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SHORT COMMUNICATION

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Identification and characterization of two novel human polymorphic STRs on the Y chromosome

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Abstract From sequence database information, we have identified two male-specific and polymorphic tetranucleotide STRs, DYS 441 (GDB:10013873) and DYS 442 (GDB: 10030304), on the Y chromosome. Analysis of 184 males allowed 7 and 5 alleles to be distinguished in the DYS 441 and DYS 442 systems, respectively, yielding 21 haplotypes. The gene diversities were 0.72 and 0.51, respectively and the haplotype diversity was 0.85.

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

Introduction

Y-chromosomal microsatellites are very useful for forensic examination and paternity testing, as well as for evolutionary studies. Over the past few years, a series of highly polymorphic Y-specific STRs have been developed [1, 2, 3] and population genetic data including haplotype databases have been accumulated [4, 5, 6, 7, 8, 9, 10]. Here we report two additional novel Y-STRs as useful markers which have a sufficient discrimination power for forensic application.

Materials and methods

Samples

Blood samples were obtained from healthy unrelated Japanese individuals, including 184 males, 3 females and 13 father/son pairs. DNA was extracted using the QIAamp DNA blood kit (Qiagen).

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PCR primers

Using the sequence database (EMBL, http://www.ebi.ac.uk/~sterk/genome-MOT/), a search was made for tetranucleotide tandem repeats from male-specific regions on the long arm of the human Y chromosome and 2 loci containing stretches of 13 CCTT (DYS 441) and 12 TATC (DYS 442) repeat units, respectively, were selected. The PCR primers were designed using OLIGO primer analysis software (National Sciences, Plymouth, Minn.). The primer sequences for DYS 441 were:

– YRE1S: 5′-AAGTTGCAGTGAGCGAAGATTG-3′ (nt 20023–20044 of GenBank AC004474)
– YRE1A: 5′-ATGTACCTGTAGCCCCAGTGAAC-3′ (nt 20406–20384 of GenBank AC004474)

Those for DYS 442 were:

– YRE2S: 5′-CCCCAAGTCCCCAAAGTGTGT-3′ (nt 48629–48649 of GenBank AC004810)
– YRE2A: 5′-AAACGCCCATCAATCAATGAGTG-3′ (nt 48933–48911 of GenBank AC004810)

The sense primers labelled with 6-Fam were used for the analyses using a capillary electrophoretic apparatus.

PCR conditions

PCR was performed in a volume of 10 µl in 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 800 µM dNTPs, 0.15 U of AmpliTaq Gold (Perkin-Elmer), 0.25 µM each primer and 10–50 ng of genomic DNA, using a GeneAmp PCR System 9700 (Perkin-Elmer). Cycling conditions for DYS 441 were: 94 °C for 15 min, 30 cycles at 94 °C for 30 s, 62 °C for 30 s, 72 °C for 20 s and 72 °C for 5 min. Those for DYS 442 were: 94 °C for 15 min, 30 cycles at 94 °C for 30 s, 68 °C for 30 s, 72 °C for 20 s and 72 °C for 5 min.

Analysis of PCR products

Aliquots of 0.4 µl of the PCR products were separated on 6% native polyacrylamide gels (200 × 300 × 0.35 mm) in TBE buffer at 1000 V for 1 h at 30 °C. The gels were stained with SYBR Green I (Molecular Probes) and the band pattern was observed using a fluorescence image analyser (FluorImager SI, Molecular Dynamics).

Aliquots of 1–3 µl of the PCR products mixed with 12 µl of deionised formamide and 0.5 µl of GeneScan size standard (GENESCAN-500 ROX) were analysed with a capillary electrophoresis apparatus (ABI PRISM 310 Genetic Analyzer, Perkin-Elmer) with a capillary (470 × 0.05 mm) and performance optimized polymer 6 (POP-6).

DNA sequence analysis

The PCR-amplified products were purified using a QIA quick PCR purification kit (Qiagen). Sequencing reactions were carried out with a BigDye terminator Cycle Sequencing FS Ready Reaction kit (Perkin-Elmer) using the PCR primers as the sequencing primers and the products were analysed using an ABI PRISM 310 Genetic Analyzer.

