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SHORT COMMUNICATION

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Characterization and haplotype analysis of the polymorphic Y-STRs DYS443, DYS444 and DYS445 in a Japanese population

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Abstract From sequence database information we have newly identified three male-specific and polymorphic tetranucleotide STRs, DYS443 (GDB: 10807127), DYS444 (GDB: 10807128) and DYS445 (GDB: 10807129) on the Y chromosome. Analysis of 190 Japanese males revealed 6, 5 and 4 alleles in the DYS443, DYS444 and DYS445 systems, with calculated STR diversities of 0.68, 0.57 and 0.53, respectively. The cumulative haplotype diversity of the five Y-STRs DYS441, DYS442, DYS443, DYS444 and DYS445 was calculated to be 0.95 and therefore application of these STRs may yield very useful information for forensic individualization.

Keywords Y chromosome · Short tandem repeat (STR) · Y haplotype · Population studies · Japanese

Introduction

Y-chromosomal STRs are a powerful tool in forensic examination, paternity testing and evolutionary studies [1, 2, 3, 4, 5, 6]. Recently, we identified two novel Y-STRs, DYS441 and DYS442, through a search of sequence database information [7]. The aim of the present study was to identify three additional novel Y-STRs, DYS443, DYS444 and DYS445 with sufficient discrimination powers and to evaluate the forensic utility of Y-haplotypes consisting of these five new markers.

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Materials and methods

Samples

Blood samples were collected from healthy unrelated Japanese individuals, including 190 males, 3 females and 13 father/son pairs after obtaining written informed consent from each participant. DNA was extracted using a QIAamp DNA blood kit (Qiagen, Chatsworth, Calif.).

PCR primers

A search for tetranucleotide tandem repeats from male-specific regions on the human Y chromosome from the sequence database was conducted according to our previous report [7] and three loci containing stretches of 13 TTCC (designated as DYS443), 14 TAGA (DYS444) and 12 TTTA (DYS445) repeat units were selected from GenBank data AC007274, AC007043 and AC009233, respectively. For PCR, we used the following primers for analysis of DYS443, DYS444 and DYS445, respectively:

- YRE43S 5′-CGCTTCCATTTACACTTCCTGTG-3′ (nt 46453–46475 of GenBank AC007274)
- YRE43A 5′-TTTCATTGGCCACCTGACATTAC-3′ (nt 46748–46726 of GenBank AC007274)
- YRE44S 5′-GTGTGAACCATTTGGCATGTTTA-3′ (nt 75785–75807 of GenBank AC007043)
- YRE44A 5′-TCTAAGGGATCCAAAGGCAGAA-3′ (nt 76092–76071 of GenBank AC007043)
- YRE45S 5′-AGTTAAGAGCCCCACCTTCCTG-3′ (nt 79252–79273 of Genbank AC009233)
- YRE45A 5′-GAGCTGAGATTATGCCACCAAAA-3′ (nt 79514–79492 of Genbank AC009233).

