TECHNICAL NOTE

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Japanese Population Study of a Y-Linked Dinucleotide Repeat DNA Polymorphism

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ABSTRACT: A polymorphic CA repeats (YCA II) was previously reported on the human Y chromosome. We have used a simple technique based on polymerase chain reaction amplification followed by native polyacrylamide gel electrophoresis to study the inheritance, the genetic stability, and the allele frequency distribution of this polymorphism in the Japanese. We found seven haplotypes which were tentatively designated as: A[(CA)19/(CA)21], B[(CA)19/(CA)22], C[(CA)19/(CA)23], D[(CA)19/(CA)19], E[(CA)21/(CA)21], F[(CA)22/(CA)22], and G[(CA)23/(CA)23]. The frequencies of these haplotypes were: A, 0.21; B, 0.29; C, 0.37; D, 0.02; E, 0.02; F, 0.07; G, 0.01. There was complete concordance with each father-son pairs. The results indicate the dinucleotide system YCA II is very useful for investigation of forensic samples, especially mixed stains in sexual offence cases.

KEYWORDS: forensic science, Y chromosome, haplotype, polymorphism, sex typing, paternity testing

The polymerase chain reaction (PCR) method is widely used for personal identification in forensic case work because PCR technology offers fast, reliable, and reproducible DNA profiles for casework evidence. Numerous PCR sex typing systems have been reported (1–3). Previously reported studies regarding Y-chromosome specific repeat sequences have not been informative due to a small number of polymorphic DNA loci on the Y chromosome (4,5).

In 1994 Mathias et al. screened a collection of 72 Y-enriched cosmids (6) with poly d(CA/GT)n and identified three cosmids containing useful Y-specific CA repeats, designated YCA I, YCA II, and YCA III (7). To explore the potential polymorphism of the site they designed flanking primers and studied the region by the polymerase chain reaction (PCR).

In the present paper, we report our results on a genetic and population study of the YCA II polymorphism in 95 Japanese males, 10 father-son pairs and three generations of males, using a simple non-isotopic technique. The objectives of this study were to evaluate the allele distribution in a larger unselected sample, and to study inheritance and genetic stability of this polymorphism.

Materials and Methods

Genomic DNA from 95 unrelated males, 50 unrelated females, 10 father-son pairs and three male generations was prepared from peripheral blood as described elsewhere (8). These blood samples were obtained from Japanese volunteers. In all of father-son pairs studied the paternity had been previously established by DNA fingerprinting with the multi-locus probes MZ1.3 (Bio test AG., Dreieigh).

PCR Conditions

The primers used for PCR were those designed by Mathias et al. (1994). These were YCA II-1: 5′TATATTAAATAGAAGTAGTGA3′ and YCA II-2: 5′TATCGATGTAATGTTATATTA3′. The PCR each reaction samples contained: 100 ng DNA template, 2.5 unit Taq DNA polymerase, 10 mM Tris-HCl pH 8.3, 16.5 mM ammonium sulfate, 10 mg mL-1 BSA, 200 μM dNTPs, 2.0 mM MgCl2, 0.5 μM of each primer. The total reaction volume was 100 μL, and each sample was overlaid with 30 μL mineral oil. The PCR was carried out in PJ 2000 Thermal Cycler (Perkin Elmer, Norwalk CT) for 30 cycles. Each cycle consisted of 1 min at 94°C for denaturation, 1 min at 55°C for primer annealing, and 1 min at 72°C for primer extension.

Electrophoresis

The PCR products were treated with Microcon 100 (TAKARA SHUZO CO., LTD., Seta) and electrophoresis performed using 8% native polyacrylamide gel in TBE buffer for 4 h at 500 V. The fragments were visualized using ethidium bromide staining. Moreover, we used the denaturing gel to confirm the complete accuracy of YCA II typing in the native gel. Microcon 100 was used to remove a rest of primer et al. and concentrate the PCR products. This treatment enabled to be detected clearly with all samples using the ethidium bromide staining.

Allele Designation

The repeat number of an allele was determined by direct DNA sequencing using Dye Terminal Cycle Sequencing FS Ready Re-

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action Kit with a 377 DNA Sequencer (Perkin Elmer, Norwalk CT). The identification of allele repeat size was determined by comparison of the amplified allele to an allelic ladder. The allelic ladder was made by mixing the PCR products of YCA II haplotype D, E, F, and G.