Results and discussion

In both the DYS 441 and DYS 442 systems, single PCR products of the expected sizes were detected in all of the samples from males, whereas no bands were detected in any of the samples from females. From routine paternity cases, 13 father/son combinations were analysed and all the alleles were found to be inherited in a regular fashion. The DNA sequences of the PCR products from several males were identical to those from the database, except for the numbers of the nucleotide repeats. The full sequences of the DYS 441 and DYS 442 loci are shown in Fig. 1. It was therefore confirmed that the DYS 441 and DYS 442 systems were male-specific STRs on the Y chromosome.

The population study performed using the capillary electrophoresis apparatus detected 7 alleles in the DYS 441 system (Fig. 2A) and 5 in the DYS 442 system (Fig. 2 B) in 184 unrelated males (Table 1), yielding 21 DYS 441/DYS 442 haplotypes (Table 2). The gene diversities were 0.72 and 0.51, respectively and the haplotype diversity was 0.85.

The present results indicate that these two novel STR systems are highly informative and suitable for forensic investigations.

DYS441 (allele 12)

primer (YRE1S)

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  1 AAGTTGCAGTGAGCGAAGATTGCACCACTGTACTCTAGCCTGGGCAACAG
 51 TGAGACTTCTCTCAAAAAAAAAAAAAAGATAACAATTCAAATTCTCAGGC
101 ATTGCAGTATTTATTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCC
151 TTCCTTCCTTCCTTCCTCCTCCTTCTCTCCCTCCTGCCTCCCTCTCTCCCTCC
201 CTCTCTCTCTTTTTCTCTCTCTTTCTCTCTTTATCTCTTTCTTTCTTTTG
251 AGACAGCTTCTTGCTCTGTCACCCAGGCTGGAGTACAGTGACACAATTAT
301 GGCTCAATGCAGCTTCTACCTCCTGGACTCAAGCAGTCCTCTCACTTCAG
351 CCTCCCAGTTCACTGGGGCTACAGGTACAT
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primer (YRE1A)

DYS442 (allele 10)

primer (YRE2S)

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  1 CCCCAAGTCCCCAAAGTGTGTTGCATCATTCTTATGCCTCTGCATCCTCA
 51 TAGCTTAGCTCCCACATATCAGTGAGAACGTATGATGTTTGGTTTTCCAT
101 TCCTGAGTTACTTCATTTAGAATAATAGCCTCCAATCTCATCCAAGCCAC
151 TGCAAATGTCATTAGCTCATTCTTTTTTGTGGCTGAGTAGTATTCCATTG
201 TATCTATCTGTCTGTCTGTCTATCTATCTATCTATCTATCTATCTATCTA
251 TCTATCTATCACAGTTTCTTTATCCACTCATTGATTGATGGGCGTTT
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primer (YRE2A)

Fig. 1 Sequences of the DYS441 and DYS442 loci. The locations of each tandem repeat (DYS441, CCTT; DYA442, TATC) are boxed

Table 1 DYS441 and DYS442 allele frequencies

Locus	Allele (bp)	Frequency	Gene diversity
DYS441 (n = 184)			
	12 (380)	0.022	0.72
	13 (384)	0.071	
	14 (388)	0.228	
	15 (392)	0.239	
	16 (396)	0.402	
	17 (400)	0.033	
	18 (404)	0.005	
DYS442 (n = 184)			
	10 (297)	0.022	0.51
	11 (301)	0.652	
	12 (305)	0.245	
	13 (309)	0.071	
	14 (313)	0.011	

Gene diversity was calculated as $1-\Sigma pi^2$ (pi = allele frequency)

