplotype diversity value of 0.9983. Therefore, a combination of the two systems should permit effective analysis with sufficient discriminatory power.

Keywords Y-STRs · Mutation · Y-haplotypes · Japanese population

Introduction

Short tandem repeats (STRs) are indispensable tools in forensic science and population genetics due to the ease of typing and high intra-population and inter-population heterogeneity. During the last decade, researchers have described highly polymorphic Y-chromosome-specific STRs (Y-STRs), which are useful in investigations of such crimes as sexual assaults and in male sibship analysis where the alleged father is deceased or missing. Y-STRs also appear to be useful for the study of genetic relationships among closely related populations [1]. Although a number of multiplex PCR-based typing systems for Y-STRs have been developed [2, 3, 4, 5], (unpublished results C.M. Ruitberg and J.M. Butler) and databases are now being constructed, there are few studies on mutations at Y-STR loci, especially in the Japanese population. Valid mutation rate estimates are necessary for paternity testing to avoid false exclusions in cases where there is an allelic discrepancy between the biological father and son due to mutation. The possibility of encountering false exclusions increases when highly polymorphic loci are used to increase discriminating power since mutations may create new alleles.

In the present study we used two multiplex PCR-based typing systems to estimate the overall mutation rate of 14 Y-STRs in the Japanese population from analysis of 161 father/son haplotype transfers in 147 pedigrees. In addition, we determined the haplotypes and the haplotype diversity value observed in our Japanese population to evaluate the discriminatory power of the 14 Y-STRs.

Materials and methods

DNA sample preparation

Blood samples or buccal swabs were collected from 307 healthy Japanese males in 147 pedigrees, and a total of 161 meioses were investigated. All relationships had been confirmed as biological father-son relationships using autosomal STR markers and/or multilocus or single-locus probe methods. Signed informed consent was obtained from all participants. Prior to collecting the samples, the study was reviewed and approved by the ethics committee of the School of Medicine, Nagoya University. DNA was extracted using a routine organic extraction method. The DNA concentration of each sample was quantified fluorometrically with bisbenziamide Hoechst 33258 trihydrochloride (Sigma, St. Louis, MO) using the DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech, San Francisco, CA).

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

K. Kamiyama
DNA-Fingerprint Division, TSL Inc.,
3-5-5 Midorigaoka, 205-0003 Hamura Tokyo, Japan

Table 1 The sequences of repetitive regions and the mutations at 14 Y-STRs in the present study

Locus	Repetitive sequence	Father	Son
DYS389I	$(CTGT)_3(CTAT)_n$	$(CTGT)_3(CTAT)_{11}$	$(CTGT)_3(CTAT)_{12}$
DYS439	$(GATA)_n$	$(GATA)_{13}$	$(GATA)_{14}$
DYS435	$(TGGA)_n$		
DYS19	$(TAGA)_3TAGG(TAGA)_n$		
DYS460	$(GATA)_n$		
Y-GATA-H4	$(GATA)_2(GGTA)_3(GATA)_n$	$(GATA)_2(GGTA)_3(GATA)_{11}$	$(GATA)_2(GGTA)_3(GATA)_{12}$
DYS391	$(TCTG)_3(TCTA)_n$	$(TCTG)_3(TCTA)_{11}$	$(TCTG)_3(TCTA)_{10}$
DYS392	$(TAT)_n$		
DYS438	$(TTTTC)_n$		
DYS437	$(TCTA)_m(TCTG)_n(TCTA)_4$		
DYS393	$(AGAT)_n$		
DYS389II	$(CTGT)_m(CTAT)_n$	$(CTGT)_4(CTAT)_{11}$	$(CTGT)_4(CTAT)_{12}$
DYS390	(TCTR)n		
DYS385	$(GAAA)_n$		

