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Journal	Bulletin of Tokyo Dental College, 40(1): 21-26
URL	http://hdl.handle.net/10130/1014
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Original Article

THE DYS19 SYSTEM IN THE JAPANESE POPULATION
AND ITS DETECTION USING TEETH
AS A SOURCE OF DNA

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Received 6 November, 1998/Accepted for Publication 21 December, 1998

Abstract

The Y-specific short tandem repeat (STR) polymorphism of the locus DYS19 was amplified by PCR and analyzed by denaturing polyacrylamide gel electrophoresis followed by silver staining. Among 119 DNA samples from Japanese males, five alleles were observed. When sequences of the products were compared, each allelic segment contained 13 to 17 GATA tetranucleotide repeats, and revealed no differences from the known allele (GenBank X77751) other than the number of tetranucleotide repeats. The most common allele in the Japanese population was allele 15, and the distribution of the alleles did not differ from the data from other regions in Japan but did differ from those of Caucasians. Amplification of the locus using 12 tooth samples as a source of DNA matched the patterns obtained from blood samples.

Key words: Short tandem repeat (STR)—DYS19—Allele distribution—PCR—Amplification from teeth

INTRODUCTION

The Y chromosome has long been regarded as mostly devoid of polymorphic content^{10,13,23}. However this view is changing as new Y chromosome-specific markers are reported and more human populations are investigated. Y-linked loci are haploid and paternally inherited and, with the exception of genes in the pseudoautosomal region, there is no recombination²⁵. Thus Y-linked polymorphisms can be expanded to a much more discriminative Y haplotype analysis^{3,9,11,12,17,20}. In addition, the male specificity

makes it useful for forensic studies, because the majority of crimes are committed by males.

DYS19 was reported by Roewer *et al.*¹⁸) as a first Y-linked polymorphic microsatellite, and it has been investigated in many human populations¹¹. The number of autosomes in any population is twice as many as the number of individuals, however the number of Y chromosomes is the same as the number of male individuals. The smaller population size of Y chromosomes compared to autosomes renders the former polymorphisms more prone to genetic drift. Therefore, in order to

calculate allele frequencies of Y-linked polymorphisms, extended regional population data should be used. More population data is necessary to examine the differences among populations and to establish the practical frequencies for individual populations in forensic casework.

In this paper, allele frequencies of DYS19 locus in the Japanese population were examined, the nucleotide sequences of the allelic products were compared, and the detection of the DYS19 locus from tooth samples was investigated.

MATERIALS AND METHODS

1. Samples

Genomic DNA was extracted from blood samples of 119 healthy unrelated Japanese males. Informed consent was given from the blood and tooth donors. Leukocyte preparations from blood were suspended in saline-EDTA (75 mM NaCl, 24 mM EDTA) containing 1% sodium dodecyl sulfate (SDS) and digested with 0.1 mg/ml of proteinase K at 55°C overnight, followed by treatment with RNase at 55°C for 2 hrs. DNA was extracted with phenol/chloroform, precipitated with ethanol, and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA at pH 7.6).

Samples of teeth and cotton used for hemostasis after extraction of teeth were obtained from 12 male patients in a dental clinic. The enamel layer of the tooth was scraped with a dental instrument and the remaining materials were crushed into powder using a "diamond mortar", a special device to crush hard tissues, followed by decalcification in 3 ml of 0.5 M EDTA for 7 days. Although not all of the tooth powder dissolved in the EDTA, it was centrifuged, washed in TE twice, and re-suspended in TE, followed by proteinase K digestion and DNA extraction as described above. Samples of cotton were washed in sterile physiological saline twice, centrifuged, suspended in TE containing 1% sodium dodecyl sulfate (SDS), and digested with 0.1 mg/ml of proteinase K at 55°C

overnight. The solution was transferred to another tube, and DNA was extracted as described above.