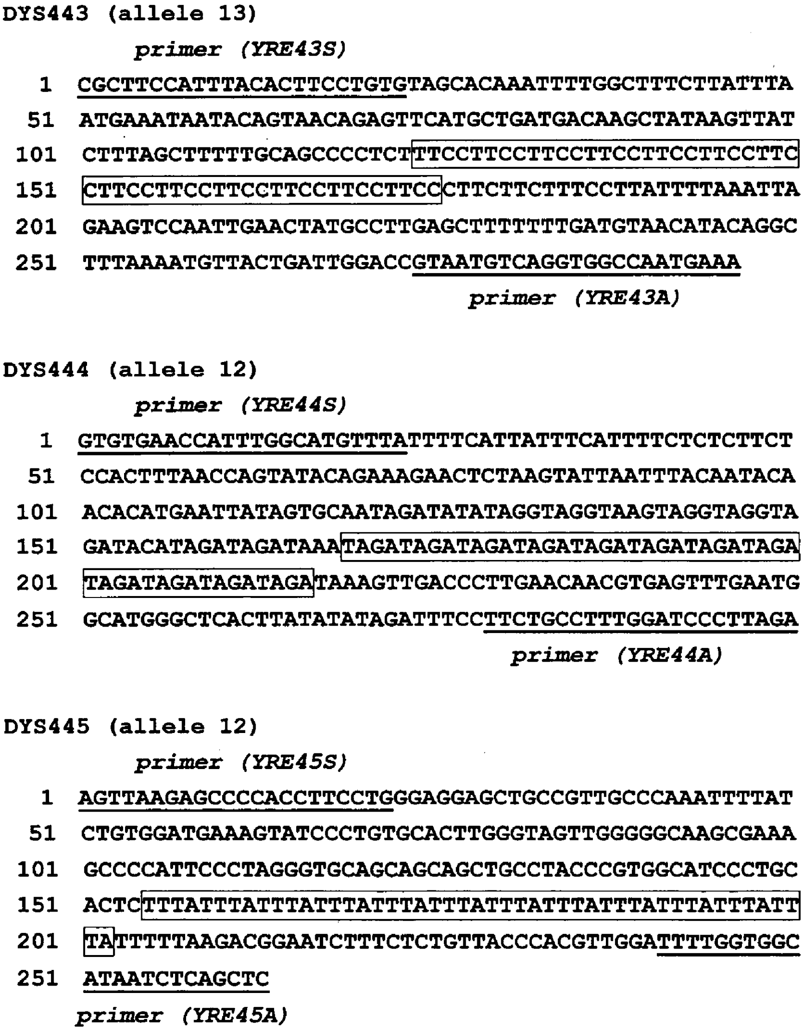
PCR conditions and analysis of PCR products

PCR was performed according to the method described in our previous paper [7] except that the annealing temperatures were 67°C for DYS443, 65°C for DYS444 and 68°C for DYS445, respectively. Electrophoresis and direct DNA sequencing were carried out as described previously [7].

Results and discussion

The DYS443, DYS444 and DYS445 systems were confirmed to be male-specific STRs on the Y chromosome

Fig. 1 Sequences of the DYS443, DYS444 and DYS445 loci. The locations of each tandem repeat (DYS443, TTCC; DYS444, TAGA; DYS445, TTTA) are boxed



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DYS443 (allele 13)
         primer (YRE43S)
  1  CGCTTCCATTTACACTTCCTGTGTAGCACAAATTTTGGCTTTCTTATTTA
 51  ATGAAATAATACAGTAACAGAGTTCATGCTGATGACAAGCTATAAGTTAT
101  CTTTAGCTTTTTGCAGCCCCTCTTTCCTTCCTTCCTTCCTTCCTTCCTTC
151  CTTCCTTCCTTCCTTCCTTCCTTCCCTTCTTCTTTCCTTATTTTAAATTA
201  GAAGTCCAATTGAACTATGCCTTGAGCTTTTTTTGATGTAACATACAGGC
251  TTTAAAATGTTACTGATTGGACCGTAATGTCAGGTGGCCAATGAAA
                                  primer (YRE43A)

DYS444 (allele 12)
         primer (YRE44S)
  1  GTGTGAACCATTTGGCATGTTTATTTTCATTATTTCATTTTCTCTCTTCT
 51  CCACTTTAACCAGTATACAGAAAGAACTCTAAGTATTAATTTACAATACA
101  ACACATGAATTATAGTGCAATAGATATATAGGTAGGTAAGTAGGTAGGTA
151  GATACATAGATAGATAAATAGATAGATAGATAGATAGATAGATAGATAGA
201  TAGATAGATAGATAGATAAAGTTGACCCTTGAACAACGTGAGTTTGAATG
251  GCATGGGCTCACTTATATATAGATTTCCTTCTGCCTTTGGATCCCTTAGA
                                     primer (YRE44A)