PCR amplification and genotyping

We analyzed a total of 14 Y-STRs with 2 multiplex PCR-based typing systems. The loci DYS389I, DYS439, DYS435, DYS19, DYS460, Y-GATA-H4, DYS391, DYS392, DYS437, and DYS438 were analyzed with a 10-plex system originally devised by Ruitberg and Butler (unpublished results, personal communication). In the present study, DYS 389I was substituted for DYS436 because the gene diversity of the latter was estimated to be low in the Japanese population [6]. The six loci, DYS393, DYS19, DYS389II, DYS390, DYS391, and DYS385 were genotyped with the commercially available kit, Y-PLEX6 (Reliagene Technologies, New Orleans, LA). DYS19 and DYS391 are included in both systems. The sequence information of the 14 Y-STRs is shown in Table 1. Alleles were named according to the recommendation by the DNA Commission of the International Society for Forensic Genetics [7]. In the 10-plex system, PCR amplification was performed using the conditions described in our previous study [6] except that the newly designed primer set (1 μM DYS389InF: ATAGATAGATTGATAGAGGGAGGGAT, 1 μM DYS389InR: CTCTCATCTGTATTATCTATGTA) for DYS389I was added to the mixture instead of those for DYS436. The PCR products were loaded onto a Genetic Analyzer 310 (PE Applied Biosystems, Foster City, CA) with GeneScan-500 (ROX) size standard (PE Applied Biosystems), and genotyped using Genotyper 2.5 software (PE Applied Biosystems). The allelic ladder markers were made by ourselves after confirming the sequences of all alleles observed in a Japanese population. In the Y-PLEX6 system, the PCR reaction and subsequent analysis of the amplified products were performed according to the manufacturer's instructions (Reliagene Technologies).

Haplotyping and sequence analysis

After genotyping all the samples with the two multiplex systems, we listed the haplotypes and checked to determine if there were any allele mismatches within the father/son pairs. In cases where an allele mismatch was observed, we performed sequence analysis.

Statistical analysis

The gene or haplotype diversity (h) value was calculated according to the formula $h=n(1-\sum x_i^2)/(n-1)$, where n is the number of individuals and x_i is the frequency of each allele or haplotype. The maximum-likelihood estimate of the mutation rate across the 14 Y-STRs in the Japanese population was computed as the number of mutations divided by the total number of allelic transfers analyzed. The number of mutation events within a given number of allelic transfers under a given mutation rate follows a binomial distribution. The 95% binomial confidence interval (CI) was determined by the Blyth-Still-Casella procedure [8]. Fisher's exact test was used to compare our mutation rate estimates with those of Brinkmann et al. [9] for 9 autosomal STRs in German and Austrian populations, Kayser et al. [10] for 15 Y-STRs in German and Polish populations, and Dupuy et al. [11] for 9 Y-STRs in a Norwegian population. We also used Fisher's exact test to examine gain versus loss of repeats mutations. Statistical calculations were all carried out with StatXact-5.0.3 (Cytel Software, Cambridge, MA).

Results

The haplotypes detected for the 14 Y-STRs in the Japanese population are listed in Table 2, where the pre-mutation allele was regarded as the pedigree allele in each haplotype with mutation events. An unusual triplicate allele was also observed instead of a duplicate allele at DYS385 between both father and son in the haplotype 71 pedigree (Fig. 1). This triplicate allele was regarded as a single haplotype at DYS385 instead of a mutated allele because there was no allelic discrepancy between them. As shown in Table 2, 138 haplotypes were observed in 147 pedigrees, yielding a haplotype diversity value of 0.9983. The values for the 10-plex and Y-PLEX6 were 0.9912 and 0.9898, respectively.

From the 161 meioses in the present 147 pedigrees, 4 mutation events consisting of 4 extra nucleotides were identified each at DYS389I in the haplotype 103 pedigree, DYS439 in the haplotype 68 pedigree, Y-GATA-H4 in 1 of the 5 haplotype 98 pedigrees and DYS389II in the haplotype 101 pedigree, and 1 mutation event consisting of 4 reduced nucleotides at DYS391 in the haplotype 33 pedigree. All the fathers in which mutations were observed were less than 50 years old. Sequence analysis revealed that all of the mutations were single-repeat gain/loss events that occurred in uninterrupted arrays of ≥11 homogeneous tetranucleotide repeats as shown in Table 1. The maximum-likelihood estimate of the mutation rate across the 14 Y-STRs in the Japanese population was 0.22%/locus/meiosis (95% C.I. 0.09–0.51%). Fisher's exact test showed that this es-

Table 2 The 138 haplotypes at 14 Y-STR loci detected in 147 Japanese father-son pedigrees