2. Detection of DYS19 locus

Segments of DYS19 were enzymatically amplified from genomic DNA using the following oligonucleotide as primers: primer 1: 5'-CTACTGGTTTCTGTTAGT-3', primer 2: 5'-ATGGCATGTAGTGAGGACA¹⁷⁾. Each amplification of DYS19 was performed in a 40 μ l mixture containing 10 ng of genomic DNA, 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 0.02% gelatin, 200 μ M of each dNTP, 500 nM of each primer and 2 units of AmpliTaq DNA Polymerase (Perkin-Elmer Cetus). Another amplification was performed in a similar mixture containing 2.0 mM MgCl₂ and 1.5 units of AmpliTaq Gold (Perkin-Elmer Cetus). The following PCR conditions were used for the first mixture: 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 51°C for 30 sec and extension at 72°C for 90 sec. After the 30th cycle, an extra step was performed at 72°C for 10 min to extend the template completely. The PCR conditions for the second mixture were 95°C for 9 min, followed by 30 cycles of denaturation at 95°C for 30 sec and annealing and extension at 54°C for 60 sec. After the 33rd cycle, an extra step was performed at 54°C for 10 min to extend the template completely.

The above-mentioned reaction mixtures were overlaid with one drop of mineral oil (SIGMA), and amplification was performed in a Perkin-Elmer Cetus DNA Thermal Cycler apparatus PJ 2000. The PCR products were electrophoresed in 6% denaturing polyacrylamide gels, followed by silver staining²⁾.

3. Sequencing analysis

Each amplified DNA fragment was eluted from silver stained gels using the "crush and soak" method¹⁹⁾, re-amplified by PCR, and directly sequenced using Dye Terminator Cycle Sequencing Kit (Perkin-Elmer Cetus); sequence analysis was obtained on an ABI 373A DNA Sequencer.

RESULTS

1. DYS19 typing and sequencing

Five kinds of different allelic products with different sizes were identified in the locus DYS19 among 119 Japanese males (Fig. 1). PCR products regarded as having the same sizes were loaded side by side on a denaturing gel and were compared for typing. All of the same allelic products showed the same migration distance, suggesting that each allele represented a 4bp difference. Five different kinds of allelic products were sequenced from both strands. Each allelic segment contained 13 to 17 GATA tetranucleotide repeats in the sequences of the 186bp, 190bp, 194bp, 198bp and 202bp fragments, respectively. These sequences were identical to the known alleles for the DYS19 locus (GenBank accession number X77751) except for the number of GATA repeats.

The allelic distribution of DYS19 locus is shown in Table 1. The predominant allele in this study was allele 15, as already observed in

other Japanese populations; allele 14 predominated in Caucasians. When allelic frequencies of the present study were compared with those from other Japanese populations (Japan-2 to Japan-9 and Japanese-9 in Table 1 and Fig. 2) with sample sizes of more than 50,

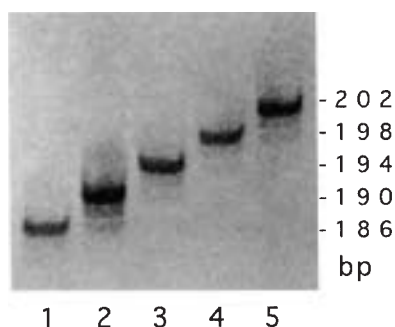


Fig. 1 Electrophoresis of DYS19 in 6% denaturing gel followed by silver staining. Only five representative allelic segments are shown. PCR amplification was conducted by using AmpliTaq Gold. The sizes of the fragments are indicated on the right. Lane 1: allele 13, lane 2: allele 14, lane 3: allele 15, lane 4: allele 16, lane 5: allele 17.

Table 1 Allele distribution of DYS19 system in various populations. The allele numbers correspond to the number of repeat units present in the amplified fragments deduced from its size.

