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SHORT COMMUNICATION

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Population genetics of the hypervariable locus D12S391 in Koreans

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Abstract The hypervariable short tandem repeat (STR) locus D12S391 was investigated in a Korean population and 34 fragments were sequenced to confirm the structure of alleles. From these sequenced fragments an allelic ladder containing 13 sequenced alleles was constructed. From 595 unrelated Koreans, 14 alleles were detected and one variant allele 19.3 was observed. The observed heterozygosity was 0.795 and no deviation from Hardy-Weinberg equilibrium was observed in the Korean population ($p = 0.606$). The allele frequency distribution in the Korean population was not similar to other racial or ethnic groups except for Egyptians, Yemenis, Japanese and Caucasoids from the Rhine area. No mutations were observed in the 702 meioses from 144 Korean families. This study demonstrates that the STR locus D12S391 is a useful tool for forensic identification and parentage testing.

Key words Short tandem repeat · D12S391 · Allele frequency · Population study · Korean population

Introduction

Since short tandem repeat (STR) markers have smaller fragment sizes than other polymorphic markers such as variable numbers of tandem repeat, STR loci can be easily amplified by PCR from small amounts of DNA and even from degraded template DNA. Analyses of STR loci are highly useful tools for forensic identification and paternity test (Clayton et al. 1995; Edwards et al. 1991). In addition, the tetranucleotide repeat loci are more useful in forensic cases, because they do not give rise to the multiple artificial bands characteristic of mono- and di-nucleotide repeats (Edwards et al. 1992). The locus D12S391 is a powerful STR marker for forensic casework. It was first described by Lareu et al. (1996a) and is a compound STR consisting of tetranucleotide repeat units (AGAT and AGAC) which vary in numbers between alleles. The heterozygosity of D12S391 is similar to that of complex STRs.

Before a new STR marker system can be applied to forensic identification or paternity testing, a study for the relevant population must be made. Since there has been no previous population study, we analysed the frequency distribution of D12S391 alleles in Koreans and carried out a family study to investigate the genetic stability of this system.

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Materials and methods

DNA extraction

Genomic DNA was extracted by the salting-out method from whole blood samples (Miller et al. 1988) and by the chelex method from hair or buccal swabs (Walsh et al. 1991). A total of 595 unrelated Koreans and 351 children from 144 unrelated Korean families participated in the population study and the mutation study, respectively. The familial relationship was confirmed by using 17 other STR loci. The quantity of DNA extracted from whole blood was estimated using a UV spectrophotometer (DU-650, Beckmann Instruments, Westbury, NY).

Amplification

The D12S391 locus was amplified using the primers described by Lareu et al.(1996b). The 5′ end of the reverse primer was labelled with 6-FAM (6-carboxyfluorescein, Genosys, USA). PCR amplification was performed using 1 ng of genomic DNA extracted from blood or aliquots of 2–4 μl of the extracts of hair and buccal swab in a 25 μl reaction volume. Reactions were carried out in 10 mM Tris-HCl (pH 8.8), 15 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 0.01% gelatin, 200 μM dNTPs, 3 mM $MgCl_2$, 0.2 μM of each primer and 0.625 U AmpliTaq DNA polymerase (PE Biosystems, Foster City, Calif.). PCR was carried out using 30 cycles of 94 °C for 1 min, 61 °C for 1 min and 72 °C 1 min in a GeneAmp PCR system 9600 (PE Applied Biosystems).

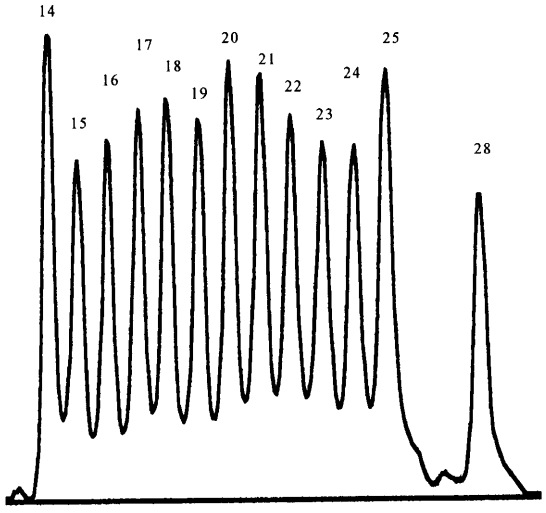

Fig. 1 Allelic ladder of the D12S391 locus containing 13 sequenced alleles from Koreans and the allele designation is based on the number of 'AGAY' (Y = C or T) repeats