Haplo-type	Speci-men	n	DYS 389I	DYS 439	DYS 435	DYS 19	DYS 460	GATA-H4	DYS 391	DYS 392	DYS 438	DYS 437	DYS 393	DYS 389II	DYS 390	DYS 385
1	B	1	10	13	11	15	11	11	10	11	10	14	13	30	25	15-17
2	M	1	11	11	11	14	9	10	10	14	11	15	12	27	23	14-19
3	B	1	11	11	11	15	11	11	10	13	13	14	13	31	22	10-20
4	B	1	11	11	12	17	10	11	10	13	10	14	12	27	26	14-18
5	B	1	11	12	11	15	11	11	10	13	13	14	13	30	22	10-20
6	M	1	11	12	11	16	11	10	11	12	10	15	12	27	23	13-16
7	B	1	11	12	11	16	11	10	11	13	13	14	13	30	23	10-19
8	M	1	12	10	12	16	11	12	10	13	10	15	12	30	24	13-18
9	B	1	12	11	11	15	9	12	10	12	10	14	12	27	23	12-16
10	B	1	12	11	11	16	9	11	9	11	10	14	13	28	25	10-12
11	B	1	12	11	11	17	10	12	10	11	10	13	12	28	24	12-19
12	M	1	12	11	12	15	10	11	10	13	14	14	12	31	24	14-20
13	B	1	12	11	12	15	11	11	10	13	10	16	12	29	24	15-21
14	B	1	12	11	12	16	11	11	10	13	10	14	12	28	25	18-19
15	B	1	12	11	12	17	10	11	10	13	10	14	12	28	24	13-17
16	B	1	12	11	13	15	10	10	10	13	10	15	12	27	24	12-16
17	B	1	12	12	11	13	12	10	10	11	10	14	13	29	23	12-16
18	B	1	12	12	11	14	10	10	10	14	11	14	12	28	24	13-20
19	B	1	12	12	11	15	10	11	11	12	10	15	12	29	23	13-19
20	M	1	12	12	11	15	11	11	10	12	10	15	13	29	23	13-18
21	M	1	12	12	11	15	11	11	10	13	10	15	12	28	24	14-18
22	B	1	12	12	11	15	11	11	10	14	10	14	13	28	23	13-13
23	B	1	12	12	11	17	11	10	11	11	10	14	13	29	25	13-18
24	B	1	12	12	12	15	10	10	10	13	10	14	12	28	24	12-20
25	B	1	12	12	12	15	10	11	10	13	10	14	12	29	25	10-15
26	M	1	12	12	12	16	11	11	10	13	10	15	12	30	25	15-21
27	M	1	12	12	12	16	12	10	10	13	10	14	12	29	24	10-20
28	M	1	12	12	12	17	10	11	10	13	10	14	12	27	25	14-19
29	B	1	12	12	12	17	10	12	11	13	10	14	12	27	25	14-18
30	B	1	12	12	12	17	12	11	10	13	10	15	12	30	24	16-21
31	B	1	12	13	11	14	9	11	10	14	11	15	12	27	24	13-18
32	B	1	12	13	11	14	10	11	10	14	11	15	12	27	24	13-18
33	B	1	12	13	11	16	9	11	11 (10)[a]	12	10	14	12	30	23	11-16
34	B	1	12	13	12	16	11	11	10	13	10	14	12	28	24	12-20
35	B	1	13	10	11	15	11	10	10	14	10	14	13	29	23	11-12
36	M	1	13	10	11	16	10	11	10	11	10	14	13	30	26	13-18
37	B	1	13	10	11	16	10	11	10	11	10	14	14	29	25	11-19
38	B	1	13	11	10	17	11	10	10	11	10	14	13	30	25	14-17
39	M	1	13	11	11	14	9	11	10	14	11	15	12	29	24	13-18
40	B	1	13	11	11	15	11	10	11	11	10	14	13	30	25	13-16
41	M	1	13	11	11	17	11	10	10	11	10	14	13	30	25	13-17
42	B	1	13	12	11	13	11	9	10	11	10	14	14	30	24	13-14
43	M	1	13	12	11	14	11	11	10	15	10	14	13	30	23	12-13
44	B	1	13	12	11	15	10	10	10	11	11	14	11	29	24	12-17
45	M	1	13	12	11	15	10	11	10	11	11	14	13	31	25	12-14
46	B	1	13	12	11	15	11	10	10	11	10	14	13	30	24	15-17
47	M	1	13	12	11	15	11	10	10	11	10	14	14	30	23	12-18
48	M	1	13	12	11	15	11	11	10	11	10	14	13	30	25	14-17
49	B	1	13	12	11	15	11	11	10	11	10	14	13	30	26	14-14
50	M	1	13	12	11	15	11	11	10	13	13	14	13	28	24	10-21
51	M	1	13	12	11	15	11	11	10	13	13	14	13	29	22	10-20
52	M	1	13	12	11	15	12	10	10	11	10	14	15	28	24	10-20
53	M	1	13	12	11	16	10	10	10	11	9	14	14	29	23	14-18
54	B	1	13	12	11	16	11	10	10	11	10	14	15	29	24	10-21
55	M	1	13	12	11	16	11	11	10	13	13	14	13	29	22	10-19
56	B	1	13	12	11	17	9	11	10	13	10	15	12	30	24	11-21

