Int J Legal Med (1998) 112:67–69
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SHORT COMMUNICATION

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Validation studies and characterization of variant alleles at the short tandem repeat locus D12S391

Received: 4 February 1998 / Received in revised form: 22 April 1998

Abstract Validation studies were carried out on the short tandem repeat (STR) locus D12S391 including the determination of the allele frequencies, forensic application and sequence analysis of variant alleles. A total of 16 alleles were found in a population survey of 158 unrelated individuals from the Rhine area, none of which exceeded the 0.20 frequency level. In 316 alleles analysed so far 18 alleles were found with an incomplete repeat unit in the 5′-end of the repeat region. The statistical values were similar to those of other European populations and no deviation from Hardy-Weinberg equilibrium (HWE) was observed.

Key words D12S391 · Population study · Forensic application · Sequence analysis · Variant alleles

Introduction

The locus D12S391 investigated by Lareu et al. (1996) is a compound STR system consisting of (AGAT) and (AGAC) repeat units. This locus is characterized not only by length, but also by sequence variants. Sequence variation is based on the variable number of the (AGAT) and (AGAC) units within the repeat region. In the past the interest has been focused on standardization and reproducibility of DNA typing and much more knowledge has been obtained on microheterogeneity within the repeat region and the influence on the electrophoretic behaviour of PCR fragments. The knowledge of these variant alleles requires electrophoresis conditions which are capable of detecting these length variants. Validation studies were carried out including the determination of allele frequencies, statistical analysis, forensic application and sequence analysis of the variant alleles.

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Materials and methods

Extraction

Human DNA was extracted from blood samples of 158 unrelated Caucasians from the Rhine area (Bonn) by the salting out method (Miller et al. 1988) and quantified using the slot-blot-technique (QuantiBlot, Human DNA Quantitation Kit, Perkin Elmer, Waye et al. 1989).

Amplification

Amplification was performed according to Lareu et al. (1996). The primers were designed as described by Lareu et al. (1996) with the reverse primer labeled with CY5 (Pharmacia Biotech, Freiburg).

Electrophoresis

PCR fragments were separated on 6% denaturing gels using a fluorescence detection system (ALFexpress Sequencer, Pharmacia Biotech, Freiburg). The fragment sizes were automatically analysed using an internal standard (114 bp, 402 bp), a system-specific allelic ladder and the ALFwin software (Version 1.1, Pharmacia Biotech, Freiburg).

Allele designation

Allele designation was according to the repeat number (ISFH recommendations 1994, 1997; Lareu et al. 1996). The allelic ladder is composed of 12 consensus alleles ranging from allele 15 to 26.

Validation studies

Sensitivity studies were carried out with different amounts of DNA ranging from 5 ng to 50 pg. Different series of experiments with the constant component being 1 ng (series I) and 5 ng (series II) and the ratios 1:1, 1:2, 1:5, 1:10, 1:20, 1:50 and 1:100 were performed for mixtures with known genotype.

Statistical analysis

The statistical values were calculated and the Hardy-Weinberg equilibrium checked according to Piccinini et al. (1997).

Solid phase sequencing

Separation, isolation and reamplification of DNA fragments from heterozygous individuals was performed as described by Möller

and Brinkmann (1994). Reamplification was carried out with primer set A (5′-M13 universal primer-D12S391 forward primer; 5′-biotin-labeled-D12S391 reverse primer) and primer set B (5′-biotin-labeled-D12S391 forward primer; 5′-M13 universal primer D12S391 reverse primer). Re-amplified PCR fragments were purified with the QUIAquick PCR Purification Kit (Quiagen, Hilden). Sequencing reactions were carried out using the Autoload-Solid-Phase-Sequencing Kit (Pharmacia Biotech, Freiburg) according to the manufactors instructions.

Results

Population studies

A total of 16 alleles were found in 158 unrelated individuals with frequencies ranging from 0.003 to 0.196 (Table 1). The Hardy-Weinberg equilibrium was tested using the exact test (Guo and Thomson 1992) and no significant deviation was detected for the locus D12S391 (p-value, exact-test: 0.51).

