TECHNICAL NOTE

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D4S43 Locus DNA Typing in the Japanese Population and Application to Teeth with Degraded DNA

REFERENCE: Hanaoka Y, Minaguch K. D4S43 locus DNA typing in the Japanese population and application to teeth with degraded DNA. J Forensic Sci 1998;43(2):406–409.

ABSTRACT: VNTR polymorphism at the D4S43 locus was investigated, and allele frequencies in the Japanese population sample were determined by the polymerase chain reaction. Eleven different alleles and 16 genotypes were observed in 131 unrelated Japanese. The most common allele was one repeat unit (60.3%), the heterozygosity index of the present study was 58.7%, and the polymorphic information content was calculated at 0.55. In addition, we found four new size variations in the one-repeat-unit allele, which was the most common allele among the Japanese population. These variations were believed to be different from variations by repeats of the basic 14-bp unit. The one-repeat-unit allele at the D4S43 locus, which comes in four sizes, was detected in all of the samples, including degraded DNA samples obtained from hard tooth tissue. Therefore, this new variation is useful in personal identification by DNA analysis using forensic scientific degraded DNA samples.

KEYWORDS: forensic science, forensic odontology, DNA typing, teeth, Japanese population, D4S43, variable number of tandem repeat, DNA, blood, dentition

Amplification of a highly polymorphic variable number of tandem repeat (VNTR) segments in human genomes by the polymerase chain reaction (PCR) is useful in race identification, and many kinds of polymorphisms at a single loci were reported (1–5). The D4S43 locus, discovered by Gilliam et al. (6), is located very close to the region responsible for Huntington's disease (7,8), and consists of a series of tandemly repeated, 14-base sequences with a number of alleles of various sizes. Horn et al. (9) detected the VNTR-polymorphism at this locus and reported the population study on Caucasian.

Based on the report by Horn et al. (9), in this study we investigated the VNTR-polymorphism at the D4S43 locus among the Japanese population. We found that, in addition to the VNTR-polymorphism of the basic 14-bp unit, there were four new variations in the 184-bp band, which is believed to be one repeat unit. Furthermore, we investigated these new variations using degraded DNA samples obtained from hard tooth tissue as forensic practical samples.

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Received 3 April 1997; and in revised form 24 June, 25 July 1997; accepted 28 July 1997.

Materials and Methods

Sample Preparation

A total of 131 blood samples were collected from healthy, unrelated Japanese individuals, and teeth (n = 20) were obtained at dental clinics with the consent of patients. DNA was isolated from blood cells and hard tooth tissue by the proteinase K digestion and phenol-chloroform extraction methods, which have previously been described as extraction methods for teeth (10,11). Isolated DNA was diluted with trisethylenediaminetetraacetic acid with pH 7.6 (TE) buffer and quantitated spectrophotometrically (1 OD_{260} = 50 μg/mL DNA) prior to PCR amplification. Ten nanograms of DNA were used for the amplification of DNA samples.

PCR Amplification

PCR was performed in a buffered solution as described by Saiki et al. (12), 200 μM each deoxynucleoside triphosphate (dNTP), but 60% of the total d GTP consisted of 7-deaza-dGTP, 2 units (U) of Amplitaq (Perkin-Elmer, New Jersey), and 0.4 μM of each of the following primers: GH437, 5′-GACCACAGAGAGCTTAGTGGAGCTT; and GH436, 5′-GACCACTTCACTGACATCCACATCT (9). The reactions were conducted in a final volume of 20 μL. The thermal cycle profiles using the DNA thermal cycler (Model Pj2000, Perkin-Elmer) were 31 cycles at 94°C for 30 sec, 51°C for 30 sec, and 72°C for 1.5 min, followed by preheating at 94°C for 5 min, then extension of the last cycle to 7 min. During the amplification process, the annealing temperature of some samples was gradually changed from 51°C to 55°C in steps.

Detection and Typing of DNA Bands

The PCR reaction products were analyzed using silver-stained (13) 10% polyacrylamide gel after electrophoresis with Vertical Gel Electrophoresis System (Bethesda Research Lab., Gaithersburg) in 100 mM of Tris-HCl, 100 mM of Boric acid, and 2 mM of EDTA, with a pH 8.3 (TBE). Further analysis of VNTR polymorphism at the D4S43 locus was carried out using silver-stained 50 cm-12% polyacrylamide gel and 6% polyacrylamide sequencing gel (50% Urea, 5.7% acrylamide, 0.3% N,N′-methylene-bisacrylamide) after electrophoresis by means of Sequi-Gen Nucleic Acid Sequencing Cell System (BIO-RAD). Sequencing gel running temperature was maintained at near 50°C. During this procedure, 1 μL of the sample was normally subjected to electrophoresis.

However, this amount was excessive in many cases, so comparisons were made following the appropriate dilution of samples. The allele syzes determined using 123 ladder marker. In addition, samples that were believed to show the same size band were subjected to electrophoresis again side by side to make sure, and the type was finally determined.