Table 2 (continued)

Haplo-type	Speci-men	n	DYS 389I	DYS 439	DYS 435	DYS 19	DYS 460	GATA-H4	DYS 391	DYS 392	DYS 438	DYS 437	DYS 393	DYS 389II	DYS 390	DYS 385
57	B	1	13	12	11	17	10	11	10	11	10	14	13	31	26	14-18
58	B	1	13	12	11	17	10	11	11	11	10	14	14	29	23	14-18
59	B	1	13	12	11	17	11	10	10	11	10	14	13	31	25	13-17
60	M	1	13	12	11	17	11	10	11	11	13	14	13	30	25	13-17
61	M	1	13	12	11	17	11	11	11	11	10	14	13	30	25	13-18
62	B	1	13	12	11	17	12	10	10	11	10	14	13	30	25	13-17
63	B	1	13	13	11	13	10	11	10	11	10	14	14	29	24	13-14
64	M	1	13	13	11	15	11	11	10	13	14	14	14	29	22	10-19
65	M	1	13	13	11	15	11	11	10	14	10	14	14	28	24	12-14
66	B	1	13	13	11	16	10	9	10	13	10	14	14	30	25	14-17
67	B	1	13	13	11	16	10	10	10	11	9	14	14	29	23	15-18
68	B	1	13	13 (14)[a]	11	16	11	11	10	12	11	14	13	31	27	12-16
69	B	1	14	11	11	13	10	11	10	15	10	14	13	29	23	12-13
70	M	1	14	11	11	14	10	10	10	14	10	14	13	31	24	11-12
71	B	1	14	11	11	14	11	11	10	13	10	14	13	31	23	11-12-13[c]
72	B	1	14	11	11	15	10	10	10	12	11	14	12	32	23	13-17
73	M	1	14	11	11	15	11	11	10	13	14	14	13	30	22	10-20
74	B	1	14	11	11	15	12	11	10	13	13	14	13	29	22	11-19
75	B	1	14	11	11	16	11	9	10	11	10	14	15	30	22	11-17
76	B	1	14	11	11	16	11	9	10	11	10	14	15	30	22	11-19
77	B	1	14	11	11	16	11	10	10	13	13	14	13	29	23	10-19
78	M	1	14	11	11	17	10	11	10	11	9	14	14	30	23	14-18
79	B	1	14	11	11	17	11	10	10	11	10	14	13	31	25	13-16
80	M	1	14	11	11	17	11	10	10	11	10	14	13	31	25	13-17
81	M	1	14	11	11	17	11	10	11	11	10	14	13	31	25	13-17
82	B	1	14	11	12	15	10	11	10	13	10	14	13	31	24	12-17
83	M	1	14	12	11	13	10	9	11	15	11	15	13	30	25	15-22
84	B	1	14	12	11	15	10	10	10	12	10	14	13	31	26	14-20
85	M	1	14	12	11	15	10	11	10	12	10	15	13	31	24	12-16
86	B	1	14	12	11	15	10	11	10	13	13	14	13	30	22	10-18
87	M	1	14	12	11	15	10	11	10	13	13	14	13	30	22	10-20
88	M	1	14	12	11	15	10	11	10	13	13	14	13	30	22	10-21
89	B	1	14	12	11	15	11	10	10	11	10	14	15	29	24	10-20
90	B	1	14	12	11	15	11	10	10	11	10	14	15	30	24	12-19
91	B	1	14	12	11	15	11	10	10	12	11	14	12	31	23	13-17
92	B	1	14	12	11	15	11	10	10	12	13	14	13	29	22	10-19
93	B	1	14	12	11	15	11	10	10	13	13	14	13	30	22	10-20
94	B	1	14	12	11	15	11	11	10	11	10	14	14	29	23	11-16
95	B	1	14	12	11	15	11	11	10	11	13	14	13	29	22	10-20
96	M	1	14	12	11	15	11	11	10	13	13	14	13	29	22	10-20
97	BB	2	14	12	11	15	11	11	10	13	13	14	13	30	22	10-19
98	BBB[d] MM	5	14	12	11	15	11	11 (12)[ab]	10	13	13	14	13	30	22	10-20
99	B	1	14	12	11	15	11	11	10	13	13	14	13	30	22	10-21
100	B	1	14	12	11	15	11	11	10	13	13	14	13	30	22	10-22
101	M	1	14	12	11	15	11	11	10	13	13	14	13	30	22	11-21
102	M	1	14	12	11	15	11	11	10	13	13	14	14	29 (30)[a]	22	10-21
103	B	1	14	12	11	15	11	11	10	14	13	14	13	29	23	10-18
104	B	1	14 (15)[a]	12	11	15	11	11	10	14	13	14	13	30 (31)[a]	22	10-18
105	B	1	14	12	11	15	11	11	11	13	13	14	13	29	21	10-19
106	M	1	14	12	11	15	12	11	10	13	12	14	13	29	22	10-19
107	B	1	14	12	11	16	11	10	10	11	10	14	13	31	24	13-17
108	B	1	14	12	11	16	11	10	10	11	10	14	13	31	26	13-17
109	B	1	14	12	11	16	11	10	10	11	10	14	13	32	26	13-17
110	B	1	14	12	11	16	11	10	11	13	13	14	12	29	23	10-18
111	B	1	14	12	11	16	11	11	10	11	10	14	13	31	25	13-14
112	B	1	14	12	11	16	12	10	10	10	13	14	13	30	23	10-17