Validation studies

Sensitivity studies revealed that the D12S391 alleles could be clearly detected and assigned to the allelic ladder at levels down to 100 pg template DNA. The phenomenon of allelic drop-out was not observed. In studies with different mixing ratios and the constant component being 1 ng (series I) and 5 ng (series II) the detection limit of the minor component was estimated at a mixture ratio of 1:10 (Fig. 1).

Sequencing data

A total of 18 variant alleles were analysed and sequenced by solid phase sequencing. The fragment length of the variant alleles were determined to be 220 bp, 224 bp, 228 bp and 240 bp respectively and assigned as 17.3, 18.3, 19.3 and 22.3 due to an incomplete unit in the repeat region (Fig. 2). In 16 out of 18 sequenced variant alleles the incomplete repeat unit was found at the 5′-end of the repeat region (Table 2). The basic sequence structure of the repeat region was determined to be : (AGAT) – (GAT) - $(AGAT)_n$ – $(AGAC)_m$ – (AGAT) for the alleles 17.3, 18.3 and 19.3. The allele 22.3 showed an T→C transition resulting in the structure (AGAT) – (GAT) – $(AGAT)_n$ – $(AGAC)_{m+1}$. Out of four alleles 19.3 two showed a different structure consisting of 5 × (AGAT), 1 × (GAT), 7 × (AGAT), 6 × (AGAC) and 1 × (AGAT).

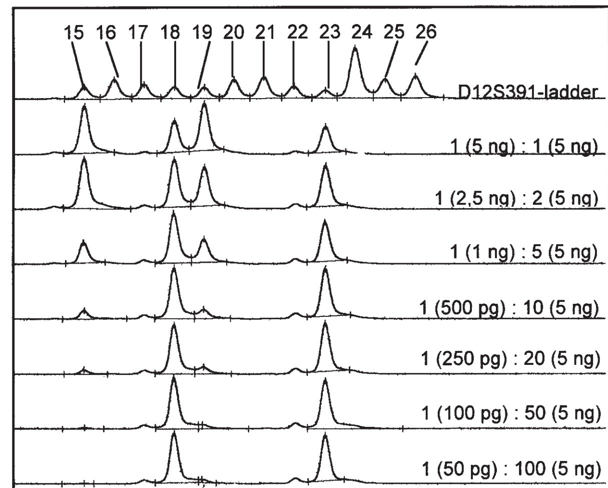

Fig. 1 Electropherogram of experimental studies with two heterozygous individuals A and B and with different mixing ratios. Separation and detection of the alleles was performed on an ALF-express Sequencer (Pharmacia Biotech, Freiburg). The allele combinations of the two individuals are: A: 18, 23 and B: 15, 19

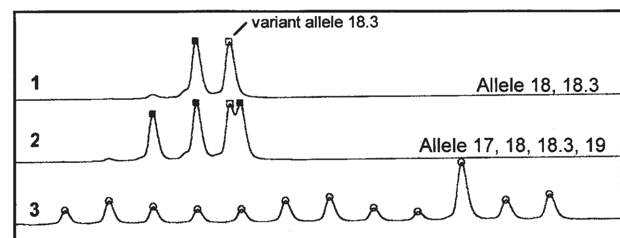

Fig. 2 Electropherogram of the separation of the variant allele 18.3 in a 6% denaturing gel system and a separation distance of 20 cm. Lane 1 Individual C with the allele combination 18, 18.3, lane 2 mixture of individual C (18, 18.3) and individual D (17, 19), lane 3 D12S391 ladder composed of 12 consensus alleles (15–26)

Table 1 Allele frequencies and statistical values of the short tandem repeat locus D12S391 (n = sample size; H = heterozygozity rate; MEC = Mean exclusion chance; DI = Discrimination index)

