Int J Legal Med (2001) 115 : 176–178

SHORT COMMUNICATION

Yoshihisa Watanabe · Atsushi Nagai
Tomohiro Takayama · Keiji Hirata · Sadao Yamada
Isao Nakamura · Yasuo Bunai · Isao Ohya

The STR locus D11S554: allele frequencies and sequence data in a Japanese population

Received: 6 December 2000 / Accepted: 31 May 2001

Abstract A population study on the short tandem repeat (STR) locus D11S554 was carried out in a sample of 362 unrelated Japanese individuals living in the Gifu Prefecture. A total of 46 different alleles ranging from 180 bp to 340 bp and 135 genotypes were revealed. Sequence analysis of alleles was carried out for 185 samples. The sequence structures of the repeat regions of the alleles were found to be complex and the alleles were classified into nine sequence types, including four new sequence types. According to the system of Adams et al. (1993), we designated the new sequence types IA^3, IA^4, IA^5 and IB^3, respectively. Out of the 46 different alleles, 11 showed sequence heterogeneity. The results of this study demonstrated that the D11S554 locus is a powerful and useful genetic marker for forensic practice in the Japanese population.

Keywords Forensic science · PCR · STR · D11S554 · Japanese population data

Introduction

Short tandem repeat (STR) loci are important genetic polymorphic markers for personal identification and paternity testing and D11S554 is one of the most polymorphic and informative of the STR systems (Phromchotikul et al. 1992; Adams et al. 1993; Dupuy and Olaisen 1997), like ACTBP2 (Rolf et al. 1997) and HumVWA (Möller et al. 1994), because it exhibits high degrees of both length and sequence polymorphism.

In the present study, we examined the allele frequencies of the STR locus D11S554 in a Japanese population and investigated the sequence structures of the alleles to evaluate the potential forensic utility of this locus.

Y. Watanabe (✉) · A. Nagai · T. Takayama · K. Hirata
S. Yamada · I. Nakamura · Y. Bunai · I. Ohya
Department of Legal Medicine,
Gifu University School of Medicine,
40 Tsukasa-machi, Gifu 500, Japan
e-mail: watayosh@sannet.ne.jp, Fax: +81-58-267-2957

Material and methods

Blood samples were obtained from 362 unrelated Japanese volunteers living in the Gifu Prefecture. DNA was extracted from blood samples by the phenol-chloroform method (Sakai et al. 1991) and DNA concentrations were determined with a UV-2200 spectrophotometer (Shimadzu, Kyoto, Japan). PCR amplification of the D11S554 locus was carried out according to the method of Phromchotikul et al. (1992). The allelic ladder marker for D11S554 typing was constructed using 27 sequenced alleles to give an almost regularly spaced and uniform allelic ladder marker (Table 1). The allelic ladder marker was prepared by re-amplification of a mixture of DNA from selected alleles. A 2 μl aliquot of the PCR product or allelic ladder marker was mixed with 2 μl of 95% formamide dye solution. The sample was heat-denatured at 95 °C for 3 min and loaded onto a 6% denaturing polyacrylamide gel (7 M urea, 300 mm long and 0.4 mm thick). Electrophoresis was carried out at 40 W and 1500 V, for 90 min, and the PCR products were visualised by silver staining (Budowle et al. 1991). When alleles were aligned with allelic ladders, the types of the alleles were determined. When alleles were not aligned with allelic ladders, the alleles were sequenced and the type of allele was determined. Alleles were arbitrarily designated by the numbers of nucleotides in the amplified fragments.

A total of 185 samples of D11S554 alleles were sequenced to confirm sequence structure. Whenever the number of samples of alleles was 5 or less, all samples were sequenced and when there were more than 5 alleles, 5 or more samples were arbitrarily chosen and sequenced. The selected alleles were sequenced with a Thermo Sequenase core sequencing kit (Amersham, Bucks., UK) using the 5′ primer (AAAG strand) labelled at the 5′ end with Texas Red (Molecular Probes, Eugene, Or.), and analysed automatically on the SQ-5500-S DNA Sequencer using SQ-5500 Ver. 2.00 software (Hitachi Electronics Engineering, Tokyo, Japan).