Table 2 (continued)

Haplo-type	Speci-men	n	DYS 389I	DYS 439	DYS 435	DYS 19	DYS 460	GATA-H4	DYS 391	DYS 392	DYS 438	DYS 437	DYS 393	DYS 389II	DYS 390	DYS 385
113	M	1	14	12	11	16	13	10	10	11	10	14	13	31	25	13-16
114	B	1	14	12	11	17	11	10	10	10	10	14	11	31	25	13-17
115	B	1	14	12	11	17	11	10	10	10	10	14	13	31	25	14-17
116	B	1	14	12	11	17	11	10	10	11	10	14	13	31	23	13-17
117	B	1	14	12	11	17	11	10	10	11	10	14	13	31	24	14-17
118	BMM	3	14	12	11	17	11	10	10	11	10	14	13	31	25	13-17
119	B	1	14	12	11	17	11	10	10	11	10	14	13	31	25	13-18
120	B	1	14	12	11	17	11	10	10	11	10	14	13	31	25	14-18
121	B	1	14	12	11	17	11	10	10	11	11	14	13	32	25	13-17
122	B	1	14	12	11	17	11	10	10	12	10	14	13	31	24	13-17
123	M	1	14	12	11	17	11	10	10	12	10	14	13	31	25	13-17
124	B	1	14	12	12	16	10	9	10	13	10	15	12	30	25	12-19
125	B	1	14	13	11	15	10	10	10	11	10	14	14	29	23	13-15
126	M	1	14	13	11	15	11	9	10	13	9	14	13	31	25	13-18
127	M	1	14	13	11	15	11	10	10	11	10	14	14	31	25	13-17
128	M	1	14	13	11	15	11	11	10	13	13	14	13	30	22	10-18
129	BBM	3	14	13	11	15	11	11	10	13	13	14	13	30	22	10-20
130	M	1	14	13	11	16	10	10	10	11	10	14	14	30	23	9-17
131	B	1	14	13	11	16	10	10	11	11	11	14	13	31	25	13-17
132	B	1	14	13	11	16	11	10	10	11	10	14	14	30	23	10-20
133	B	1	14	13	11	16	11	10	11	13	13	14	14	30	23	10-18
134	M	1	14	13	11	16	11	11	10	14	13	14	13	29	23	10-18
135	M	1	14	13	11	17	11	10	11	11	10	14	13	31	24	12-17
136	M	1	14	13	12	16	9	10	10	12	10	15	11	32	25	15-20
137	B	1	14	14	11	15	11	11	10	13	13	14	13	29	22	10-21
138	B	1	15	12	11	15	11	11	10	13	13	14	13	30	22	10-21