Duplex Amplification

The possibility of a duplication, or deletion, polymorphism was determined by the thermal asymmetric interlaced (TAIL) PCR method (9). The primers used for PCRs were those designed: SP-1: 5′AATAGAAGTAGTGAAAACAGGCA3′ for the first, SP-2: 5′GCTCTTAGAGTAAATCTGTCAG3′ for the second, and the set of random primers. Each PCR was carried out in PJ 2000 Thermal Cycler (Perkin Elmer Norwalk, CT) and the PCR conditions are described in Table 1. Electrophoresis of the PCR products was performed using 2% agarose gel in TBE buffer for 25 min at 100 V. The fragments were visualized using ethidium bromide staining.

Results and Discussion

In 95 males, YCA II was amplified as a single or double band with a variable size around 150 bp. Two Y-derived products were observed in 83 males. The lower amplified allele showed the same migration with a band size of 148 bp. We could observe the upper 3 alleles, 2 bp apart, with sizes varying from 152 to 156 bp (Fig. 1, lanes 1 to 3). A single band was observed in 12 males, and we could resolve 4 alleles with sizes varying from 148, 152 and 154 to 156 bp, respectively (Fig. 1, lanes 4 to 7). The two amplified products synthesized by the YCA II primers were identical to the previously reported allele sizes by Mathias et al. (7). They designated the upper allele as YCA II a and the lower allele YCA II b, and reported that 5 alleles were seen for YCA II a and 2 alleles for YCA II b. The lower allele sequenced contained 19 copies of the CA repeats (148 bp). Then, we considered the lower amplified allele was monomorphic in all males. The upper middle allele had a sequence of 21–23 CA repeats (152,154,156 bp). The YCA II show one or two male specific PCR products after amplification. Using TAIL PCR method, two PCR products were amplified, and the YCA II CA repeated sequences were duplicated on the Y chromosome, with identified flanking sites allowing independent loci (Fig. 2). Probably the single band was two products of the same size. Because of bilocal nature of these Y-STRs (2Y loci) the frequencies in the population refer to allelic classes and not to single alleles, and so we designated the allelic pairs at the respective loci as classes of alleles. No amplification product was seen in 50 females. In the 95 males studied, we found seven haplotypes. Those were tentatively designated as A[(CA)19/(CA)21], B[(CA)19/(CA)22], C[(CA)19/(CA)23], D[(CA)19/(CA)19], E[(CA)21/(CA)21], F[(CA)22/(CA)22] and G[(CA)23/(CA)23]. The frequencies of these haplotypes were: A, 0.21; B, 0.29; C, 0.37; D, 0.02; E, 0.02; F, 0.07; G, 0.01 (Table 2). Kayser et al. reported the Y chromosomal STRs (YCA

TABLE 1—*Cycling conditions used for TAIL-PCR.*

Reaction	Cycle No.	Thermal Condition
First	1	94°C (2min), 95°C (1min)
	5	94°C (1min), 62°C (1min), 72°C (3min)
	1	94°C (1min), 25°C (3min), 72°C (3min)
	15	94°C (30sec), 65°C (1min), 72°C (3min) 94°C (30sec), 65°C (1min), 72°C (3min) 94°C (30sec), 44°C (1min), 72°C (3min)
	1	72°C (5min)
Secondary	12	94°C (30sec), 55°C (1min), 72°C (3min) 94°C (30sec), 55°C (1min), 72°C (3min) 94°C (30sec), 44°C (1min), 72°C (3min)
	1	72°C (5min)

TABLE 2—*YCA II haplotype frequences in 95 unrelated Japanese males.*

Haplotype	Combination of (CA)n	Frequency (%)
Type A	19/21	21.11
Type B	19/22	29.47
Type C	19/23	36.84
Type D	19/19	2.11
Type E	21/21	2.11
Type F	22/22	7.37
Type G	23/23	1.05