DYS445 (allele 12)
         primer (YRE45S)
  1  AGTTAAGAGCCCCACCTTCCTGGGAGGAGCTGCCGTTGCCCAAATTTTAT
 51  CTGTGGATGAAAGTATCCCTGTGCACTTGGGTAGTTGGGGGCAAGCGAAA
101  GCCCCATTCCCTAGGGTGCAGCAGCAGCTGCCTACCCGTGGCATCCCTGC
151  ACTCTTTATTTATTTATTTATTTATTTATTTATTTATTTATTTATTTATT
201  TATTTTTAAGACGGAATCTTTCTCTGTTACCCACGTTGGATTTTGGTGGC
251  ATAATCTCAGCTC
    primer (YRE45A)
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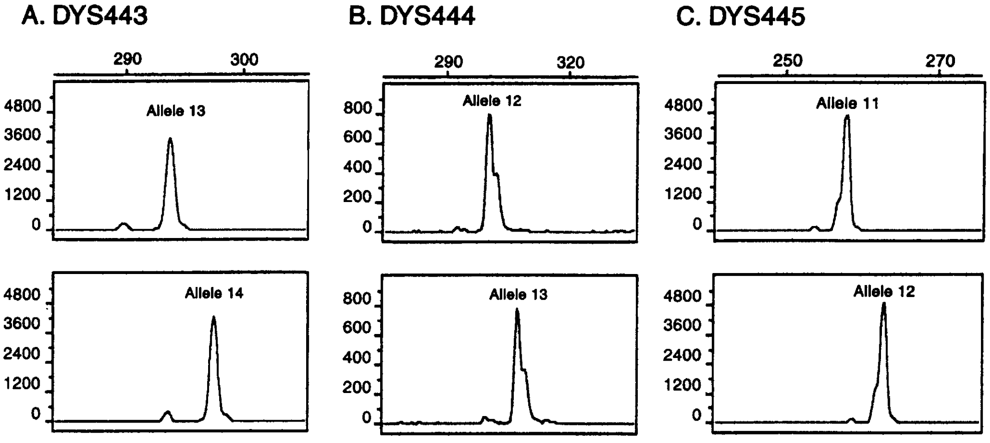
Fig. 2 Fluorescent DNA typing analysis of **A** DYS443, **B** DYS444 and **C** DYS445 loci. Two examples of the most common alleles are shown respectively. The PCR products were run on an ABI PRISM 310 genetic analyzer. The results were analysed using GeneScan analysis software



based on the electrophoretic detection of single PCR product bands of the expected sizes from all of the male DNA samples, whereas no bands were detected in the female samples and the fact that the DNA sequences of the PCR products from several males were completely identical to those from the GenBank database, except for the numbers of nucleotide repeats. From routine paternity cases 13 father/son combinations were analysed and all the alleles were found to be inherited in a regular fashion. The full sequences of the DYS443, DYS444 and DYS 445 loci are shown in Fig. 1.

Table 1 DYS443, DYS444 and DYS445 allele frequencies in a Japanese population

Locus	Allele (bp)	Frequency	STR diversity
DYS443 (*n*=190)	12 (292)	0.032	0.68
	13 (296)	0.437	
	14 (300)	0.247	
	15 (304)	0.258	
	16 (308)	0.021	
	17 (312)	0.005	
DYS444 (*n*=190)	11 (296)	0.042	0.57
	12 (300)	0.579	
	13 (304)	0.300	
	14 (308)	0.074	
	15 (312)	0.005	
DYS445 (*n*=190)	10 (255)	0.005	0.53
	11 (259)	0.421	
	12 (263)	0.537	
	13 (267)	0.037	

STR diversity was calculated as $1\text{-}\Sigma pi^2$ (pi = allele frequency)