	Total No.	Alleles					
		13 (186bp)	14 (190bp)	15 (194bp)	16 (198bp)	17 (202bp)	Others
Japan-1 (Present study)	119	3 (2.5)	12 (10.1)	57 (47.9)	25 (21.0)	22 (18.5)	0
Japan-2 (Honshuu) ⁸⁾	88	7 (8.0)	3 (3.4)	48 (54.5)	18 (20.5)	12 (13.6)	0
Japan-3 (Gunma) ¹⁾	120	4 (3.3)	9 (7.5)	65 (54.2)	30 (25.0)	12 (10.0)	0
Japan-4 (Shizuoka) ⁸⁾	61	5 (8.2)	1 (1.6)	35 (57.4)	13 (21.3)	7 (11.5)	0
Japan-5 (Osaka) ¹¹⁾	150	20 (13.0)	7 (5.0)	74 (49.0)	33 (22.0)	16 (11.0)	0
Japan-6 (Fukui) ¹⁾	131	7 (5.3)	10 (7.6)	53 (40.5)	30 (22.9)	31 (23.7)	0
Japan-7 ²⁶⁾	258	17 (6.6)	17 (6.6)	109 (42.2)	59 (22.9)	55 (21.3)	1 (0.4)
Japan-8 ²⁴⁾	200	10 (5.0)	14 (7.0)	92 (46.0)	38 (19.0)	44 (22.0)	2 (1.0)
Japanese-9 (in Brazil) ²¹⁾	136	10 (7.4)	4 (2.9)	66 (48.5)	33 (24.3)	22 (16.2)	1 (0.7)
Slovakia ¹⁴⁾	81	4 (4.9)	17 (21.0)	16 (19.8)	27 (33.3)	17 (21.0)	0
Germany-1 (West Saxony) ²¹⁾	251	14 (5.6)	115 (45.8)	56 (22.3)	43 (17.1)	23 (9.2)	0
Germany-2 ^{6,14)}	306	21 (6.9)	136 (44.4)	74 (24.2)	59 (19.3)	16 (5.2)	0
Spain ¹⁵⁾	77	5 (6.5)	61 (79.2)	9 (11.7)	2 (2.6)	0	0
Greece ⁵⁾	84	18 (21.4)	28 (33.4)	21 (25.0)	16 (19.0)	1 (1.2)	0
Italy (Southern) ⁵⁾	101	12 (11.9)	54 (53.5)	29 (28.7)	6 (5.9)	0	0
Brazil-1 ²²⁾	100	19 (19.0)	49 (49.0)	24 (24.0)	7 (7.0)	1 (1.0)	0
Brazil-2 ²¹⁾	252	41 (16.3)	135 (53.6)	59 (23.4)	14 (5.5)	2 (0.8)	1 (0.4)

Note: Numbers in parentheses indicate the allelic frequency in each population.

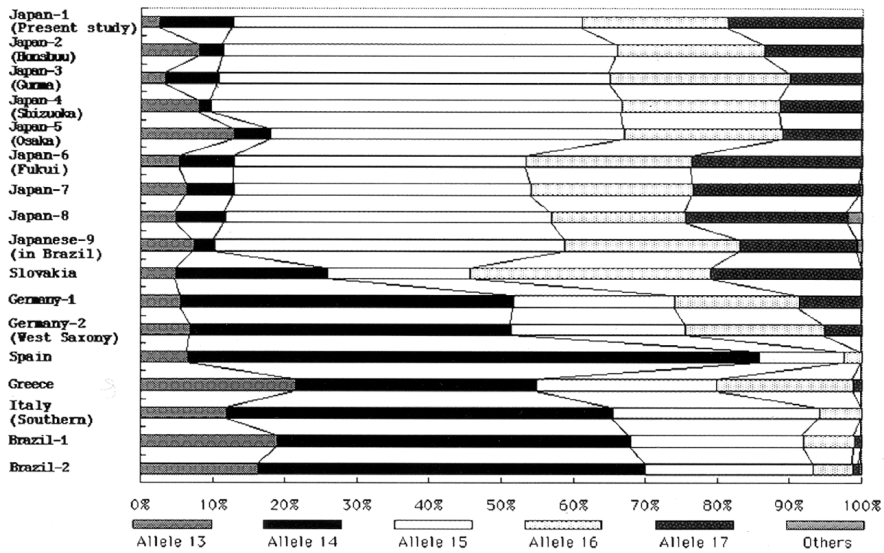


Fig. 2 Comparison of the DYS19 allele distribution from various populations. The numbers attached to the country names are the same as those in Table 1.

