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SHORT COMMUNICATION

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Sequence variation of a variable short tandem repeat at the D18S535 locus

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Abstract A short tandem repeat in the D18S535 locus was sequenced in 25 selected alleles. A total of 8 different alleles were found which can be designated according to the total number of repeats. This STR is a simple hypervariable STR consisting of blocks of (GATA) repeats with a basic sequence structure $(GATA)_1(GACA)_1(GATA)_1(GAT)_1(GATA)_{9–16}$. Population data showed that this is a highly polymorphic STR with a heterozygosity of more than 0.80, a simple structure and small size (130–158 bp) which makes this an interesting DNA polymorphism for forensic and genetic purposes.

Key words Short tandem repeats · Human genome · DNA sequencing · DNA polymorphisms

Introduction

Genetic typing of short tandem repeats (STR) has become a robust and efficient tool in forensic genetics (Edwards et al. 1992; Kimpton et al. 1992; Urquhart et al. 1994; Pestoni et al. 1995). The most commonly used STRs only exhibit a reduced number of alleles with a relatively low discrimination power. On the other hand complex hypervariable STRs (such as ACTBP2) are being introduced, although, due to their complexity, they are prone to problems in the identification and assignment of alleles (Kimpton et al. 1995). In addition these highly informative STRs are normally relatively long, which causes difficulties in their application for the analysis of degraded material (Alvarez-García et al. 1996).

STRs are abundant in the human genome (Weber and May 1989) and only a few of them have already been evaluated for forensic purposes. It might therefore be possible to find new, more suitable STRs. Ideal STRs for forensic purposes should combine characterististics such as hypervariability, low mutation rate, robustness, easy mutiplexing, low stutter characteristics and small size.

Using a GATA probe, a short tandem repeat was found in the locus D18S535 (also know as: CHLC-GATA13.91, G00-208-299, G07985 GenBank accession). Preliminary population data indicate the potential usefulness of this system for forensic purposes. The nucleotide sequences of all the common alleles of this system have not been determined. We report here the sequence of eight different alleles, isolated from the Galician population (NW Spain). In addition a nomenclature for this system is proposed and population data from Galicia is reported.

Material and methods

Genomic DNA was isolated from human blood as described by Valverde et al. (1993) and amplified using the following primers: forward primer 5′ TCA TGT GAC AAA AGC CAC AC, reverse primer *5′ AGA CAG AAA TAT AGA TGA GAA TGC A (*fluorescein labeled at the 5′ end). PCR was performed using 5 ng of genomic DNA in a 50 µl reaction volume, 10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.25 µM each primer and 1.25 U AmpliTaq DNA polymerase (Cetus, Emerville, Calif.). The PCR conditions were: 30 cycles denaturation 94 °C for 45 s, annealing 60 °C for 60 s and extension 72 °C for 60 s, in a Perkin Elmer 480A thermocycler (Perkin Elmer Foster City, Calif.). The size of the PCR products were first determined in a 6% PAGE gel in an automated sequencer (A.L.F. Pharmacia, Uppsala, Sweden) and then purified from a PAGE gel after silver staining. DNA sequences were obtained using a PCR Fentomol Sequencing Kit (Promega, Madison, Wis.). Cycle sequencing was carried out using the same cycle conditions as the first PCR. The resultant PCR products were denatured and applied to a 6% PAGE DNA sequencing gel.

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Results

Nomenclature

Allelic designation was done according to the recommendations of the DNA Commission of the International Society for Forensic Haemogenetics (Bär et al. 1997). The allele designation is based on the number of repeats in the repeat unit and therefore it has been defined for (GATA)n, and eight different allelic groups were found with a total number of repeats ranging from $(GATA)_9$ to $(GATA)_{16}$.