The Hardy-Weinberg equilibrium was investigated by forming 8 allelic groups consisting of alleles 180–213, 216–221, 224–225, 227–229, 230–233, 234–237, 241–250 and 253–340, according to the method of Katsumata et al. (1999): a bin in the database was pooled with adjacent bins so that all probabilities of the homozygosity test, the likelihood ratio test, and the exact test indicated more than 5%. The statistical properties of this locus were calculated as previous described (Nagai et al. 1996).

Table 1 Allele frequencies for D11S554 in 362 unrelated Japanese subjects (*n* number of observed)

Allele	*n*	Frequency	Allele	*n*	Frequency	Allele	*n*	Frequency
180	1	0.001	227	1	0.001	257[a]	1	0.001
192[a]	8	0.011	229[a]	128	0.177	269	1	0.001
196[a]	6	0.008	230	5	0.007	270[a]	1	0.001
200[a]	33	0.046	232	7	0.010	274[a]	3	0.004
204[a]	22	0.030	233[a]	91	0.126	278[a]	1	0.001
205	3	0.004	234	2	0.003	282[a]	8	0.011
208[a]	6	0.008	236	1	0.001	286[a]	3	0.004
209	2	0.003	237[a]	57	0.079	290[a]	6	0.008
212	8	0.011	241[a]	20	0.028	293	1	0.001
213[a]	34	0.047	242	1	0.001	294[a]	5	0.007
216	3	0,004	245[a]	8	0.011	298[a]	1	0.001
217[a]	46	0.064	246	1	0.001	302[a]	2	0.003
220	3	0.004	249[a]	4	0.006	305	1	0.001
221[a]	82	0.113	250	4	0.006	306[a]	1	0.001
224	11	0.015	253[a]	3	0.004	340	1	0.001
225[a]	87	0.120						

[a] alleles selected for the allelic ladder marker
Homozygosity test $P = 0.744$
Likelihood ratio test $P = 0.129$
Exact test $P = 0.089$

Results and discussion

In a sample of 362 unrelated Japanese individuals living in the Gifu Prefecture, a total of 46 different alleles ranging from 180 bp to 340 bp, and 135 genotypes were revealed (Table 1). The most common alleles were alleles 221, 225, 229 and 233, with frequencies of 0.113, 0.120, 0.177, and 0.126, respectively. No significant deviations from the Hardy-Weinberg equilibrium were found by the homozygosity test, the likelihood ratio test or the exact test. Table 2 summarises the statistical parameters of forensic interest calculated for D11S554 which indicate that it is a powerful genetic marker for individual identification and paternity testing in the Japanese population.

Table 2 Forensic efficiency values for D11S554 in Japanese population sample (*H-exp* expected heterozygosity, *SE* standard error, *MEC* mean exclusion chance, *PD* power of discrimination)

Statistical properties	
H-exp ± SE	0.908 ± 0.015
MEC	0.817
PD	0.985

A total of 185 samples of alleles were sequenced in this study and 9 sequence types, including 4 new sequence types, were found (Table 3). No sequence variation was observed in the flanking regions (data not shown). The sequence data confirm that D11S554 has a highly complex sequence structure, as reported by Adams et al. (1993) and Dupuy and Olaisen (1997). In accordance with the system of Adams et al. (1993), we designated the new sequence types in the present study IA^3, IA^4, IA^5 and IB^3, respectively. Presumably these sequence types were derived from the consensus types, IA and IB, by mutational events. Type IA^3 may have arisen as a result of a G insertion or an AAA deletion in AAAG repeats at the 3′ segment of consensus type IA. Type IA^4 may have been caused by an AAGG insertion into the AAAG repeats, or by an A to G transition in AAAG repeats at the 3′ segment of consensus type IA. Type IA^5 may be attributable to a simple deletion of an AG at the 3′ segment of consensus type IA, and type IB^3 may be due to a simple deletion of an AAAG at the 3′ segment of consensus type IB.