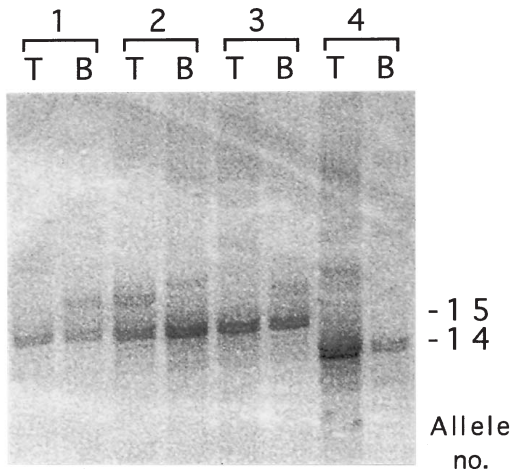


Fig. 3 Electrophoresis of the DYS19 amplified from tooth samples (T) and blood samples (B) obtained from four individuals. Amplification was conducted by using usual AmpliTaq DNA polymerase and not by AmpliTaq Gold. Note the high background compared with the electrophoretogram in Fig. 1.

samples obtained from both tooth and blood samples from 12 donors. PCR amplification was successful in all the samples (Fig. 3). The products amplified using AmpliTaq Gold showed lower backgrounds than those using usual AmpliTaq DNA polymerase under the conditions described in this text. Even after the dual PCR using AmpliTaq Gold and the same primer pair from the samples with inefficient amplification, the background did not increase, and target bands were intensified. The patterns obtained from teeth and blood were identical, which supported the use of teeth as a source of DNA for personal identification.

DISCUSSION

Several reports have examined the frequencies of DYS19 in various populations^{1,5,6,8,11,12,14,15,21,22,24,26}. Distinct differences in allelic distribution have been observed between Japanese and Caucasians, and also within several ethnic groups of Caucasians (Table 1, Fig. 2)¹¹. In addition, overwhelming predominances of one or two alleles have

the present population data exhibited the highest frequency of allele 14 and the lowest frequency of allele 13 (Table 1, Fig. 2).

2. Detection using tooth DNA

The DYS19 locus was amplified from DNA

been found in several populations^{11,21}). Race identification is one of the important problems awaiting solution in forensic science. Race discrimination in Asian populations is especially difficult. The Alu polymorphic (YAP) element is present or absent at a specific site on the Y chromosome. Population studies have shown that the insertion of YAP element occurred only once during human evolution in Africa^{7,23}). The element is present in 42% of Japanese, and absent in Taiwanese, suggesting an irregular distribution of this polymorphism in Asia⁸). Although the differences among Japanese populations were not extreme in the DYS19 system, recent haplotype analysis of the Y chromosome has the possibility of finding more regional differences^{4,8,9,11,17,20}).

PCR product sizes of DYS19 are about 200bp, which is a suitable size for detection using degraded DNA. The DNA samples from teeth used in this study were fairly degraded. In half of the samples, amplification of the VNTR locus D1S80 with product sizes ranging from 353bp to 961bp was unsuccessful or revealed additional artifact products within the range of common allele sizes. In contrast, the expected DYS19 segments were successfully amplified from those samples. Since Y-linked STRs were not amplified from female DNA (In this study DYS19 of 10 samples from females were not amplified), the analysis has advantages for rape and sexual assaults in forensic applications. Unlike autosomes, even high amounts of female DNA do not inhibit the amplification of Y-specific alleles¹⁶). This approach does not require the time-consuming differential lysis step usually used to separate male DNA from female DNA and reduces the risk of contamination. Although Y chromosome markers are very useful for forensic studies, we have to establish a practical database that can be used for the calculation of the probability of identity from the results of Y-STRs analysis in consideration of the characteristics of Y chromosome polymorphisms.

ACKNOWLEDGEMENTS

This study was supported by the Grant-in-Aid for Scientific Research (C) (09672120) from the Ministry of Education, Science, Sports and Culture of Japan.

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