Electrophoresis

The samples were denatured at 95 °C for 2 min before being loaded and electrophoresis was carried out on a 4% polyacrylamide sequencing gel on an ABI 377 Genetic Analyzer (PE Biosystems) using the internal size standard Genescan 500 labelled with ROX (6-carboxyrhodamin) for 2 h at a constant 3000 V with a fixed temperature of 51 °C. Fragment sizes were determined automatically using Genescan software ver 2.1 (PE Applied Biosystems), and typed by comparison with the allelic ladder using Genotyper software ver 2.1 (PE Biosystems).

Allelic ladder and allelic designation

A total of 13 sequenced alleles from 14–28 were selected to construct the allelic ladder (Fig. 1). The allelic designation was made according to the recommendations of the DNA commission of the International Society for Forensic Haemogenetics (Bär et al. 1997).

Sequencing

After polyacrylamide gel electrophoresis and silver staining, the DNA fragments were eluted from the gel as described by Möller and Brinkmann (1994). Eluted DNA fragments were reamplified for sequencing. The sequencing reactions for both strands were performed by using the BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems). Electrophoresis was carried out on a 4.5% polyacrylamide sequencing gel on an ABI 377 Genetic Analyzer (PE Applied Biosystems) at a constant 3000 V for 2 h. Sequences were analysed using the sequencing analysis software ver 2.1 (PE Applied Biosystems).

Statistical analysis

The expected and observed heterozygosity, the power of discrimination (PD), the mean exclusion chance (MEC) and polymorphic information content (PIC) and tests for Hardy-Weinberg expectations were calculated using the program 'DNA View' (Charles Brenner, Berkeley, Calif.). The allele frequencies in the Korean population were compared with those of other populations by the exact test using a StatXact software ver 3.1 (Cytel Software Corporation, Cambridge, MD).

Table 1 Allele frequency and statistical parameters at the D12S391 locus in Koreans (n = 595)

Allele	Frequency
14	0.001
15	0.024
16	0.006
17	0.108
18	0.282
19	0.223
19.3	0.001
20	0.145
21	0.102
22	0.058
23	0.029
24	0.013
25	0.007
28	0.001
Heterozygosity expected	0.822
Heterozygosity observed	0.795
Power of discrimination	0.946
Mean exclusion chance	0.652
Polymorphism information content	0.811

Results and discussion

Population data

A total of 14 different alleles at the D12S391 locus were identified in 595 unrelated Koreans. The frequency distribution of the observed alleles and genotypes is shown in Table 1. Allele 18 was the most common, as found in other populations (Gene et al. 1998; Glock et al. 1997; Junge and Madea 1999; Klintschar et al. 1998; Lareu et al. 1996 a; Phillips et al. 1998 b; Waiyawuth et al. 1998) and 52 genotypes were found with the genotype 18/19 being the most frequent.

Sequence analysis

After sequencing 33 alleles, 20 different sequence variants were found which could be grouped into 13 allelic groups based on the total number of repeat units. The sequence structures of the repeat region in these alleles are shown in Table 2. Additionally, three new sequence variants were identified with varying compositions of the number of (AGAT) and (AGAC) repeats. The first "A" in the second (AGAT) repeat unit was deleted in an incomplete variant allele 19.3. The allele 28 previously identified only in Japanese was also detected in Koreans and the repeat unit structure was identical to that of the Japanese (Waiyawuth et al. 1998). According to Lareu et al. (1996 a, b), small alleles from allele 15–18 only show variations in the (AGAT) repeating unit. However, the allele 16 in the Korean population also showed variation in the (AGAC) repeat unit.

