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DXS10011: a hypervariable TTTC/GAAA repeat marker on human chromosome Xq27-q28

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Abstract

We mapped human STS UT413 on human chromosome Xq27-q28, renamed it DXS10011 and developed an easy method of analysis using capillary electrophoresis. The probability of discrimination was 0.954 from 1198 chromosomes in the Japanese population. DXS10011 is a hypervariable and stable marker on the human X chromosome.

Keywords: DXS10011; X chromosome; STR; Capillary electrophoresis; Japanese population data

1. Introduction

DXS10011 was first reported as human STS UT413 (HUMUT413) [1] and formerly registered as DYS384 in the Genome Database (GDB). We analyzed this locus extensively, confirmed it to be a hypervariable polymorphic marker localized not on the human Y chromosome but the X chromosome, and renamed it DXS10011 [2]. Here, we describe its typing by capillary electrophoresis, assignment of the chromosomal localization, the exact sequence of two types of DXS10011 [3] and Japanese population genetic data.

2. Materials and methods

2.1. Sample collection and DNA purification

EDTA blood samples were collected from 550 unrelated Japanese (358 male and 192 female) for a population genetics study. DNA was extracted by the Chelex-100 method [4] or QIAamp blood kit (QIAGEN, Hilden, Germany) [5].

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2.2. PCR primers

Primer 1 (GAAA strand): 5′-6Fam-CCAGCCAGGGCAACAAGAGTGAA-3′
Primer 2 (TTTC strand): 5′-GCGCGATGGGAGAACGTTTGAAG-3′

2.3. PCR conditions

PCR was performed in a total reaction volume of 5 μl containing 1–20 ng genomic DNA, 0.05 mM each dNTP, 0.25 μM each primer, 1.5 mM $MgCl_2$, 0.25 U AmpliTaq Gold (AB: Applied Biosystems Japan, Tokyo). Amplification was carried out using a GeneAmp PCR System 9700 (AB), preheating for 16 min at 95 °C, for 30–40 cycles with denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, extension for 30 s at 72 °C and extra-extension for 10 min at 72 °C.

2.4. Analysis of PCR products

Capillary electrophoresis was carried out on denatured polymer POP6 in a 47-cm long capillary with GS-500 ROX as a size standard by ABI PRISM 310 Genetic Analyzer and GeneScan software (AB). Some PCR products from male samples were directly sequenced with a BigDye Terminator Cycle Sequencing FS kit (AB) [5].

2.5. Determination of chromosomal localization

PCR analysis of DNA from human–mouse or human–chinese hamster hybrid panel cells was performed using Somatic Cell Hybrid Mapping Panel #2 (version 3), regional mapping panel for chromosome #X (Coriell Cell Depositories, Camden, NJ) and human monochromosomal hybrid cell DNA (The Japanese Collection of Research Bioresources, Tokyo).

3. Results and discussion

Chromosomal localization was analyzed by PCR amplification of the target sequence in the panel cell DNAs. The PCR product of the target sequence was detected when sample numbers NA06318C (human X chromosome DNA) and NA1MR91 (parent human male cell DNA) from Somatic Cell Hybrid Mapping Panel #2 (version 3), and JCRB2223 (chromosome #X DNA of a human monochromosomal hybrid cell) from the Japanese Collection of Research Bioresources were amplified. No PCR product was detected when 25 other samples including NA06317 (chromosome #Y) and other parent cell DNAs were analyzed. These results indicated that the DXS10011 marker is located on the human X chromosome.

In the experiment, to determine the fine localization of DXS10011 on chromosome X, the PCR product of the target sequence was detected when sample numbers NA12510, NA11172, NA13492, NA07298A, NA10501, NA11100, NA13339 and NA10063 from the Regional Mapping Panel for Chromosome #X were amplified, and no PCR product

was detected when NA10663, NA11099 and NA09142 were amplified. These results indicated that DXS10011 was localized specifically to Xq27-q28.

Table 1 shows the expected and observed sizes of the DXS10011 alleles and allele frequencies from 1198 Japanese chromosomes including 742 chromosomes analyzed in Fukui (present study, column *N*) and 456 in Yamagata (cited from Ref. [6]).

Table 1
Expected size and observed size of DXS10011 alleles and allele frequencies in the Japanese population

Allele	(An/Bn)	*N*	Total *	Frequency	Fragment size (bp)	
					Expected	Observed
16	A16	1	1	0.0008	201	197.1
17	A17	3	6	0.0050	205	200.9
18	A18	8	10	0.0084	209	204.9
19	A19	16	27	0.0225	213	208.7
20	A20	22	46	0.0384	217	212.6
20.2	B12	3	5	0.0042	219	214.8
21	A21	25	45	0.0375	221	216.5
21.2	B13	19	28	0.0234	223	218.7
22	A22	37	63	0.0526	225	220.4
22.2	B14	20	29	0.0242	227	222.6
23	A23	31	60	0.0501	229	224.3
23.2	B15	49	64	0.0534	231	226.5
24	A24	30	52	0.0434	233	228.2
24.2	B16	66	106	0.0885	235	230.4
25	A25	38	53	0.0442	237	232.1
25.2	B17	27	39	0.0326	239	234.3
26	A26	37	66	0.0551	241	235.9
26.2	B18	7	11	0.0092	243	237.5
27	A27	33	57	0.0476	245	239.9
27.2	B19	2	2	0.0017	247	241.9
28	A28	37	66	0.0551	249	243.8
29	A29	51	84	0.0701	253	247.7
30	A30	34	62	0.0518	257	251.6
30.2	B22	0	1	0.0008	259	
31	A31	39	59	0.0493	261	255.6
32	A32	31	42	0.0351	265	259.6
33	A33	23	28	0.0234	269	263.4
34	A34	13	17	0.0142	273	267.3
35	A35	11	18	0.0150	277	271.2
36	A36	4	8	0.0067	281	274.9
37	A37	6	10	0.0084	285	278.9
38	A38	3	7	0.0058	289	282.8
39	A39	5	7	0.0058	293	286.7
40	A40	6	11	0.0092	297	290.9
41	A41	1	2	0.0017	301	294.5
43	A43	4	5	0.0042	309	302.4
45	A45	0	1	0.0008	317	
	Total	742	1198	1.0002		

Column "Total * " means combined number of data in Fukui (present study, column *N*) and Yamagata [6].

Type An: -TCCTTC- (TTTC)$_n$-TTCTTT-

Type Bn: -TCCTTC- (TTTC)$_n$-*TCTC- (TTTC)$_6$-TCTTTC* -TTCTGT-

Fig. 1. Sequences of allele type A and type B of DXS10011. The sequences underlined correspond to the polymorphic tetranucleotide repeat region and those in italic type to an additional 34-bp sequence of type B.

In this study, 37 alleles including nine inter-alleles with different sequences (Fig. 1) [3] were detected. The probability of discrimination was 0.954 in the 1198 Japanese chromosomes. The DXS10011 system is a supervariable and stable marker on the human X chromosome and will be valuable for forensic and human genetic analyses [7].

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