ADDITIONAL VARIABILITY AT THE D21S11 LOCUS: SEQUENCING EVIDENCE OF A NEW ALLELE D21S11*33.1

Anita DETTLAFF-KĄKOL[1], Ryszard PAWŁOWSKI[1, 2]

[1] *Department of Forensic Medicine, Medical University of Gdańsk, Gdańsk, Poland*

[2] *Institute of Forensic Research, Cracow, Poland*

ABSTRACT: Analysis of short tandem repeats (STR) is rapidly growing method of individual identification in forensic testing. The highly polymorphic STR locus D21S11 was investigated in a Polish population sample by PCR amplification using commercially available AmpFISTR Profiler Plus Kit (Perkin Elmer) and capillary electrophoresis on ABI Prism 310 Genetic Analyzer. For fragment sizing, PCR amplification product and allelic ladders were run with an internal size standard ILS400 labelled with CXR dye. During routine testing of reference samples a new variant of allele 33 was found. GeneScan™ analysis showed the presence of off-ladder allele (218.53 bp) exactly one nucleotide shorter than allele 33.2 (219.60 bp). The variant allele was sequenced in both directions and the following repetitive sequence was found: $(TCTA)_5$ $(TCTG)_6$-CR-$(TCTA)_{13}$-A-TCTA. According to the ISFG recommendations the allele was designated 33.1. Frequencies of D21S11 alleles in a population sample of over 900 unrelated individuals originating from Polish population are presented.

KEY WORDS: D21S11; Short tandem repeats; DNA polymorphism; Sequence structure.

Problems of Forensic Sciences, vol. XLVII, 2001, 314–321
Received 22 December 2000; accepted 15 September 2001

INTRODUCTION

Short tandem repeat loci (STR, microsatelites) are higly polymorphic regions of the human DNA consisting of tandemly repeated sequences of 2–6 base pairs which can easily be analysed using the polymerase chain reaction (PCR).

STR systems are valuable markers which have become widely used in human identification, particularly in criminal cases and mass disasters and paternity testing. One of the very useful STR systems is D21S11 locus which is used by many laboratories worldwide.

STR loci are divided into 3 categories on the basis of their variable sequence: (1) simple repeats, (2) compound repeats and (3) complex repeats. Because of the high degree of polymorphism and structural variants D21S11 falls into the third category.

D21S11 system is located on chromosome 21 at position q11.2–q21 with the basic sequence of $(TCTA)_{4-6}$ $(TCTG)_{5-6}$ $(TCTA)_3$ TA $(TCTA)_3$ TCA $(TCTA)_2$ TCCA TA $(TCTA)_n$. This structure is composed of three variable units: the first $(TCTA)_{4-6,}$ the second $(TCTG)_{5-6,}$ and the last $(TCTA)_{n,}$ and the internal constant region $(TCTA)_3$ TA $(TCTA)_3$ TCA $(TCTA)_2$ TCCA TA. The typing of STR loci is faciliated by the ability to amplify several loci simultaneously in a multiplex PCR using commercially available kits. One of them, Profiler Plus™ (Perkin Elmer) enable co-amplification of the amelogenin locus and nine STR loci: D3S1358, VWA, FGA, D5S818, D13S317, D7S820, D8S1179, D21S11 and D18S51 which are subsequently typed using capillary electrophoresis (ABI Prism 310 Genetic Analyzer, Perkin Elmer).

During routine testing of references samples we have found a new variant of allele 33 at the D21S11 locus. The distribution of alleles and genotype frequencies of D21S11 in the population from northern Poland are also presented.

MATERIALS AND METHODS

DNA samples preparation

Blood samples were obtained from 911 unrelated persons of both sexes living in Poland. DNA was extracted by phenol-chloroform method.

The amount of DNA was quantified by slot blot hibridization using a primate-specific alpha satelite probe, D17Z1 (QuantiBlot Kit, Perkin Elmer, USA) with chemiluminescences detection.