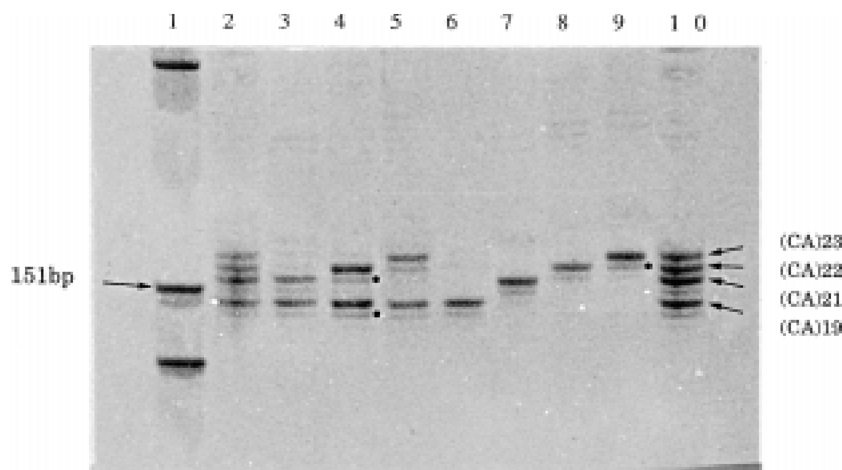

FIG. 1—*YCA II haplotypes in a Japanese male. Lane 1 is the molecular weight standard, lanes 2 and 10 allelic ladders, lane 3 Type A [(CA)19/(CA)21], lane 4 Type B [(CA)19/(CA)22], lane 5 Type C [(CA)19/(CA)23], lane 6 Type D [(CA)19/(CA)19], lane 7 Type E [(CA)21/(CA)21], lane 8 Type F [(CA)22/(CA)22] and lane 9 Type G [(CA)23/(CA)23]. ●: stutter band.*

