Int J Legal Med (1996) 108:256–258

ORIGINAL ARTICLE

F. De Stefano · L. Casarino · M. G. Costa · G. Bruni
A. Mannucci · M. Unseld · R. Hiesel · M. Canale

Analysis of a short tandem repeat locus on chromosome 19 (D19S253)

Received: 11 September 1995 / Received in revised form: 30 November 1995

Abstract A tetranucleotide tandem repeat locus on chromosome 19 (D19S253) was analysed. PCR products were detected by denaturing polyacrylamide gels with fluorescent-based technology. This study has confirmed a polymorphism with 9 alleles ranging from 209 to 241 bp with a simple repeat structure arranged from 7 to 15 repeats. Family studies confirmed mendelian inheritance of alleles. The efficiency on DNA extracted from bloodstains and cigarette butts has been evaluated. The protocol has shown sensitivity and reproducibility.

Key words DNA typing · PCR · STRs · D19S253

Introduction

Short tandem repeat (STR) loci are highly represented in the human genome and, among them, tetranucleotide tandem repeat sequences are considered very useful for individual identification and paternity testing. As suggested by Urquhart et al. [1], they conform to an incremental repeat pattern, and are classified as simple, compound or complex according to the variability of the repeat blocks. The knowledge of microheterogeneity in both the repeat and the flanking regions allows a precise definition of the allelic ladder [2].

The STR locus D19S253 was reported by Weber et al. [3]. Our analyses of cell lines corresponding to CEPH family individuals 1331-01 and 1331-02 confirmed heterozygosity of samples but gave alleles 3 bp shorter than those assigned by Weber et al. [3] when separated by a fluorescent-based technology. In our experience, this marker locus shows the presence of 9 alleles arranged from 7 to 15 repeats in a range from 209 to 241 bp and can be classified as a simple repeat containing GATA units without variations, as shown by analysed sequences of alleles in the allelic ladder. PCR product sizes were determined automatically by a fluorescent-based technology (Genescan 672 software, Applied Biosystems) with an internal lane standard GS2500 employing the local Southern method. Fragment size values of alleles in the ladder were confirmed by sequencing.

F. De Stefano (✉) · L. Casarino · M. G. Costa · G. Bruni
A. Mannucci · M. Canale
Istituto di Medicina Legale,
Università degli Studi di Genova via De Toni 12,
I-16132 Genova, Italy

M. Unseld · R. Hiesel
Institut für Genbiologische Forschung Berlin GmbH,
Ihnestrasse 63, D-14195 Berlin, Germany

Materials and methods

DNA extraction and preparation

DNA was extracted from fresh blood samples (volunteer blood donors and families) using a phenol/chloroform protocol. A rapid Chelex extraction was used for 30 bloodstains on different substrates and 53 cigarette butts. DNA quantification was carried out using a fluorometer TK100 (Hoefer Instruments). Extracted DNAs from CEPH family cell lines corresponding to individuals 1331-01 and 1331-02 were kindly supplied by Dr. L. Varesco from the Laboratory of Immunogenetics of the National Institute for Cancer Research, Genoa.

PCR conditions

Primer sequences as described by Weber et al. [3] were:

5′ ATAGACAGACAGACGGACTG (primer 1)
5′ GGGAGTGGAGATTACCCCT (primer 2)

Primer 1 was labelled with the dye TAMRA (Applied Biosystems). Up to 10 ng of DNA (or 10 µl of Chelex-extracted samples) was amplified in a total volume of 50 µl consisting of 0.1 µM of each primer, 200 µM dNTPs, 1.25 units of *Taq* polymerase, 50 mM KCl, 10 mM Tris-HCl pH 8.3 and 1.5 mM $MgCl_2$. Amplification conditions were as follows: 95° C for 2 mins; 28 cycles of 94° C for 45 s, 55° C for 30 s and 72° C for 30 s, and a final extension at 72° C for 10 mins on a 9600 GeneAmp PCR System (Perkin Elmer).

Ladder

The construction of a ladder followed isolation of single alleles from the gel after silver staining and reamplification under the same conditions. A mix of PCR products was resuspended to ob-

tain a 10^{-6} dilution, and 1–5 µl of this solution was reamplified in a total volume of 50 µl.

Detection systems

Aliquots of 0.5–3 µl of PCR products (0.5–2 µl of the ladder) were combined with an internal lane standard (GS2500, Applied Biosystems) and loaded onto a 6% polyacrylamide denaturing gel. Samples were electrophoresed at constant power (36 W) for 5 h 30 min on an Applied Biosystems automated DNA sequencer 373A Leon and automatically sized by GeneScan 672 software (Applied Biosystems) employing the local Southern method.