[a]Numbers in parentheses indicate the mutated allele in the son.
[b]The mutation was observed in 1 of the 5 pedigrees.
[c]Triplicate alleles.
[d]In one of the 3 blood specimens, the mutation was observed.
B Blood sample.
M Buccal mucosa sample.

timate did not differ significantly (p>0.05) from those reported by Brinkmann et al. [9], Kayser et al. [10] and Dupuy et al. [11]. The test did not reveal a significant bias toward gain versus loss-repeat mutations (p>0.05). With the exception of DYS389II, 4 of the 5 mutated alleles were either as long as or longer than the most frequent allele. In addition, all the loci with a mutation event followed a unimodal allele frequency distribution, and 4 out of the 5 loci were “compound” STRs as shown in Table 1.

Discussion

In the present study, 138 haplotypes at 14 Y-STR loci were identified in 147 pedigrees. The allele frequency distribution at each locus and the diversities were not significantly different from those of the Honshu-Japanese in our previous study [6].

With regard to mutation studies on autosomal STRs, various strategies have been adopted such as computer simulations [12, 13, 14], analysis of non-human species [15, 16, 17], and genotype comparisons of family members using blood [9, 13] or cell lines [18, 19]. Such studies revealed the following: there is a positive correlation between the mutation rate and the number of homogeneous repeats [9, 16, 17]; that interspersed irregular repeats have an inhibitory effect on mutation [9, 15]; that the average mutation rate is approximately 0.2%/locus/meiosis [9, 18, 19]; that the majority of mutations comprise one-repeat gain/loss [9, 13, 18, 19]; and one major mechanism for creating a new allele is slipped-strand mispairing [9, 12, 20, 21]. Brinkmann et al. [9] analyzed 23 cases of mutation among 10,844 parent/child allelic transfers for 9 autosomal STRs and reported that no mutations were observed in loci where the allele length versus frequency distribution was not unimodal.

Alternatively, a few studies on Y-STR mutations have been reported previously. Heyer et al. [22] estimated an average mutation rate of 0.21%/locus/meiosis (95% CI, 0.06–0.49%) among 9 Y-STRs in multigenerational pedigrees. Bianchi et al. [23] observed 2 mutations within 1,743 meioses in cell lines but attributed these to somatic, not germline, mutation events. Kayser et al. [10] identified 14 mutations among 4,999 allelic transfers of 15 Y-STRs in German and Polish father/son pairs; the estimated overall mutation rate was 0.28%/locus/meiosis (95% C.I. 0.17–0.43%). Dupuy et al. [11] also identified 5 mutations among 1,200 allelic transmissions of 8 Y-STRs in Norwegian father/son pairs; the estimated overall mutation rate was 0.42%/locus/meiosis (95% C.I. 0.14–0.97%). Al-