PCR amplification and detection with capillary electrophoresis

Amplification of ten loci included in the Profiler Plus™ Kit (Perkin Elmer, USA) was carried out in accordance with manufacturer's instructions on 2400 Thermal Cycler (Perkin Elmer, USA) or 877 Robotic Station (Perkin Elmer, USA).

Detection of PCR products using capillary electrophoresis was carried out on the ABI Prism 310 Genetic Analyzer (Perking Elmer, USA). From the PCR product 1 μl was mixed with 12 μl deionised formamide and 0.3 μl of internal size standard ILS400 labeled with CXR dye. Before electrophoresis the samples were denatured for 3 min at 95°C and subsequently snap-cooled on ice. The samples were run on a capillary 47 cm in length filled with the de-

naturing polymer POP4. The separation was conducted for 24 min at 15 kV, 9 mA and 10 μW. The analysis of the fragments was carried out using the computer program GeneScan v.2.1.

Sequencing analysis

After fragments size determination genomic DNA was again amplified by PCR with unlabelled PCR primers. The PCR product was electrophoresed in 6% nondenaturing gel for 3 h. Bands were visualized by silver staining. The stained bands were cut out from the gel and eluted with 50 μl steriled water (HPLC Water, Backer) at 56°C overnight. Aliquots of 5–10 μl eluate were reamplified and the reamplified product purified twice over Centricon 100 (Amicon) spin column. The variant allele was sequenced in both directions using unlabelled D21S11 primers. Sequencing using the BigDye Terminator Sequencing Kit (Perkin Elmer, USA) was performed in 20 μl containing 3.2 pmol of forward or reverse primer according to the manufacturer's instruction. Sequencing products were purified by absolute ethanol and 3M sodium acetate (pH 5.8) precipitation. Electrophoresis of the sequencing products was carried out on ABI 310 Genetic Analyzer. The obtained data were analysed using ABI Prism Sequencing Analysis Software v.2.1 (Perkin Elmer).

Statistical calculations

Statistical analysis of the results was performed using the following tests. The relative frequency of each allelic class was obtained from the allele counts. Test χ^2 was used in order to define the compatibility of the frequency of the phenotypes with the Hardy-Weinberg equation. The exact test, LR test, power discrimination (PD) and the polymorphic information content (PIC) were calculated as described earlyer.

RESULTS AND DISCUSSION

Distribution of D21S11 alleles in the Polish population sample

The highly polymorphic STR D21S11 locus was investigated in the Polish population sample by PCR-amplification.

The population sample included 911 randomly chosen unrelated people, among whom there was detected the presence of 15 alleles forming 64 out of 120 theoretically possible phenotypes (Figures 1 and 2). Among the observed alleles there were detected 7 interalleles, differing by 1 or 2 bp from the alleles with exclusively tetranucleotide repetitive units.

Figure 1 and 2 show the allele and phenotype frequencies distribution for D21S11 locus observed for 911 unrelated individuals. In the analysed population sample 56 heterozygotes and 8 homozygotes were observed.

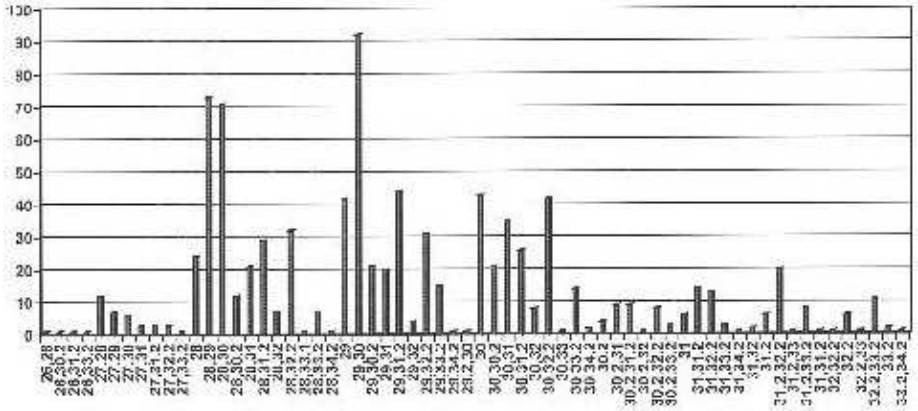

Fig. 1. Frequencies of D21S11 genotypes in Polish population samples.