Table 2 Sequence variations at the D12S391 locus in Koreans

Allelic group	Size (bp)	Sequence in the repeat region	No. of sequenced alleles
14	205	$(AGAT)_7(AGAC)_6(AGAT)_1$	1
15	209	$(AGAT)_8(AGAC)_6(AGAT)_1$	2
16	213	$(AGAT)_9(AGAC)_6(AGAT)_1$	1
	213[a]	$(AGAT)_8(AGAC)_7(AGAT)_1$	1
17	217	$(AGAT)_{10}(AGAC)_6(AGAT)_1$	2
18	221	$(AGAT)_{11}(AGAC)_6(AGAT)_1$	5
	221	$(AGAT)_{10}(AGAC)_7(AGAT)_1$	1
19	225	$(AGAT)_{12}(AGAC)_6(AGAT)_1$	4
	225[a]	$(AGAT)_{11}(AGAC)_8$	1
19.3	228	$(AGAT)GAT(AGAT)_{10}(AGAC)_7(AGAT)_1$	1
20	229	$(AGAT)_{13}(AGAC)_6(AGAT)_1$	3
	229	$(AGAT)_{12}(AGAC)_7(AGAT)_1$	1
21	233	$(AGAT)_{12}(AGAC)_8(AGAT)_1$	2
	233	$(AGAT)_{12}(AGAC)_9$	1
22	237	$(AGAT)_{15}(AGAC)_6(AGAT)_1$	1
	237	$(AGAT)_{13}(AGAC)_9$	2
23	241[a]	$(AGAT)_{13}(AGAC)_9(AGAT)_1$	1
	241	$(AGAT)_{14}(AGAC)_9$	1
24	245	$(AGAT)_{14}(AGAC)_9(AGAT)_1$	1
25	249	$(AGAT)_{15}(AGAC)_9(AGAT)_1$	1
28	261	$(AGAT)_{19}(AGAC)_8(AGAT)_1$	1

[a] Additional new sequence variants of the D12S391 alleles that have not been identified in previously published reports

Statistical analysis

The genotype distribution showed no deviation from Hardy-Weinberg equilibrium in the Korean population ($p = 0.652$, exact test). The values for expected and observed heterozygosity, power of discrimination, mean exclusion chance, and polymorphism information content are shown in Table 1.

A quantitative comparison of allele frequencies between the Korean and other populations is shown in Table 3. No significant differences were detected between Koreans and Egyptians, Yemenis, Japanese or Caucasoids from the Rhine area (Junge and Madea 1999; Klintschar et al. 1998; Shigeta et al. 1999; Waiyawuth et al. 1998) ($p > 0.01$). However, the allele distributions of the Korean population were significantly different from those of Caucasoids from different areas, Chinese, Thais, Mozambicans, and Portuguese (Corte-Real et al. 1999a, b; Gene et al. 1998; Glock et al. 1997; Junge and Madea 1999; Klintschar et al. 1998; Lareu et al. 1996a; Phillips et al. 1998b; Waiyawuth et al. 1998).

Mutation rates

Samples from 351 children from 144 families were analysed to test the genetic stability of the locus D12S391. The age distribution of the parents at the birth of the child is shown in Table 4. The youngest father and mother were 28 and 27 years old, respectively and the oldest father and mother were 75 and 69, respectively. Brinkmann et al. reported 1 mutation in 562 meioses of Caucasoids living in Germany or Austria (1998), but no mutations in this study were detected in a total number of 702 meioses.

In conclusion, the STR locus D12S391 is a powerful tool for forensic identification and paternity testing in the Korean population.

Table 3 A comparison of allele frequencies of the Korean and other racial/ethnic groups

Populations	n[a]	p value[b]
Caucasoids, Austria (Glock et al. 1997)	150	< 0.0001
Caucasoids, Austria (Klintschar et al. 1998)	100	< 0.0001
Caucasoids, Germany (Lareu et al. 1996b)	188	< 0.0001
Caucasoids, Germany (Waiyawuth et al. 1998)	124	< 0.0001
Caucasoids, Germany (Junge and Madea 1999)	158	0.1857
Caucasoids Italy (Klintschar et al. 1998)	152	0.0011
Caucasoids, Spain (Lareu et al. 1996b)	166	< 0.0001
Caucasoids, Spain (Gene et al. 1998)	167	0.0006
Caucasoids, UK (Phillips et al. 1998a)	260	< 0.0001
China (Waiyawuth et al. 1998)	222	< 0.0001
Egypt (Klintschar et al. 1998)	100	0.3744
Japan (Waiyawuth et al. 1998)	100	0.0590
Japan (Shigeta et al. 1999)	350	0.3388
Mozambique (Corte-Real et al. 1999a)	103	< 0.0001
Portugal (Corte-Real et al. 1999b)	142	0.0056
Thai (Waiyawuth et al. 1998)	154	< 0.0001
Yemen (Klintschar et al. 1998)	104	0.1065