Out of 46 alleles 11 showed sequence heterogeneity due to structural differences in repeat region composition (Table 4). Among the alleles showing sequence heterogeneity, alleles 209, 217, 225, 245, 249, 253 and 290 were

Table 3 Sequence types of D11S554 found in the present study (*n* number of observed)

Sequence type	Repeat region sequence	*n*
IA	(AAAG)$_3$(AG)$_4$AAAGGAGAAAGG(AAAG)$_5$GAAAGG(AAAG)$_{10-22}$----------(AG)$_1$AAAAAT	68
IA^1	(AAAG)$_3$(AG)$_4$AAAGGAGAAAGG(AAAG)$_5$G-----(AAAG)$_{4-19}$ ----------(AG)$_1$AAAAAT	9
IA^3[a]	(AAAG)$_3$(AG)$_4$AAAGGAGAAAGG(AAAG)$_5$GAAAGG(AAAG)$_1$ ---G(AAAG)$_{15-18}$ (AG)$_1$AAAAAT	4
IA^4[a]	(AAAG)$_3$(AG)$_4$AAAGGAGAAAGG(AAAG)$_5$GAAAGG(AAAG)$_{13-21}$AAGG(AAAG)$_4$ (AG)$_1$AAAAAT	6
IA^5[a]	(AAAG)$_3$(AG)$_4$AAAGGAGAAAGG(AAAG)$_5$GAAAGG(AAAG)$_{16}$ ----------AAAAAT	1
IB	(AAAG)$_3$(AG)$_4$AAAGGAGAAAGG(AAAG)$_{4-6}$AA(AAAG)$_{7-20}$(AAGG)$_{13-17}$AAG(AAAG)$_1$(AG)$_2$AAAAAT	31
IB^3[a]	(AAAG)$_3$(AG)$_4$AAAGGAGAAAGG(AAAG)$_5$ AA(AAAG)$_{16}$ (AAGG)$_{15}$ AAG-------(AG)$_2$AAAAAT	1
IIA	(AAAG)$_3$(AG)$_9$AAAGGAG(AAAG)$_{8-48}$---------AAAAAGAAAAAT	39
IIA^3	(AAAG)$_3$(AG)$_9$AAAGGAG(AAAG)$_{12-36}$ A(AAAG)$_3$AAAAAGAAAAAT	26

[a] new types found in the present study. Sequence types have been designated according to Adams et al. (1993). Differences from the respective consensus sequence (IA, IB, or IIA) are underlined

Table 4 Sequence types of alleles showing sequence heterogeneity

Allele	Sequence type
209	IA, IIA^3
217	IA, IIA^3
225	IA, IIA^3
237	IA, IA^4, IIA^3
245	IA, IIA^3
249	IA, IA^4
253	IA, IA^4
282	IB
286	IB
290	IB, IB^3
294	IB
302	IB

composed of 2 sequence types, and allele 237 was composed of 3 sequence types. Alleles 282, 286, 294, and 302, on the other hand, were classified into one sequence type, IB which has 2 repeat units, (AAAG) and (AAGG). Thus, sequence heterogeneity can occur if the numbers of individual types of repeat unit differs, even when the total number of repeat units is the same. For example, in allele 286, 2 samples contained $(AAAG)_5$... $(AAAG)_{14}(AAGG)_{15}$ and the other sample contained $(AAAG)_5$...$(AAAG)_{16}(AAGG)_{13}$.

In the previous study by Phromchotikul et al. (1992), D11S554 alleles showed regular increments, whereas the studies by Adams et al. (1993) and by Dupuy and Olaisen (1997) did not show regular increments in the intervals between the D11S554 alleles. The AMPFLP analysis of the alleles in the present study also failed to detect any regular increments, but did not find any sequence variation in the flanking region and all alleles varied by increments of 4 bp within the same sequence type (Fig. 1). The D11S554 locus is composed of various sequence types and thus the regular increments of 4 bp were masked in the AMPFLP analysis.