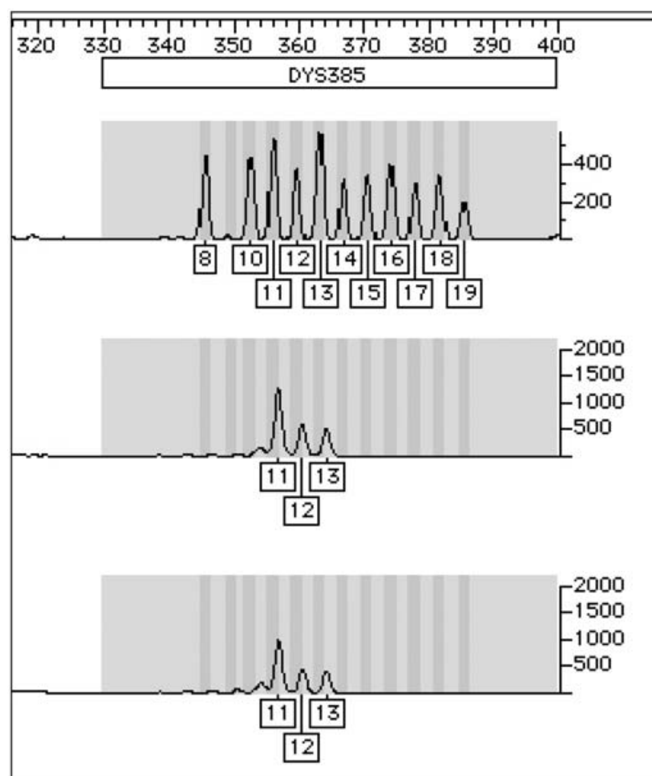

Fig. 1 Genotyper plot of a triplicate allele at DYS385 locus in No. 71 haplotype shown in Table 1. *Upper column* allelic ladder marker, *middle column* father, *lower column* son

though accumulation of data is still not enough, the rate estimates and characteristics of Y-STR mutations reported so far were similar to those of autosomal STRs, which suggests that the general mechanism for both autosomal and Y-STR mutations is slipped-strand mispairing, and is independent of recombination [10]. Kayser et al. [10] also noted that mutations always occurred in either the most frequent alleles or in alleles longer than the most frequent ones, and that the majority of mutations (13 out of 14) were observed at "compound" microsatellites.

The best way to obtain an accurate understanding of mutations at STRs is to investigate allelic transfers within families with biological parental relationships confirmed using other DNA markers [10]. We followed this approach to study Y-STR mutations in the Japanese population, and compared our results to the studies using the same approach [9, 10, 11]. There were no statistically significant differences between our mutation rate (0.22%) and those of other authors, and the characteristics of the mutation events observed in the present study were similar.

With regard to the direction (gain/loss) of Y-STR mutations, there appears to be a bias toward allele expansion mutations. The ratio of increased repeats to decreased repeats was 4:1; that of Kayser et al. [10] was 10:4, but neither of the differences was statistically significant. However, it was impossible to draw any conclusions because the sample sizes and the number of loci analyzed were extremely small compared to the study by Xu et al. [24], who examined 236 mutations in 122 autosomal STR loci and found neither bias despite the fact that the rate of contraction mutations increased exponentially with allele size.

It is generally assumed that different loci have different mutation rates and that different alleles of the same locus have different mutation rates. Because allele frequencies differ among populations, allele-specific or locus-specific mutation rates can vary among them. Recently, Rolf et al. [25] advocated a method for calculating the probability of paternity using the overall mutation rate of each locus, however, we feel it is best to use population, locus, and allele-specific Y-STR mutation rate estimates for accuracy in forensic analyses and population studies. Furthermore, it is noticeable that some Y-STRs are slowly mutating and that such loci are useful for phylogenic studies on humans [26]. The accumulation of data will make it possible to construct alternative combinations of Y-STRs for different purposes.

The allele triplication at DYS385 was observed in one father/son pair in the present study. Since the same event was also reported by Kayser et al. [10], such an event may not be rare in this extremely polymorphic locus. The allele duplications at DYS19, DYS390, and DYS391 have also been reported previously [27, 28, 29]. Consequently, such additional alleles should been taken into consideration in forensic practice because they can be wrongly interpreted as a mixed profile.

For forensic purposes, further studies on Y-STRs, including the construction of a worldwide database with more loci and mutation events at each locus are necessary.

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References

1. Roewer L, Kayser M, Dieltjes P, Nagy M, Bakker E, Krawczak M, Knijff P de (1996) Analysis of molecular variance (AMOVA) of Y-chromosome-specific microsatellites in two closely related human populations. Hum Mol Genet 5:1029–1033
2. Prinz M, Boll K, Baum H, Shaler B (1997) Multiplexing of Y chromosome specific STRs and performance for mixed samples. Forensic Sci Int 85:209–218
3. Redd AJ, Clifford SL, Stoneking M (1997) Multiplex DNA typing of short-tandem-repeat loci on the Y chromosome. Biol Chem 378:923–927
4. Gusmão L, González-Neira A, Pestoni C, Brión M, Lareu MV, Carracedo A (1999) Robustness of the Y STRs DYS19, DYS389I and II, DYS390 and DYS393: optimization of a PCR pentaplex. Forensic Sci Int 106:163–172
5. Butler JM, Schoske R, Vallone PM, Kline MC, Redd AJ, Hammer MF (2002) A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. Forensic Sci Int 129: 10–24
6. Uchihi R, Yamamoto T, Usuda K et al. (2003) Haplotype analysis with 14 Y-STR loci using 2 multiplex amplification and typing systems in 2 regional populations in Japan. Int J Legal Med 117:34–38
7. 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