[a] Number of individuals analysed from published population studies
[b] Exact test using Monte-Carlo method

Table 4 The age distribution of the parents at the birth

Father		Mother	
Age	No. of meioses	Age	No. of meioses
< 19	0	< 19	6
19–24	20	19–24	98
25–30	230	25–30	366
30–34	300	30–34	152
35–39	88	35–39	24
≥ 40	12	≥ 40	4
Unknown	52	Unknown	52

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References

Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA recommendations. Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. International Society for Forensic Haemogenetics (editorial). Int J Legal Med 110: 175–176

Brinkmann B, Klintschar M, Neuhuber F, Huhne J, Rolf B (1998) Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. Am J Hum Genet 62: 1408–1415

Clayton TM, Whitaker JP, Maguire CN (1995) Identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci. Forensic Sci Int 76: 7–15

Corte-Real F, Andrade L, Vieira DN, Carracedo A, Vide MC (1999a) Allele distribution of three STRs in a population from Mozambique. Int J Legal Med 112: 345–347

Corte-Real F, Souto L, Anjos MJ, Carvalho M, Vieira DN, Carracedo A, Vide MC (1999b) Population distribution of six PCR-amplified loci in Madeira Archipelago (Portugal). Forensic Sci Int 100: 93–99

Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49: 746–756

Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12: 241–253

Gene M, Carracedo A, Huguet E, Perez-Perez A, Moreno P (1998) Population genetics of the D12S391, CSF1P0 and TPOX loci in Catalonia (Northeast Spain). Int J Legal Med 111: 52–54

Glock B, Dauber EM, Schwartz DW, Mayr WR (1997) Additional variability at the D12S391 STR locus in an Austrian population sample: sequencing data and allele distribution. Forensic Sci Int 90: 197–203

Junge A, Madea B (1999) Validation studies and characterization of variant alleles at the short tandem repeat locus D12S391. Int J Legal Med 112: 67–69

Klintschar M, Ricci U, al Hammadi N, Reichenpfader B, Ebner A, Uzielli ML (1998) Genetic variation at the STR loci D12S391 and CSF1PO in four populations from Austria, Italy, Egypt and Yemen. Forensic Sci Int 97: 37–45

Lareu MV, Pestoni C, Barros F, Salas A, Carracedo A (1996a) Sequence variation of a hypervariable short tandem repeat at the D12S391 locus. Gene 182: 151–153

Lareu MV, Pestoni C, Schurenkamp M, Rand S, Brinkmann B, Carracedo A (1996b) A highly variable STR at the D12S391 locus. Int J Legal Med 109: 134–138

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215

Möller A, Brinkmann B (1994) Locus ACTBP2 (SE33). Sequencing data reveal considerable polymorphism. Int J Legal Med 106: 262–267

Phillips CP, Carracedo A, Lareu MV (1998a) Manual electrophoretic methods for genotyping amplified STR loci. Methods Mol Biol 98: 181–192

Phillips CP, Syndercombe Court D, Lareu MV, Hasskamp J, Carracedo A (1998b) Band shift analysis of three base-pair repeat alleles in the short tandem repeat locus D12S391. Forensic Sci Int 93: 79–88

Shigeta Y, Yamamoto Y, Doi Y, Miyaishi S, Ishizu H (1999) Polymorphism of the D12S391 microsatellite in a Japanese population sample. Forensic Sci Int 102: 61–66

Waiyawuth W, Zhang L, Rittner C, Schneider PM (1998) Genetic analysis of the short tandem repeat system D12S391 in the German and three Asian populations. Forensic Sci Int 94: 25–31

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506–513