TECHNICAL NOTE

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The Structure, Frequency, and Forensic Application of the STR Locus D16S543 in the Japanese Population

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ABSTRACT: D16S543 is a complex STR locus consisting of five types of repeat units. The frequency distribution and genetic characteristics of this locus in Japanese were investigated using blood samples from 124 unrelated Japanese and 15 families. Alleles were detected using denatured polyacrylamide gels followed by automated analysis on an ABI 373 sequencer using Genescan software 672. Twenty-one alleles were identified, ranging in size from 281 to 489 bp. An allelic ladder containing the 21 alleles was constructed and used as a typing standard. The repeat unit arrays allowed the 21 alleles to be classified into three distinct groups, including alleles 1 to 7 in group I, alleles 8 to 14 in group II, and alleles 15 to 22 in group III. The alleles in group II were characterized by the insertion of one repeat unit of CAGG, one of AAAG, and three of AAGG, while the group III alleles differed from those of groups I and II by the insertion of a total of 32 repeat units ranging in 5 types. Within each group, the alleles differed from each other only in one 5′ side tetranucleotide AAGG. The power of discrimination (Pd) and the estimated heterozygosity were calculated to be 0.989 and 0.934, respectively. Typing of this locus was successfully applied in four old forensic materials. The study presented herein demonstrates that D16S543 is a highly polymorphic and applicable locus in Japanese.

KEYWORDS: forensic science, D16D543 (wg1f2), sequence structure, frequency distribution, Japanese, forensic materials

Ideal short tandem repeat (STR) loci for forensic purposes should be hypervariable, short in length, and of low mutation rates (1–4). In recent years, an increasing number of such STR loci have been validated and applied in forensic practice, especially in the typing of old, degraded forensic materials (5–7). New STR systems that demonstrate a high power of discrimination are stably inherited and ideal genetic markers for forensic paternity and crime scene DNA profiling.

D16S543 (wg1f2) is a locus isolated by Armour et al. (1994) together with 23 other trimeric or tetrameric tandem repeat loci (8). To clarify its genetic characteristics in Japanese and evaluate its applicability in forensic practice, we investigated the frequency distribution and genetic characteristics of this locus using blood samples from 124 unrelated Japanese and 15 families. D16S543 DNA typing was used on crime scene forensic materials.

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Materials and Methods

DNA Extraction

DNA from blood, nail, hair, bone, and dental pulp of a tooth were extracted using the phenol/chloroform method (9). Blood samples were obtained from 124 unrelated healthy donors from a blood bank and 15 families. DNA extracted from a piece of nail and a single hair of 5 cm in length, which were collected from a crime scene and stored in a desiccator for five years, was concentrated using Centricon 100 devices (Amicon Co., MA) after phenol/chloroform extraction. DNA from a humorous bone, collected during an autopsy and stored at −20°C for five years, and the dental pulp of a tooth sample collected from another autopsy, stored at −20°C for seven years, were obtained after powdering the samples in a freezer mill (Speck Inc., NJ). The concentration of DNA from all these extracts was quantified by means of optical density at 260 nm (OD_{260}) minus OD_{320}, and their purity was checked by means of the ratio OD_{260}/OD_{320}.

PCR

PCR was performed in a 25 μL reaction mixture containing 10 pmol of each primer, 0.2 mM of each dNTP, 2.5 U Taq polymerase, and a corresponding buffer (TaKaRa, Japan). Fifty ng of DNA from blood and more than 10 ng of DNA from nail, hair, bone, and dental pulp were used as templates. Primer sequences (Armour et al.) were as follows:

wg1f2c: 5′-GATCTTTAACTTAACTACATTTGCAAG-3′
wg1f2d: 5′-ACAGTGTGAGACCCTGTCAAGG-3′
The wg1f2c primer was labeled with 6-FAM amidide.

Amplification was carried out in a TaKaRa PCR Thermal Cycler MP for 30 cycles at 94°C for 60 s, 62°C for 60 s, 72°C for 120 s, and a final extension at 72°C for 5 min.

Allele Typing

Samples were automatically analyzed on an ABI 373A sequencer using Genescan software 672, following electrophoresis on 6% denatured PAG (acrylamide/bisacrlyamide 19:1, containing 8.3M urea) in 1xTBE buffer. TAMRA-labeled GS500 and an allelic ladder labeled with TET fluorescent dye were used as internal markers.

Sequencing

The target alleles were separated through 12% polyacrylamide gel electrophoresis. Each allele was excised from the gel and allowed to recover DNA using the Centrifugal filter devices ultrafree-DA (Millipore Co., MA). The sequencing reaction was performed using the Dye Terminator Cycle Sequencing Ready Reaction mix (Perkin-Elmer) with the purified alleles as templates, and primer wg1f2c or wg1f2d as the primer for sequencing the forward or reverse strand, respectively.