8. Casella G (1987) Refining binomial confidence intervals. Can J Stat 14:113–129
9. Brinkmann B, Klintschar M, Neuhuber F, Hühne J, Rolf B (1998) Mutation rate in human micro-satellites: influence of the structure and length of the tandem repeat. Am J Hum Genet 62:1408–1415
10. Kayser M, Roewer L, Hedman M et al. (2000) Characteristics and frequency of germline mutations at microsatellite loci from the human Y chromosome, as revealed by direct observation in father/son pairs. Am J Hum Genet 66:1580–1588
11. Dupuy BM, Andreassen R, Flønes AG et al. (2001) Y chromosome variation in a Norwegian population sample. Forensic Sci Int 117:163–173
12. Valdes AM, Slatkin M, Freimer NB (1993) Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics 133:737–749
13. Di Rienzo A, Petersion AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. Proc Natl Acad Sci U S A 91:3166–3170
14. Chakraborty R, Kimmel M, Stivers DN, Davison LJ, Deka R (1997) Relative mutation rates at di-, tri-, and tetranucleotide microsatellite loci. Proc Natl Acad Sci U S A 94:1041–1046
15. Petes TD, Greenwell PW, Dominska M (1997) Stabilization of microsatellite sequences by variant repeats in the yeast *Saccharomyces cerevisiae*. Genetics 146:491–498
16. Wierdl M, Dominska M, Petes TD (1997) Microsatellite instability in yeast: dependence on the length of the microsatellite. Genetics 146:769–779
17. Schlötterer C, Ritter R, Harr B, Brem G (1998) High mutation rate of a long microsatellite allele in *Drosophila melanogaster* provides evidence for allele-specific mutation rates. Mol Biol Evol 15:1269–1274
18. Sajantila A, Lukka M, Syvänen AC (1999) Experimentally observed germline mutation at human micro- and minisatellite loci. Eur J Hum Genet 7:263–266
19. Weber JL, Wong C (1993) Mutation of human short tandem repeats. Hum Mol Genet 2:1123–1128
20. Ohta T, Kimura M (1973) The model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a genetic population. Genet Res 22:201–204
21. Levinson G, Gutman GA (1987) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol 4:203–221
22. Heyer E, Puymirat J, Dieltjes P, Bakker E, Knijff P de (1997) Estimating Y chromosome specific microsatellite mutation frequencies using deep rooting pedigrees. Hum Mol Genet 6: 799–803
23. Bianchi NO, Catanesi CI, Bailliet G, Martinez-Margnac VL, Bravi CM, Vidal-Rioja LB, Herrera RJ (1998) Characterisation of ancestral and derived Y-chromosome haplotypes of new world native populations. Am J Hum Genet 63:1862–1871
24. Xu X, Peng M, Fang Z, Xu X (2000) The direction of microsatellite mutations is dependent upon allele length. Nat Genet 24:396–399
25. Rolf B, Keil W, Brinkmann B, Roewer L, Fimmers R (2001) Paternity testing using Y-STR haplotypes: assigning a probability for paternity in cases of mutations. Int J Legal Med 115: 12–15
26. Forster P, Röhl A, Lünnemann P, Brinkmann C, Zerjal T, Tyler-Smith C, Brinkmann B (2000) A short tandem repeat-based phylogeny for the human Y chromosome. Am Hum Genet 67: 182–196
27. Santos FR, Gerelsaikhan T, Munkhtuja B, Oyunsuren T, Epplen JT, Pena SDJ (1996) Geographic differences in the allelic frequencies of the human Y-linked tetranucleotide polymorphism DYS19. Hum Genet 97:39–313
28. Kayser M, Caglià A, Corach D et al. (1997) Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 110:125–133
29. Redd AJ, Clifford SL, Stoneking M (1997) Multiplex DNA typing of short-tandem-repeat loci on the Y chromosome. Biol Chem 378:923–927