Sequencing variation

The STR in the D18S535 locus was sequenced in 25 selected alleles, including at least 2 individuals from each allelic group (except for allele 16) and eight different alleles were found. The sequence composition of the D18S535 alleles is displayed in Table 1. No variation was found in the constant regions. D18S535 is a simple hypervariable STR consisting of blocks of (GATA) repeats with a basic sequence structure $(GATA)_1(GACA)_1(GATA)_1(GAT)_1(GATA)_{9–16}$.

Population data

A total of eight different allelic groups (GATA) were found in a total of 129 healthy unrelated individuals from Galicia (NW Spain). Allele and genotype frequencies are given in Table 2 and frequencies range from 0.0039 (alleles 16) to 0.330 (allele 13). The system is in Hardy-Weinberg equilibrium and the exact test (Guo and Thompson 1992) gave a *P* value of 0.59. The discrimination power (Fisher 1951) is 0.918% and the heterozygosity (Nei and Roychoudhury 1974) 0.80.

Table 1 Sequence composition of the D18S535 STR alleles

Allelic group	n°alleles sequenced	Sequence
9 (130 bp)	4	$(GATA)_9$
10 (134 bp)	2	$(GATA)_{10}$
11 (138 bp)	2	$(GATA)_{11}$
12 (142 bp)	4	$(GATA)_{12}$
13 (146 bp)	5	$(GATA)_{13}$
14 (150 bp)	4	$(GATA)_{14}$
15 (154 bp)	3	$(GATA)_{15}$
16 (158 bp)	1	$(GATA)_{16}$

Complete sequence: TCATGTGACAAAAGCCACACCCATAACTTTTTGCCTCTAGATAGACAGATAGAT $(GATA)_{9–16}$ TAGATTCTCTTTCTCTGCATTCTCATCTATATTTCTGTCT

Table 2 Allele frequencies of D18S535 in the Galician population (n:129)

Allele	Frequency	Allele	Frequency	Allele	Frequency
9	0.1063	12	0.1535	15	0.1496
10	0.0079	13	0.3307	16	0.0039
11	0.0150	14	0.2323		

Hardy-Weinberg equilibrium: Exact test: p = 0.59
Heterozygosity: 80% CE: 0.57 PD: 0.91

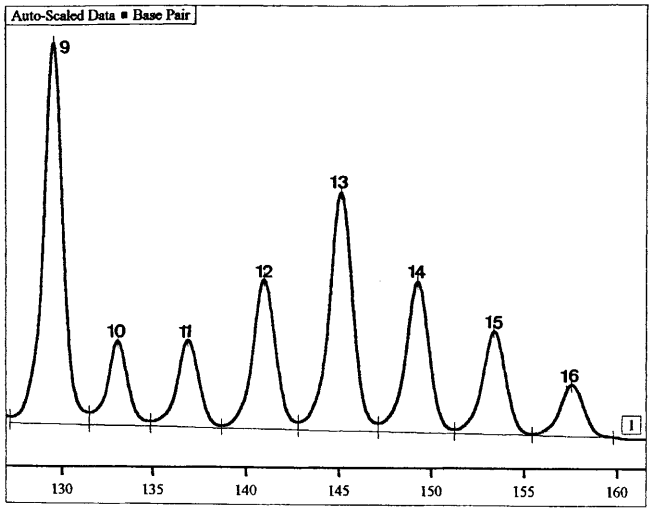

Fig. 1 Representation of D18S535 allelic ladder composed of 8 sequenced alleles. The allele designation is based on the number of (GATA) repeats

Additional information

An allelic ladder was constructed with the allele types 9, 10, 11, 12, 13, 14, 15 and 16 (Fig. 1) which is freely available from the authors.

The system seems to be robust and it has very low stutter characteristics. Stutter bands are in the range observed for other STRs used in forensic investigations.

In conclusion the STR at the D18S535 locus is a highly polymorphic STR with a simple structure. The characteristics of this system, including easy amplification, high heterozygosity, small size and sequence simplicity make this STR an interesting DNA polymorphisms for forensic purposes.

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