Results and Discussion

Compared with the method of Horn et al. (9), less template DNA was used for analysis in the amplification method used in the present study, but the annealing temperature was 4°C lower. Consequently, although amplification was successful in all of the samples tested, many non-specific bands also appeared near the target band, particularly near alleles 5 and 9, and near the origin side of allele 11. As result, the annealing temperature was gradually increased from 51°C to 55°C. As shown in Fig. 1, by elevating the temperature to 54°C, the target band was gradually clarified, and the non-specific bands disappeared. Therefore, during amplification process, samples with non-specific bands that could confuse typing of VNTR polymorphism at the D4S43 locus were annealed at 54°C and then compared and analyzed.

The Japanese Allele Frequencies

VNTR polymorphism at the D4S43 locus was investigated in 131 unrelated Japanese individuals, and 11 alleles and 16 genotypes were found. The product of each allele ranged from approximately 184 bp-369 bp (Fig. 2). These 11 alleles were numbered from 1–11 from the anodic side, and the frequency of each allele was calculated. As shown in Fig. 3, the frequencies of alleles 1, 5, and 9 were over 10%, and the most common allele was number 1 (184 bp). In addition, the distributions of each genotype were

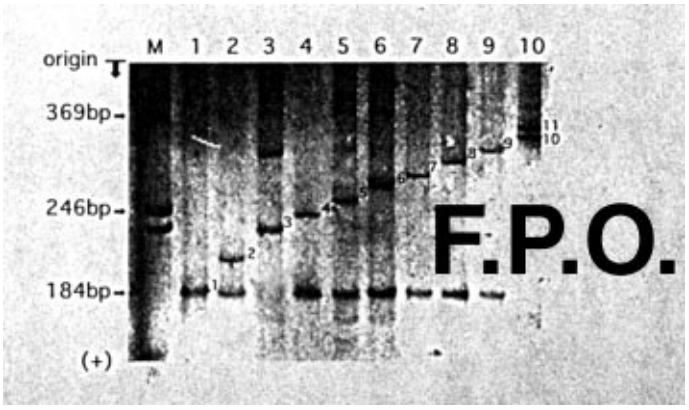
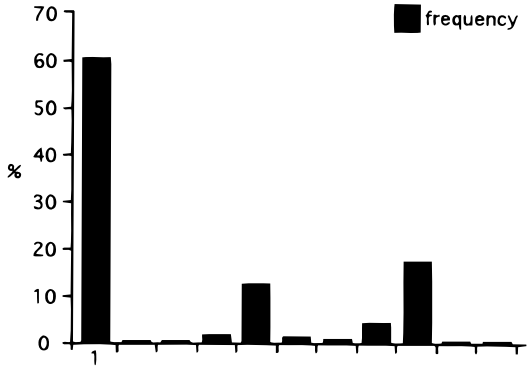


FIG. 2—*Allele products of D4S43 observed by silver-stained 10% polyacrylamide gel electrophoresis. Lane 1-genotype 1-1; Lane 2-1-2; Lane 3-3-9; Lane 4-1-4; Lane 5-1-5; Lane 6-1-6; Lane 7-1-7; Lane 8-1-8; Lane 9-1-9; Lane 10-10-11.*



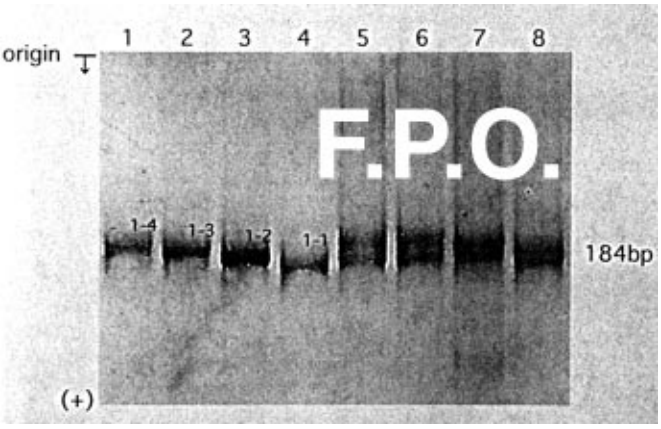


FIG. 4—*50-cm 12% polyacrylamide gel electrophoresis. Size variation observed in allele 1. Lanes 1, 2, 3, and 4-homozygote; Lanes 5, 6, 7, and 8-heterozygote.*

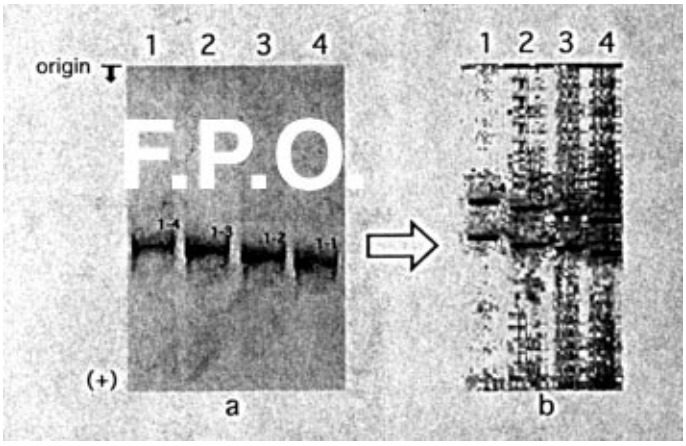


FIG. 5—*The four new variations. (a) 50-cm 12% polyacrylamide gel electrophoresis. (b) 6% polyacrylamide sequencing gel. The four new variations were confirmed by silver-stained 6% polyacrylamide sequencing gel. Lane 1-1-4; Lane 2-1-3; Lane 3-1-2; Lane 4-1-1.*