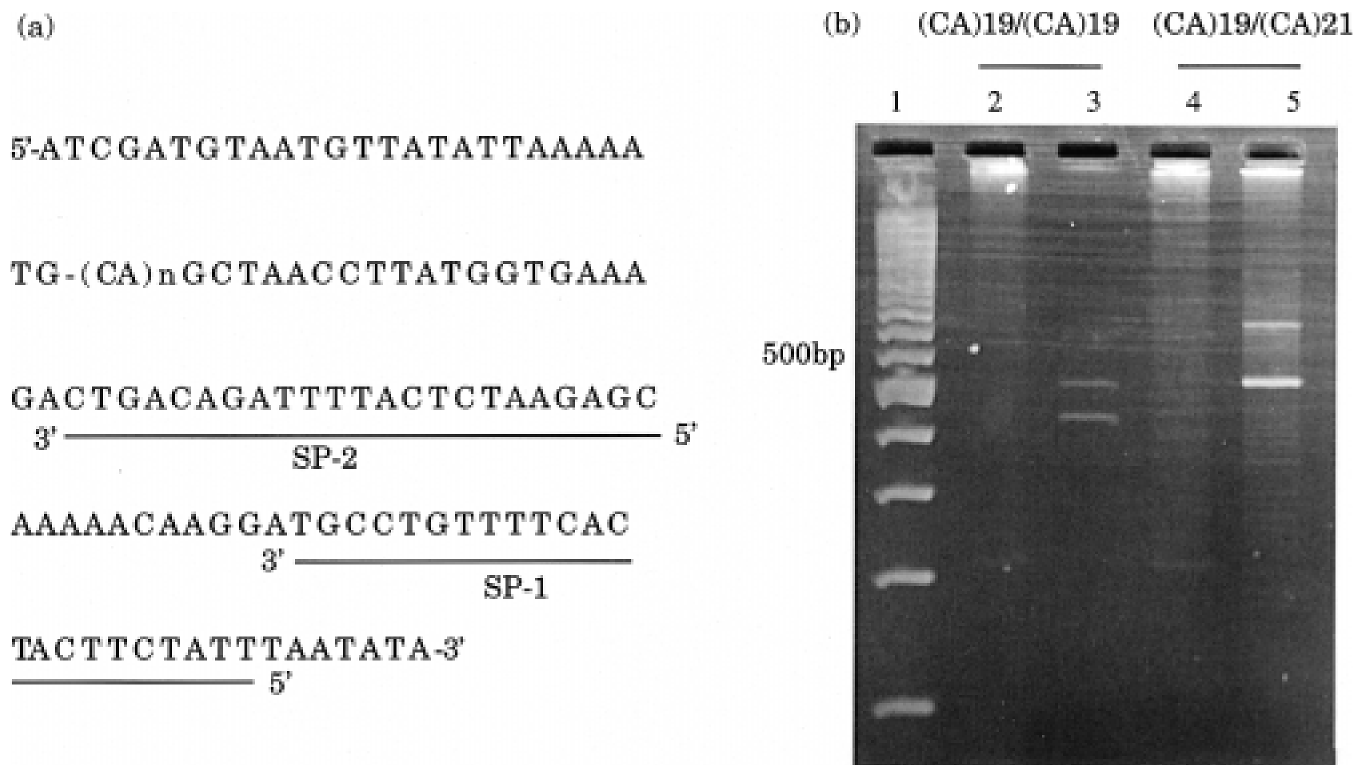

FIG. 2—*(a) Schematic representation of the specific primer for TAIL PCR. (b) TAIL PCR amplification of (CA)19/(CA)19 and (CA)19/(CA)21. Lane 1 is the molecular weight standard, lanes 2 and 4 the first PCR products 3, lanes 3 and 5 the second PCR products.*