Table 2 The 85 haplotypes detected in 190 unrelated Japanese males

Haplotype	DYS441	DYS442	DYS443	DYS444	DYS445	Frequency
1	12	11	14	13	10	0.005
2	12	11	15	12	12	0.005
3	12	12	13	12	11	0.005
4	12	12	14	13	12	0.005
5	13	10	13	13	12	0.005
6	13	11	13	11	11	0.016
7	13	11	13	12	11	0.011
8	13	11	13	13	11	0.021
9	13	12	14	13	12	0.011
10	13	12	14	13	13	0.005
11	13	12	15	14	12	0.005
12	14	11	12	12	11	0.005
13	14	11	13	11	12	0.005
14	14	11	13	13	11	0.005
15	14	11	15	12	12	0.095
16	14	11	15	13	12	0.016
17	14	11	16	12	12	0.005
18	14	12	13	12	12	0.005
19	14	12	14	12	12	0.016
20	14	12	14	13	12	0.016
21	14	12	14	14	12	0.005
22	14	12	15	12	11	0.005
23	14	12	15	12	12	0.016
24	14	12	15	13	12	0.011
25	14	12	15	14	12	0.005
26	14	12	16	12	12	0.005
27	14	13	13	13	12	0.005
28	14	13	14	13	12	0.005
29	14	13	14	14	12	0.005
30	14	13	15	12	12	0.005
31	15	10	15	13	13	0.005
32	15	11	13	11	12	0.005
33	15	11	13	12	11	0.021
34	15	11	13	12	12	0.005
35	15	11	13	14	11	0.005
36	15	11	13	14	12	0.005
37	15	11	13	15	11	0.005
38	15	11	14	12	11	0.005
39	15	11	14	12	12	0.011
40	15	11	14	13	11	0.005
41	15	11	14	13	12	0.005
42	15	11	14	14	11	0.005
43	15	11	15	12	12	0.011
44	15	11	15	13	12	0.021
45	15	11	15	14	12	0.005
46	15	11	16	12	12	0.005
47	15	11	16	13	12	0.005
48	15	11	17	12	12	0.005
49	15	12	13	13	11	0.005
50	15	12	13	13	12	0.005
51	15	12	14	12	12	0.016
52	15	12	14	13	12	0.021
53	15	12	14	13	13	0.005
54	15	12	15	12	12	0.011
55	15	13	13	12	12	0.005
56	15	13	14	13	12	0.005
57	15	13	14	13	13	0.016
58	16	10	12	12	11	0.005
59	16	10	12	14	11	0.005
60	16	11	12	12	11	0.011
61	16	11	12	13	11	0.005
62	16	11	13	12	11	0.174
63	16	11	13	12	12	0.011
64	16	11	13	13	11	0.042
65	16	11	13	14	11	0.016
66	16	11	14	11	12	0.005
67	16	11	14	12	12	0.042
68	16	11	14	13	12	0.005
69	16	11	15	12	12	0.005
70	16	12	13	11	12	0.011
71	16	12	13	12	11	0.016
72	16	12	13	14	11	0.005
73	16	12	14	13	12	0.011
74	16	12	15	12	12	0.011
75	16	12	15	13	12	0.005
76	16	13	15	12	12	0.005
77	16	13	15	13	12	0.005
78	16	14	14	14	12	0.005
79	16	14	15	13	12	0.005
80	17	11	13	12	11	0.011
81	17	11	13	13	11	0.005
82	17	11	14	12	12	0.005
83	17	12	15	12	13	0.005
84	17	13	14	12	12	0.005
85	18	11	13	13	11	0.005

Haplotype diversity ($1\text{-}\Sigma qi^2$ where qi = haplotype frequency) was 0.95

The population study performed using capillary electrophoresis detected six alleles in the DYS443 system (Fig. 2A), five in the DYS444 system (Fig. 2B) and four in the DYS445 system (Fig. 2C) in 190 unrelated males (Table 1), with STR diversities of 0.68, 0.57 and 0.53, respectively. Combining the present data with those of our previous study on DYS441 and DYS442 [7] allowed the distinction of 85 haplotypes (Table 2) with a haplotype diversity of 0.95. Thus the five new Y-STRs described in this study will provide much additional information for forensic and anthropological analyses.

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