Sequencing

After isolation and purification of fragments from the silver-stained gel, reamplifications using the described conditions were carried out in two independent reactions using either primer 1 or primer 2 biotinylated at the 5′ end. PCR products were purified with QiaQuick Spin Columns (Qiagen) according to the supplier's instructions. Of the purified products 10–20% was attached to M-280 Streptavidin Dynabeads (Dynal) to purify single-stranded DNA. Solid-phase sequencing was performed on both strands using the unlabelled PCR primers. The Fluore-dATP labelling mix and the AutoRead sequencing kit from Pharmacia were used. Sequences were run on the ALF automated sequencing system (Pharmacia).

Table 1 **a** D19S253 alleles assigned as molecular weight (bp) and number of repeats; size range for each allele present in the ladder. **b** Consensus sequence

a

Allele (bp)	Repeats	Number of sequenced alleles	Size range	
			Min.	Max.
241	15	1	240.91	241.59
237	14	3	236.85	237.82
233	13	3	232.89	234.29
229	12	4	229.01	230.40
225	11	2	224.47	225.83
221	10	1	220.38	221.46
217	9	1	216.82	217.22
213	8	2	212.82	213.14
209	7	5	208.40	210.12

b

ATAGACAGACAGACGGACT-(GATA)$_n$
GATGTAGATATTGATATCCTATTAATTCTATTACTCTG-
GAGAAATAAATACATTTCCCAGGAGG
AGCCACAGCAGGTCCCACTGCAGGCTTTAGAGGTG-
GCACAGCCTCTGCAATGAACAGCATG
TCCAGATTCTGCGCACAT-**AGGGGTAATCTCCACTCCC**

Results

A total of 22 alleles were sequenced and all showed simple repeat structures with 7–15 regular GATA repeats (Table 1). The sequenced fragments were consistent with the base pair values of alleles in the ladder obtained by GeneScan 672 software analysis (Fig. 1), and alleles from CEPH family individuals 1331-01 and 1331-02 were found to be 3 bp shorter than those reported by Weber et al. [3]. The results were: 1331-01 = 233/229 bp (13/12 repeats) and 1331-02 = 225/209 bp (11/7 repeats).

Allele designations were based on repeat units present in the sequenced fragments. The window of variation in size range for each allele (Table 1) was determined by analysis of 14 samples and 12 allelic ladders run 40 times across 10 gels.

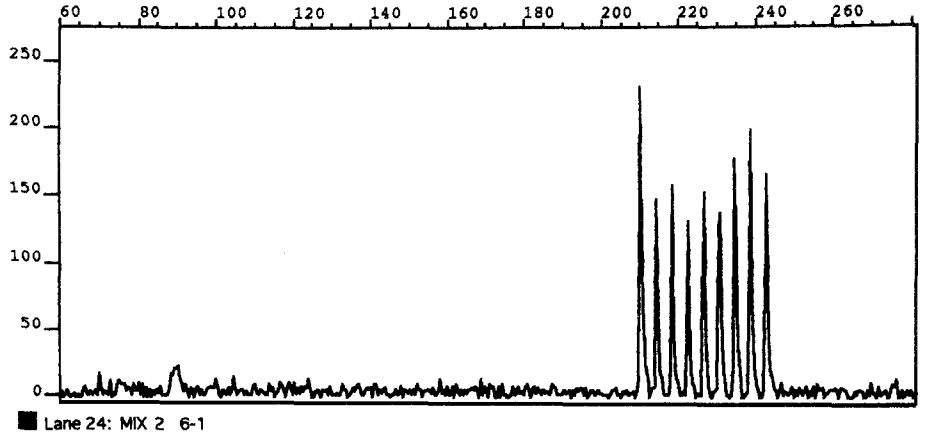

Fig. 1 Electropherogram of the allelic ladder for the D19S253 locus. Separation of 1.5 µl of PCR products from 1 µl of a 10^{-6} dilution. Regular intervals among alleles can be identified

Peak/Lane	Min.	Size	Peak Height	Peak Area	Scan #
1Y, 24	247	209.08	269	2086	2478
2Y, 24	251	213.10	155	1150	2519
3Y, 24	256	217.14	160	1164	2560
4Y, 24	260	221.10	149	1105	2600
5Y, 24	264	225.65	191	1348	2642
6Y, 24	268	229.97	204	1563	2683
7Y, 24	272	233.95	212	1647	2724
8Y, 24	276	237.42	200	1585	2765
9Y, 24	280	241.01	172	1333	2806

This marker is located in a fragment size range where allelic drop-out may occur. In our experience, with primers suggested by Weber et al. [3] and using constant quantities of DNA between 3 and 10 ng, such a phenomenon was not observed in over 200 fresh blood and 83 stain samples. The analysis of a control sample kindly supplied by Dr. C. Kimpton of the Forensic Science Service, Birmingham, where a deletion of an AGAC block at the primer site was described [4], constantly gave evidence of heterozygosity with an allele intensity ratio corresponding to 1.5 : 1.