TABLE 1—*Allele frequency distribution in 124 Japanese.*

Allele	*n*	%
1	2	0.81
2	3	1.21
3	15	6.05
4	23	9.27
5	27	10.89
6	20	8.06
7	6	2.42
8	4	1.61
9	22	8.87
10	12	4.84
11	31	12.5
12	11	4.44
13	4	1.61
14	2	0.81
15	6	2.42
16	5	2.02
17	21	8.47
18	19	7.66
19	9	3.63
21	3	1.21
22	3	1.21
Total	248	100
DP	0.989	
HET	0.934	
MEC	0.847	
PIC	0.920	

Results and Discussion

Twenty-one alleles, ranging in size from 281 to 489 bp, were identified in 124 healthy and unrelated donors. An allelic ladder containing the 21 alleles was constructed and used as a typing standard (Table 1). In Table 1, each component allele differs from its neighboring alleles by a regular 4 bp, except alleles 14 and 15, which differ from each other by 128 bp. Between alleles 19 and 21, the 481 bp allele was not observed. No interalleles or variants that did not match the allelic ladder were found in this investigation.

Figure 1 shows the allele frequency distribution of this locus in 124 Japanese. The alleles formed three clusters with expressed centers, flanked by decreasing frequencies. The central alleles within the three clusters were alleles 5 (0.1089), 11 (0.1250), and 17 (0.0847), respectively. The Pd values, the estimated heterozygosity, the mean exclusion chance, and the polymorphic information content of this locus were calculated to be 0.989, 0.934, 0.847, and 0.920, respectively. D16S543 allele frequency distributions met Hardy-Weinberg equilibrium expectations as determined through an exact test using the GENEPOP software package (10,11). The typing result of the fifteen family samples revealed that the alleles were inherited steadily in a co-dominant mode. No paternal or maternal mutation was observed.

Sequence data of the alleles composing the allelic ladder are shown in Table 2. All alleles contained five types of repeat units (a: AAGG, b: AGAA, c: AAAG, d: CAGG, e: TAGG, f: GAGG) in their central repeat regions. Consistent with the frequency distribution characteristics, the repeat unit arrays also allowed the 21 alleles to be classified into three groups, i.e., group I: alleles 1 to 7, group II: alleles 8 to 14, and group III: alleles 15 to 22. The alleles in group II were characterized by the insertion of one repeat unit of d (CAGG), one of c (AAAG), and three of a (AAGG). The group III alleles differed from the previous two groups by the insertion of a total of 32 repeat units ranging in 5 types. Within each group, the alleles differed from each other only in one 5′ side tetranucleotide AAGG. The rest of the repeat units within each group remained the same.

The EDNAP recommended that STR alleles should be named according to the total number of repeat units present (12–14), but it was difficult to accommodate the complex D16S543 locus to this recommendation. Variations in the number of the 5′ side AAGGs accounted only for the intra-group size increment, not the inter-group ones, so they could not be used directly to designate alleles. The rest of the repeat units exhibited no contribution to the intra-group size increment, although they were responsible for the inter-group size increment. Thus, they were not considered appropriate

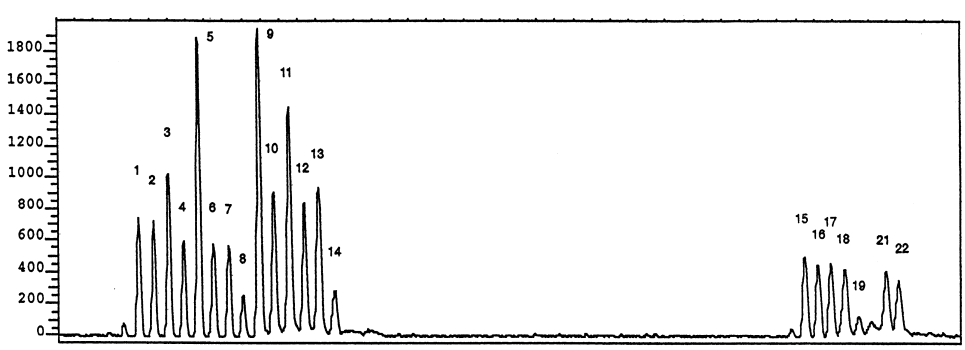

FIG. 1—*An electropherogram of a constructed allelic ladder containing 21 alleles.*