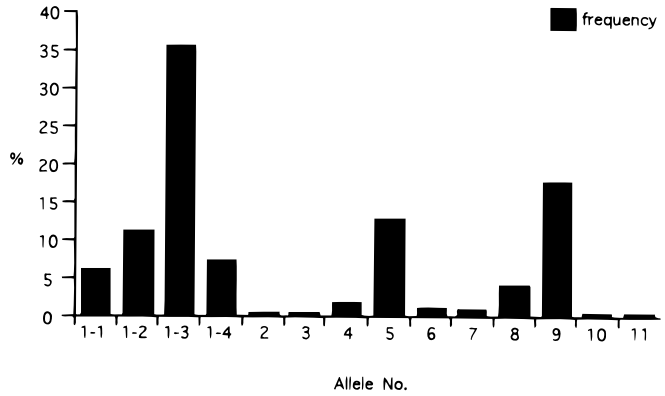


FIG. 6—*D4S43 allele frequencies including the subdivision of allele 1 in the Japanese population. The frequencies of alleles 1-1, 1-2, 1-3, 1-4, 5, and 9 were over 5%.*

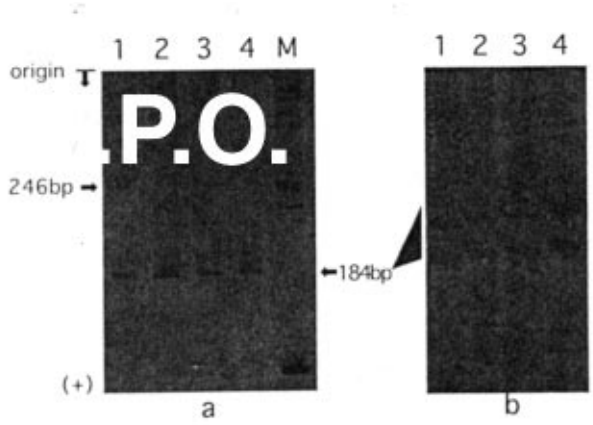


FIG. 7—*VNTR analysis at the D4S43 locus using DNA obtained from hard tooth tissue. (a) 10% polyacrylamide gel electrophoresis. Only a one-repeat-unit allele was efficiently amplified in all the samples; (b) 6% polyacrylamide sequencing gel. Four types of fragments of different sizes were clearly detected.*

total number of alleles to 14. Therefore, the frequency of each allele was calculated again, and Fig. 6 show the results of this calculation. The frequency of 1-3 was the highest at 35.4%, and the frequencies of six alleles (1-1, 1-2, 1-3, 1-4, 5, and 9) were greater than 5%. The heterozygosity increased to 80.7%, and the PIC was calculated at 0.78.

Detection of the Four New Variations Using Degraded DNA Obtained from Hard Tooth Tissue

Degraded DNA obtained from hard tooth tissue has previously been described (10,11). In the VNTR analysis conducted at the D4S43 locus using DNA obtained from hard tooth tissue, no large sized alleles were detected in any of the samples tested. However, only a one-repeat-unit allele was efficiently amplified in all the samples (Fig. 7A). Subsequently, these samples were further analyzed using 6% polyacrylamide sequencing gel. As a result, four fragments of different sizes were clearly detected (Fig. 7B).

Due to the differences in electrophoretic mobility, the natures of these four variations were believed to be different from the variations in the 14-base repeated sequence. Consequently, to investigate the cause of these four variations, based on the nucleotide sequence of Horn et al. (9), homozygous PCR products were digested with Hae III, and electrophoresis was performed. Subsequently, two fragments weighing approximately 110 bp and 70 bp were obtained (not shown). While the different electrophoretic mobilities were shown in the 110-bp fragments, the mobilities of the 70-bp fragments were the same. Therefore, the cause of the four variations was believed to exist near the 5′ terminus of the 110-bp fragments. Although no definite conclusions have been reached concerning differences in the nucleotide sequence, similar variations may exist in other alleles.

These four new variations could be detected from DNA obtained from hard tooth tissue, as shown in present study. Therefore, this size variation is useful for personal identification by DNA analysis using forensic scientific degraded DNA samples.

Conclusions

We investigated the D4S43-locus VNTR-polymorphism among the Japanese population. Eleven different alleles and 16 genotypes were observed. The most common allele was one repeat unit, and a difference in allele distribution from Caucasians was suggested. In addition, we found four new variations in the one-repeat-unit

allele. Because the one-repeat-unit allele was detected in all of the degraded DNA samples tested that were obtained from hard tooth tissue, the four new variations in the one-repeat-unit allele were useful for personal identification by DNA analysis, especially using degraded DNA samples.

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