Allele	Caucasians (Rhine area, n = 158)
15	0.063
16	0.025
17	0.127
17.3	0.022
18	0.196
18.3	0.009
19	0.111
19.3	0.009
20	0.098
21	0.123
22	0.098
22.3	0.003
23	0.076
24	0.025
25	0.009
26	0.003
H (obs.)	0.85
MEC	0.78
DI	0.97

Discussion

Population studies and sequence analysis were performed for the STR system D12S391 which was first described by Lareu et al. (1996). The allele frequencies were less than 0.20 indicating that this system will be a powerful marker in forensic science. The heterozygosity rate, discrimination index (DI) and other forensic parameters (e.g. sensitivity) are comparable with those of other well established systems (Möller et al. 1994, Rolf et al. 1997a). A

Table 2 Sequence structure of the repeat region at the D12S391 locus for variant and consensus alleles

Variant allele	No. of sequenced alleles	Fragment length (bp)	Sequence structure of the repeat region $5' \rightarrow 3'$
17.3	8	220	(AGAT) – (GAT) – $(AGAT)_8$ – $(AGAC)_7$ – (AGAT)
18.3	5	224	(AGAT) – (GAT) – $(AGAT)_9$ – $(AGAC)_7$ – (AGAT)
19.3	2	228	$(AGAT)_5$ – (GAT) – $(AGAT)_7$ – $(AGAC)_6$ – (AGAT)
19.3	1	228	(AGAT) – (GAT) – $(AGAT)_9$ – $(AGAC)_8$ – (AGAT)
19.3	1	228	(AGAT) – (GAT) – $(AGAT)_{10}$ – $(AGAC)_7$ – (AGAT)
22.3	1	240	(AGAT) – (GAT) – $(AGAT)_{12}$ – $(AGAC)_9$
Consensus allele			
18	1	221	$(AGAT)_{10}$ – $(AGAC)_7$ – (AGAT)
18	2	221	$(AGAT)_{11}$ – $(AGAC)_6$ – (AGAT)
19	1	225	$(AGAT)_{11}$ – $(AGAC)_7$ – (AGAT)
19	1	225	$(AGAT)_{12}$ – $(AGAC)_6$ – (AGAT)
20	1	229	$(AGAT)_{12}$ – $(AGAC)_7$ – (AGAT)
21	1	233	$(AGAT)_{12}$ – $(AGAC)_9$
21	1	233	$(AGAT)_{13}$ – $(AGAC)_8$
22	2	237	$(AGAT)_{13}$ – $(AGAC)_9$
23	2	241	$(AGAT)_{14}$ – $(AGAC)_9$
23	1	241	$(AGAT)_{14}$ – $(AGAC)_8$ – (AGAT)
24	1	245	$(AGAT)_{15}$ – $(AGAC)_8$ – (AGAT)

total of 18 variant alleles were found, all differing by 1 bp from the neighbouring consensus allele. Rolf et al. (1997a) also found variant alleles, but with lower frequency than found in this study. Gene et al. (1998) found no variant alleles in a population survey of 167 unrelated individuals from Catalonia (NE Spain). Reproducible detection of the variant alleles was possible using denaturing electrophoresis conditions in combination with a laser fluorescence detection system and a separation distance of 20 cm. The mean deviation of the sample probes to the corresponding ladder allele was calculated to be within the ± 0.5 bp-window. The mean value of the standard deviations was 0.11 bp. Application of the strategy proposed by Gill et al. (1996) for the designation of variant alleles the band shifts measurements were always < 0.5. Sequence analysis revealed that the variant alleles arose from an incomplete tetrameric unit within the repeat region and not from a 1 bp deletion in one of the flanking regions as known for the STR locus HumACTBP2 (Möller and Brinkmann 1994; Rolf et al. 1997b). The knowledge of these modifications requires electrophoresis systems which are capable of separating and detecting variant alleles. If the occurrence of variant alleles at the D12S391 locus is known and standardized electrophoresis conditions are applied, this STR system will be a valuable marker in forensic science.

Acknowledgements We would like to thank the Institute of Legal Medicine Münster for helpful assistance in the statistical calculations. We would like to thank M. Steevens for technical assistance.

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