TABLE 2—*Sequence structure of the 21 alleles at the D16S543 locus.*

Allele	Repeat Region (5'–3')						Length	Group
1	– ***a***$_{11}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$		e a e a$_4$ c a$_4$ e a$_4$ e d a^3 *d* d$_7$ –		281	I
2	– ***a***$_{12}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$		e a e a$_4$ c a$_4$ e a$_4$ e d a^3 *d* d$_7$ –		285	I
3	– ***a***$_{13}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$		e a e a$_4$ c a$_4$ e a$_4$ e d a^3 *d* d$_7$ –		289	I
4	– ***a***$_{14}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$		e a e a$_4$ c a$_4$ e a$_4$ e d a^3 *d* d$_7$ –		293	I
5	– ***a***$_{15}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$		e a e a$_4$ c a$_4$ e a$_4$ e d a^3 *d* d$_7$ –		297	I
6	– ***a***$_{16}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$		e a e a$_4$ c a$_4$ e a$_4$ e d a^3 *d* d$_7$ –		301	I
7	– ***a***$_{17}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$		e a e a$_4$ c a$_4$ e a$_4$ e d a^3 *d* d$_7$ –		305	I
8	– ***a***$_{13}$ b a$_2$ c a d$_4$	d	a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_3$ d$_7$ –		309	II
9	– ***a***$_{14}$ b a$_2$ c a d$_4$	d	a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_3$ d$_7$ –		313	II
10	– ***a***$_{15}$ b a$_2$ c a d$_4$	d	a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_3$ d$_7$ –		317	II
11*	– ***a***$_{16}$ b a$_2$ c a d$_4$	d	a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_3$ d$_7$ –		321	II
12	– ***a***$_{17}$ b a$_2$ c a d$_4$	d	a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_3$ d$_7$ –		325	II
13	– ***a***$_{18}$ b a$_2$ c a d$_4$	d	a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_3$ d$_7$ –		329	II
14	– ***a***$_{19}$ b a$_2$ c a d$_4$	d	a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_3$ d$_7$ –		333	II
15	– ***a***$_{11}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_2$ d$_3$ a d$_4$ a$_4$ c a$_3$ c a$_3$ c a$_3$ e a e a$_4$ c a$_4$ e d	a$_3$*d* d$_7$ –	461	III
16	– ***a***$_{12}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_2$ d$_3$ a d$_4$ a$_4$ c a$_3$ c a$_3$ c a$_3$ e a e a$_4$ c a$_4$ e d	a$_3$*d* d$_7$ –	465	III
17	– ***a***$_{13}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_2$ d$_3$ a d$_4$ a$_4$ c a$_3$ c a$_3$ c a$_3$ e a e a$_4$ c a$_4$ e d	a$_3$*d* d$_7$ –	469	III
18	– ***a***$_{14}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_2$ d$_3$ a d$_4$ a$_4$ c a$_3$ c a$_3$ c a$_3$ e a e a$_4$ c a$_4$ e d	a$_3$*d* d$_7$ –	473	III
19	– ***a***$_{15}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_2$ d$_3$ a d$_4$ a$_4$ c a$_3$ c a$_3$ c a$_3$ e a e a$_4$ c a$_4$ e d	a$_3$*d* d$_7$ –	477	III
21	– ***a***$_{16}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_2$ d$_3$ a d$_4$ a$_4$ c a$_3$ c a$_3$ c a$_3$ e a e a$_4$ c a$_4$ e d	a$_3$*d* d$_7$ –	485	III
22	– ***a***$_{18}$ b a$_2$ c a d$_4$		a$_3$ c a$_3$ c a$_3$	ca$_3$	e a e a$_4$ c a$_4$ e d *f* a$_2$ d$_3$ a d$_4$ a$_4$ c a$_3$ c a$_3$ c a$_3$ e a e a$_4$ c a$_4$ e d	a$_3$*d* d$_7$ –	489	III

a: AAGG, b: AGAA, c: AAAG, d: CAGG, e: TAGG, f: GAGG; *: EMBL (x75556). The shadowed parts of repeat units denote the insertions in groups II and III. The italicized and underlined characters demonstrate the differences among groups I, II, and III.

