BRIEF COMMUNICATION

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Sequence Variation of New Alleles at the Short Tandem Repeat D19S253 Locus

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ABSTRACT: This paper reports the sequences of two new alleles identified in a population database study on the short tandem repeat D19S253 locus. A Portuguese Caucasian population and a Portuguese African population were studied. Forty-four selected alleles were sequenced and 11 different alleles were found. All the sequenced alleles shown to possess a simple tetranucleotide GATA repeat region structure. The two new alleles, alleles 6 and 16, follow the simple repeat pattern. During paternity investigation casework, 1028 meiosis were analyzed and five isolated genetic incompatibilities detected. In one case, a non-detectable allele with the used set of primers could be the explanation. In the other four cases, single-step mutations could be considered. The mutation rate obtained for this locus was 3.89×10^{-3}.

KEYWORDS: forensic science, DNA typing, short tandem repeat, paternity, D19S253, sequencing, mutation, new alleles

Short tandem repeat (STR) loci are highly polymorphic and sensitive markers for human identification and paternity investigation casework. Their accessibility to amplification using the polymerase chain reaction (PCR) has seen their increasing use in forensic identity testing.

One of these locus, D19S253 locus was first described by Weber et al. (1) and validated for forensic proposes by De Stefano et al. (2). This locus is a tetranucleotide STR with simple GATA repeats and initially nine alleles (alleles 7 to 15) were characterized. In this paper, two other alleles were observed and sequenced. Moreover, a Portuguese Caucasian population and a Portuguese African population were evaluated in order to obtain data concerning the application of D19S253 locus in paternity investigation.

Methods

A total of 973 healthy unrelated Portuguese Caucasian individuals, obtained from 737 routine paternity investigation cases, were studied. All individuals and their parents were natives in Portugal, predominantly from the South region. Moreover, 140 Portuguese African individuals (natives from Portuguese speaking African countries) were also studied.

Blood samples were collected and stored using UltraStain card (Fitzco). DNA was extracted by the Chelex method, previously described by Singer-Sam et al. (3).

PCR amplification conditions were carried out as described by De Stefano et al. (2) in a GeneAmp PCR System 9600 (Perkin-Elmer). Fragment separation was carried out on a 5.75% polyacrylamide denaturing DNA sequencing gel (Long Ranger Singel™ Packs, Type: Pharm, FMC) on an Automated Laser Fluorescent (ALF) DNA Sequencer (Pharmacia), and analyzed by AlleleLinks™ Version 1.00 software (Pharmacia, Biotech).

The distribution of allele frequencies was determined in the studied populations. Minimum allele frequencies were estimated according to the formula proposed by Budowle et al. (4). The potential usefulness of the studied locus for forensic studies in the Portuguese and African populations was assessed (5–7).

Gel purification of individual STR alleles, before DNA sequencing, was carried out by horizontal gel electrophoresis in 5.75% polyacrylamide gel stained with Silver Nitrate (Silver Sequence™ DNA Staining Reagents, Promega). DNA bands were excised and purified by using the "crush and soak" method, essentially as described by Sambrook et al. (8). An aliquot of the recovered PCR product was reamplified using the same conditions of the first round PCR. Amplified products were purified either by Centricom 100 filters (Amicon) or MicroSpin™ S-200 HR Columns (Pharmacia Biotech).

Solid phase sequencing of both strands was carried out using an ABI PRISM™ Dye Terminator Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS (PE Applied Biosystems). Unincorporated labeled terminators were removed with DYNAPURE™ Dye Terminator Removal (DYNAL®) according to manufacture's instructions.

DNA sequences were separated in a 36-cm well-to-read plate using an ABI PRISM 377 DNA Sequencer (PE Applied Biosystems) and 5% polyacrylamide denaturing sequencing gel (Long Ranger Singel™ Packs, Type: 377-36-cm WTR, FMC). Sequence data were analyzed automatically on the DNA Sequencer using the Data Collection software (PE Applied Biosystems).

Routinely, in our laboratory, several loci are studied in order to confirm paternity (9–12).

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Results and Discussion

A Portuguese Caucasian population and a Portuguese African population were evaluated to obtain data concerning the application of the D19S253 locus in paternity investigation. The observed allele frequency distributions and the forensic statistical parameters of the D19S253 locus for the two studied populations are listed in Table 1.