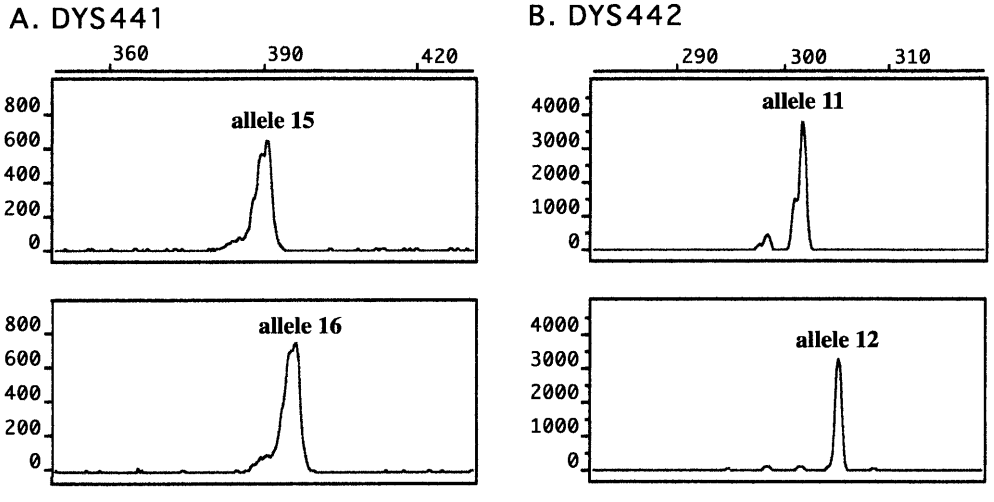

Fig. 2 Fluorescent DNA typing analysis of the **A** DYS441 and **B** DYS442 loci and two examples of the most common alleles are shown (PCR products were run on an ABI PRISM 310 Genetic Analyzer and the results were analysed using GeneScan analysis software)

Table 2 DYS441/DYS442 haplotype frequency

Haplotype	DYS441/ DYS442	Frequency	Haplotype diversity
1	12/11	0.011	0.85
2	12/12	0.011	
3	13/10	0.005	
4	13/11	0.049	
5	13/12	0.016	
6	14/11	0.125	
7	14/12	0.082	
8	14/13	0.022	
9	15/10	0.005	
10	15/11	0.136	
11	15/12	0.065	
12	15/13	0.033	
13	16/10	0.011	
14	16/11	0.304	
15	16/12	0.065	
16	16/13	0.011	
17	16/14	0.011	
18	17/11	0.022	
19	17/12	0.005	
20	17/13	0.005	
21	18/11	0.005	

Haplotype diversity was calculated as $1-\Sigma qi^2$ (qi = haplotype frequency)

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References

1. Kayser M, Caglia A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, Knijff P de, Roewer L (1997) Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 110:125–133
2. White PS, Tatum OL, Deaven LL, Longmire JL (1999) New, male-specific microsatellite markers from the human Y chromosome. Genomics 57:433–437
3. Ayub Q, Mohyuddin A, Qamar R, Mazhar K, Zerjal T, Mehdi SQ, Tyler-Smith C (2000) Identification and characterization of novel human Y-chromosomal microsatellites from sequence database information. Nucleic Acids Res 28:e8
4. Knijff P de, Kayser M, Caglia A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, Roewer L (1997) Chromosome Y microsatellites: population genetic and evolutionary aspects. Int J Legal Med 110:134–140
5. Aoyama M, Matsuki T, Kishi K, Iida R, Tsubota E (1997) DYS19 marker in Japanese populations. Int J Legal Med 110:112–113
6. Rossi E, Rolf B, Schürenkamp M, Brinkmann B (1998) Y-chromosome STR haplotypes in an Italian population sample. Int J Legal Med 112:78–81
7. Pestoni C, Cal ML, Lareu MV, Rodriguez-Calvo MS, Carracedo A (1998) Y chromosome STR haplotypes: genetic and sequencing data of the Galician population (NW Spain). Int J Legal Med 112:15–21
8. Sasaki M, Shiono H (1999) The polymorphisms of DYS388 and DYS392 on the Y chromosome in Japanese and German populations. Int J Legal Med 112:132–133
9. Csete K, Szabo A, Varga T (1999) The Y-STRs DYS19 and DYS390 in a south-east Hungarian (Szeged area) population. Int J Legal Med 112:207–208
10. Anslinger K, Keil W, Weichhold, Eisenmenger W (2000) Y-chromosomal STR haplotypes in a population sample from Bavaria. Int J Legal Med 113:189–192