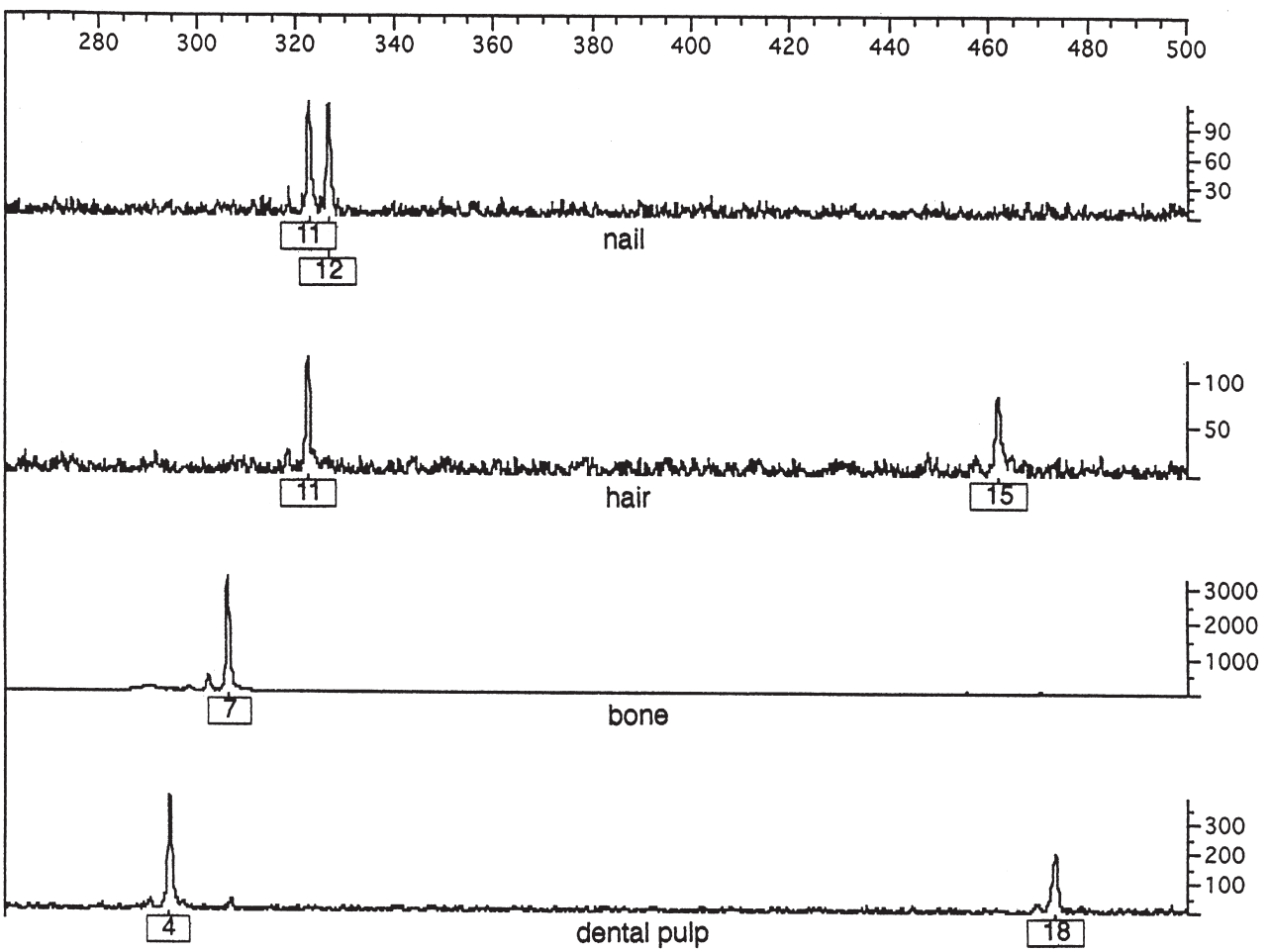

FIG. 2—*Typing of D16S543 locus in nail, hair, bone, and dental pulp. The numerals shown in □ denote the typing results.*

for use in allele designation. Until we find a reasonable way to accommodate the nomenclature, we tentatively designate the present alleles using consecutive numerals from 1 to 21, with 1 representing the shortest allele.

It is interesting to note that there was an insertion of 32 repeat units between group II and group III alleles. This kind of insertion of such a large fragment within consecutive alleles of a STR locus has rarely been reported. According to the frequency distribution, the group III alleles appeared to constitute 26.6% of all alleles, indicating that they were not variant alleles produced by insertion, but major component alleles of this locus. The frequencies of the bordering alleles between groups II and III (i.e., alleles 14 and 15) were low, suggesting that even if new alleles appear between these two groups, the incidence rate will be very low.

As shown in Fig. 2, typing of this locus was successfully applied in four old forensic materials: hair, nail, bone, and dental pulp. The sensitivity of detection for this locus may be high, as the allele peaks were detectable with 10 ng of template DNA using the Genescan technique.

In summary, our present study clarified the sequence characteristics of D16S543 alleles found in Japanese, and indicated that the D16S543 locus is a well-distributed, highly polymorphic, and stable genetic marker in the Japanese population. It also revealed that the D16S543 locus had high detection sensitivity and was robust in application for forensic purposes.

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