A total of 11 different alleles were found with repeats units (GATA) ranging from 6 to 16. Forty-four alleles of the D19S253 STR system were sequenced. As described in Table 2, at least two samples of each allele were sequenced. No variation was found in the flanking regions when compared with the D19S253 sequence structure described by De Stefano et al. (2). Two new allele structures were observed, $(GATA)_6$ and $(GATA)_{16}$. The allelic designation was based on the number of GATA repeat units according to the recommendations of the DNA Commission of the International Society for Forensic Haemogenetics (13)

TABLE 1—*Observed allele frequency distributions and forensic statistical parameters for D19S253 locus in the Portuguese Caucasian population (*N *= 973) and in the Portuguese African population (*N *= 140).*

Allele	Allele Freq.	Allele Freq.
6	…	0.0108
7	0.2467	0.2238
8	0.0396	0.0578
9	0.0118	0.0433
10	0.0247	0.0361
11	0.1182	0.1155
12	0.3119	0.2888
13	0.1814	0.1516
14	0.0570	0.0505
15	0.0087	0.0181
16	0.0005	0.0036
Minimum Allele Frequency	0.0031	0.0213
Heterozygosity	0.7897	0.8236
Probability of Exclusion	0.6175	0.6733
Probability of Discrimination	0.9257	0.9458

TABLE 2—*Sequence composition of the D19S253 STR alleles.*

Allele	No. of Sequenced Alleles	Sequence	Base Pairs (bp)
6	2	$(GATA)_6$	205
7	9	$(GATA)_7$	209
8	2	$(GATA)_8$	213
9	2	$(GATA)_9$	217
10	3	$(GATA)_{10}$	221
11	4	$(GATA)_{11}$	225
12	5	$(GATA)_{12}$	229
13	7	$(GATA)_{13}$	233
14	4	$(GATA)_{14}$	237
15	4	$(GATA)_{15}$	241
16	2	$(GATA)_{16}$	245

In paternity investigations studied, five isolated genetic incompatibilities in D19S253 locus were detected (Table 3). All alleles from these five incompatibility cases were sequenced. Paternity was confirmed by other loci and accepted with paternity probability higher than 99.99%, including mutations in the D19S253 locus.

According to Weber and Wong (14), the majority of mutations observed in STRs involved the gain or the loss of a single repeat unit. The observed incompatibilities were interpreted in this way. In all the analyzed cases, a simple repeat unit gain or loss could explain the observed incompatibilities. Moreover, our results confirm that gains are more frequent than losses as referred by Weber and Wong (14).

In the first case, a mother-child incompatibility was observed (Table 3). The mother and the child were homozygous for this locus. Probably, there is an allele not detected with the used set of primers. In the second and third case, two alleged father-child incompatibilities were observed with mutations leading to one repeat unit gain. In the two other cases, the origin of the mutation (mother or alleged father) could not be confirmed, although in one of these cases a gain of one repeat unit was detected. In the fifth case, three possibilities can explain the observed incompatibility. If the alleged father transmits the allele 15 to the child, a gain or a loss of one repeat unit concerning the mother allele could be possible. Conversely, if the mother transmits the allele 15, a loss of one repeat unit of the father allele could be the explanation.

Considering a total of 1028 maternal and paternal meiosis, the mutation rate for D19S253 locus is 3.89×10^{-3}. The mutation rate observed within the Portuguese African population (alleged father in Case 2 and alleged father in Case 4) is higher (7.35×10^{-3} or 1.47×10^{-2} depending on the origin of mutation in Case 4) than that observed in the Portuguese Caucasian population. In this last population, depending on the origin of mutation in Case 4, we have observed a mutation rate of 2.24×10^{-3} (mutation origin-alleged father allele) or 3.36×10^{-3} (mutation origin-mother allele).

Paternal mutation rate ranged from 4.35×10^{-3} to 8.71×10^{-3} depending on the origin of mutation in the cases studied (Table 3). These values are higher than those obtained for maternal mutations (0 to 3.5×10^{-3}) as described also by other authors (15).

In conclusion, this study confirms that D19S253 is a suitable locus for paternity investigation.

TABLE 3—*Observed D19S253 genetic incompatibilities in paternity investigation casework.*

Alleged Father	Mother	Child	Origin	Mutation	Gain/Loss	Observations
8–12	7–7	12–12	m	…	…	Allele probably not detected with the used set of primers
8–11	7–12	12–12	af	11⇒12	Gain	
12–14	13–14	13–15	af	14⇒15	Gain	
7–13	7–13	7–14	m/af	13⇒14	Gain	
7–15	13–15	14–15	m	13⇒14	Gain	
			m/af	15⇒14	Loss	

NOTE: m: mother, af: alleged father.

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