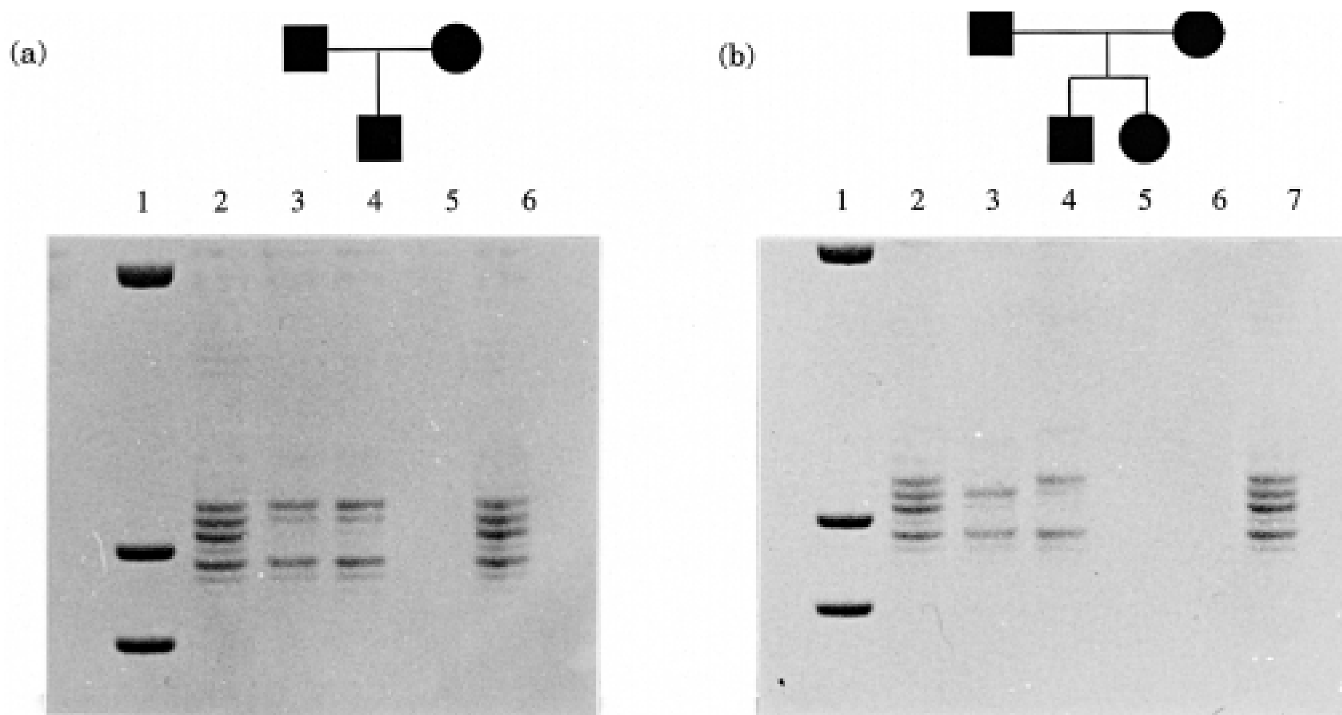

FIG. 3—*YCA II haplotypes in two cases of paternity testing. (a) The father-son-mother trios with paternity established by multi-locus DNA fingerprinting. Lane 1 is the molecular weight standard, lanes 2 and 6 allelic ladders, lanes 3 to 5 PCR-pattern of individual family members. The products of amplification of YCA II are only seen in the male (■). There are complete agreement of the haplotypes (type C) between father and son. (b) The paternity case negated by multi-locus DNA fingerprinting. Lane 1 is the molecular weight standard, lanes 2 and 7 allelic ladders, lanes 3 to 6 PCR-pattern of individual family members. The products of amplification of YCA II are only seen in the male (■). There is different size of haplotypes, type B and C between man and child.*

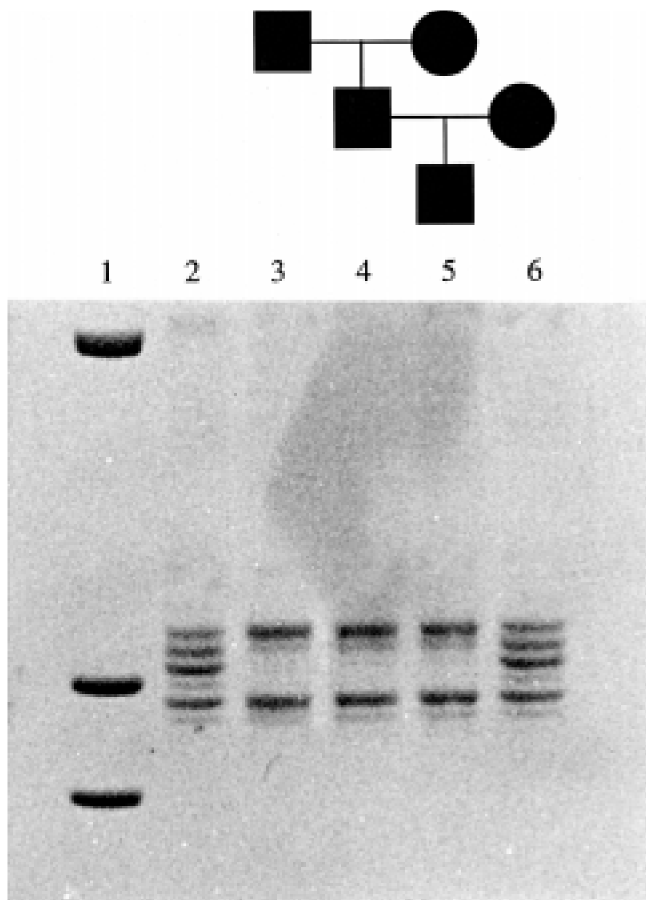

FIG. 4—*YCA II haplotypes of the three male generations. Lane 1 is the molecular weight standard, lanes 2 and 6 allelic ladders, lanes 3 to 5 PCR-pattern of individual family members. There was complete agreement of the haplotypes (type C) among the grandfather, father and son (■).*

II) as a highly polymorphic marker in Caucasians (10). They could find 28 haplotypes, and the size of the PCR segment were 147–165 bp. We found only seven haplotypes and the size of PCR products were 148–156 bp. These difference between our result and theirs might be caused by the condition of analytical method (PCR condition and electrophoretic system). In the 10 cases of paternity testing, 8 cases were affirmative and there was complete agreement of the alleles between father and son (Fig. 3a). On the other hand, there was a different pattern between the man and child in two negative cases (Fig. 3b). In the three generations of males, there was complete agreement of the alleles (type C) between grandfather, father and son (Fig. 4). Our results show that YCA II polymorphism is extremely useful for paternity testing, especially in the father-son case. The result are applicable to forensic identification particularly in rape cases, where their utilization avoids confusion between the victims and the assailant's DNA. Furthermore in conjunction with other YSTRs, they are suitable for inferring phylogenetic relationships between human populations.

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