Analyses were conducted on 20 families. Paternity exclusions were confirmed by other markers, no false maternal exclusions were observed, and homozygous parents always transmitted the detected allele.

Analyses of stained materials gave constantly good results for bloodstains, and clear PCR products were obtained in 50 of 53 cigarette butts. Identified genotypes from aged samples were identical to reference fresh samples.

Discussion

As recommended by the DNA Commission of the International Society for Forensic Haemogenetics [5], definition of the allelic ladder and sequencing of each allele have to precede adoption of a new marker for routine forensic examinations.

The D19S253 STR locus was analysed to generate an allelic ladder of known sequence and obtain more information about its precise structure. According to the repeat blocks identified in our sequences, D19S253 can be defined as a simple repeat locus, and no variability in the 3′ flanking region was detected in 22 sequenced alleles. Some problems could arise in sequencing alleles because, as shown in Table 1, primer 1 ends in the first G of the GATA repeat. In our experience, no mistyping of PCR products occurred for samples (CEPH family individuals 1331-01 and 1331-02 above all) amplified several times.

This marker is robust and easy to handle, and the amplification protocol can be adapted for both single amplification [3] and multiplex reaction (data not shown).

It can be presumed that allelic drop-out could occur, as shown by Lygo et al. [6], but in our experience, by using 3–10 ng of DNA such a phenomenon can be prevented. Attention must be paid to defining the annealing temperature, as temperatures higher than 55° C could result in allelic drop-out and loss of heterozygosity.

Samples with an AGAC deletion at primer 1 site have been reported [4]. Such a mutation could occur in 1% of samples. In our experience, annealing temperatures of 55° C can prevent allelic drop-out, preserving identification of GATA repeat blocks and flanking regions between primers. Moreover, the described ratio of allelic intensity (1.5 : 1) is similar to what can randomly happen for other STR markers, such as HUMFES/FPS.

Analysis of 20 families showed mendelian inheritance of alleles. Paternity exclusions were confirmed by other markers, and no false maternal exclusions occurred. Homozygous parents always transmitted the observed allele to siblings (data not shown).

Amplification of DNA extracted from bloodstains and cigarette butts produced good results, and in only 3 of 83 cases were no amplification products observed.

In conclusion, it must be stressed that this marker can be considered a useful tool for individual identification, paternity testing and genetic studies because of the sensitivity and robustness of the described protocol.

Acknowledgements We thank Dr. Eduardo Thüroff (Tib Mol-Biol Italia) for practical support, Dr. Liliana Varesco, who kindly supplied DNA from CEPH families 1331-01 and 1331-02, and Dr. Colin Kimpton for reference DNA. This investigation has been partially supported (40%) by the project „Innovazioni Tecnologiche in Medicina Legale“ of the Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

References

1. Urquhart A, Kimpton CP, Downes TJ, Gill P (1994) Variation in short tandem repeat sequences – a survey of twelve microsatellite loci for use as forensic identification markers. Int J Legal Med 107 : 13–20
2. Moeller A, Meyer E, Brinkmann B (1994) Different types of structural variation in STRs: HumFES/FPS, HumVWA and D21S11. Int J Legal Med 106 : 319–323
3. Weber JL, Wang Z, Hansen K, Stephenson M, Kappel C, Salzman S, Wilkie PJ, Keats B, Dracopoli NC, Brandriff BF, Olsen AS (1993) Evidence for human meiotic recombination interference obtained through construction of a short tandem repeat-polymorphism linkage map of chromosome 19. Am J Hum Genet 53 : 1079–1095
4. Urquhart A, Chiu CT, Clayton T, Downes T, Frazier R, Jones S, Kimpton C, Lareu MV, Millican E, Oldroyd N, Thompson C, Watson S, Whitaker J, Gill P (1995) Multiplex STR systems with fluorescent detection as human identification markers. Proceedings of the Fifth International Symposium on Human Identification 1994. Promega Corporation pp 73–83
5. DNA Commission of the International Society for Forensic Haemogenetics (1994) DNA recommendations – 1994 report concerning further recommendations of the DNA Commission of the ISFH regarding PCR-based polymorphisms in STR (short tandem repeat) systems. Forensic Sci Int 69 : 103–104
6. Lygo JE, Johnson PE, Holdaway DJ, Woodroffe S, Whitaker JP, Clayton TM, Kimpton CP, Gill P (1994) The validation of short tandem repeat (STR) loci for use in forensic casework. Int J Legal Med 107 : 77–89