References

Adams M, Urquhart A, Kimpton C, Gill P (1993) The human D11S554 locus: four distinct families of repeat pattern alleles at one locus. Hum Mol Genet 2:1373–1376

Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC (1991) Analysis of the VNTR locus D1S80 by the PCR followed by high resolution PAGE. Am J Hum Genet 48:137–144

Chakraborty R, Pornage M, Guegue R, Boerwincle E (1991) Population genetics of hypervariable loci: Analysis of PCR based VNTR polymorphisms within population. In: Burke T, Dolf G, Jeffreys AJ, Wolf R (eds) DNA fingerprinting: approaches and applications. Birkhauser, Berlin, pp 127–143

Dupuy BM, Olaisen B (1997) A dedicated internal standard in fragment length analysis of hyperpolymorphic short tandem repeats. Forensic Sci Int 86:207–227

Katsumata Y, Mizutani M, Nozawa H, Uchihi R, Yamamoto T, Tamaki K (1999) Tests for Hardy-Weinberg equilibrium in Japanese population. Nippon Hoigaku Zasshi 50: 231–236

Möller A, Meyer E, Brinkmann B (1994) Different types of structural variation in STRs: HumFES/FPS, HumVWA and HumD21S11. Int J Legal Med 106:319–323

Nagai A, Yamada S, Watanabe Y, Bunai S, Ohya I (1996) Analysis of the STR loci HUMF13A01, HUMFXIIIB, HUMLIPOL, HUMTH01, HUMTPOX and HUMVWFA31 in Japanese population. Int J Legal Med 109:34–36

Phromchotikul T, Browne D, Litt M (1992) Microsatellite polymorphisms at the D11S554 and D11S569 loci. Hum Mol Genet 1:214

Rolf B, Schürenkamp M, Junge A, Brinkmann B (1997) Sequence polymorphism at the tetranucleotide repeat of the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2) locus. Int J Legal Med 110:69–72

Sakai I, Kasai K, Yoshida K, Mukoyama H (1991) An improved method for DNA isolation from human bloodstains and typing of the isolated DNA using single locus VNTR probes. Rep Nat Res Inst Police Sci 44:36–49

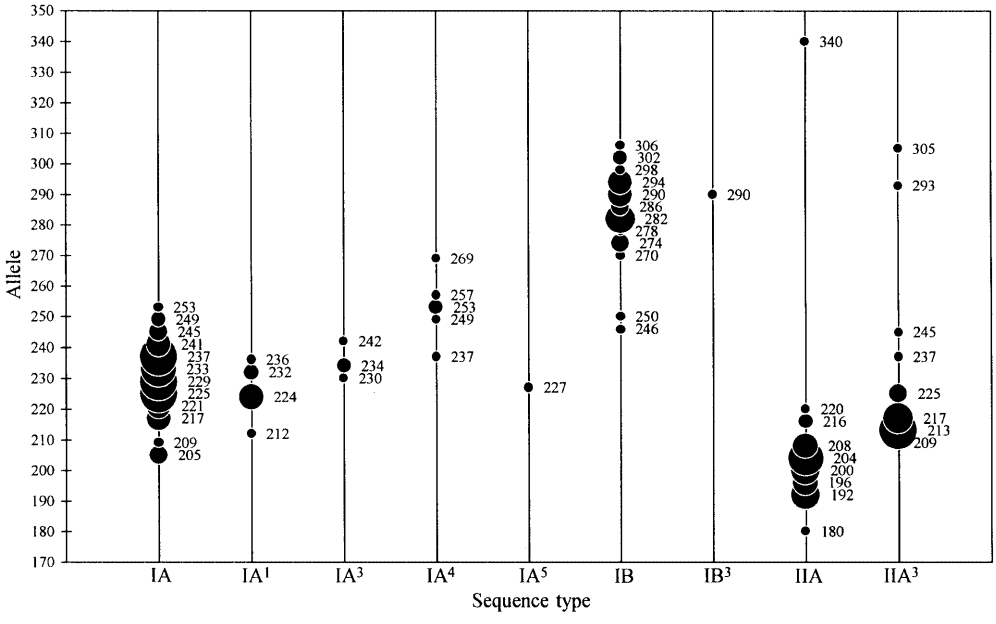

Fig. 1 Sequence types of 185 alleles sequenced in the present study *(The name of the alleles is shown beside the circles, the numbers of the alleles found are indicated by the size of the circles)*