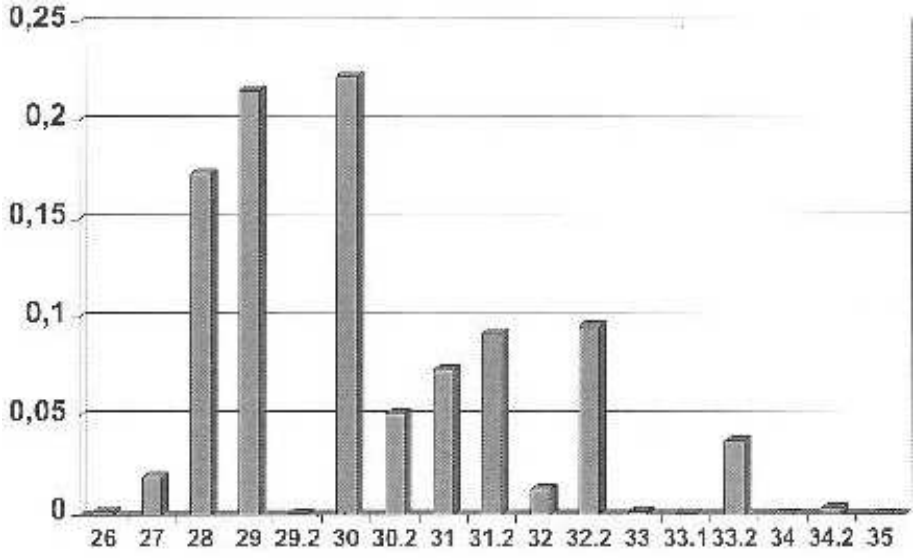

Fig. 2. Allele frequencies distribution for D21S11 locus in Polish population samples.

The most frequently occurring phenotypes were 29/30 (10.1%), 28/29 (8%) and 28/30 (7.8%) (Table I).

TABLE I. FREQUENCIES OF D21S11 PHENOTYPES IN POPULATION SAMPLES FROM POLAND (N = 911)

Genotype	Number observed	Genotype	Number observed	Genotype	Number observed	Genotype	Number observed
26 / 28	1	30 / 32	8	29 / 30.2	21	30.2 / 33.2	3
27 / 28	12	30 / 33	1	29 / 31.2	44	31 / 31.2	14
27 / 29	7	31 / 31	6	29 / 32.2	3	31 / 32.2	13
27 / 30	6	31 / 32	2	29 / 33.2	15	31 / 33.2	3
27 / 31	3	26 / 30.2	1	29 / 34.2	1	31 / 34.2	1
28 / 28	24	26 / 31.2	1	29.2 / 30	1	31.2 / 31.2	6
28 / 29	73	26 / 33.2	1	30 / 30.2	21	31.2 / 32.2	20
28 / 30	71	27 / 31.2	3	30 / 31.2	26	31.2 / 33	1
28 / 31	21	27 / 32.2	3	30 / 32.2	42	31.2 / 33.2	8
28 / 32	7	27 / 33.2	1	30 / 33.2	14	31 / 31.2	1
29 / 29	42	28 / 30.2	12	30 / 34.2	2	32 / 32.2	1
29 / 30	92	28 / 31.2	29	30.2 / 30.2	4	32.2 / 32.2	6
29 / 31	20	28 / 32.2	32	30.2 / 31	9	32.2 / 33	1
29 / 32	4	28 / 33.1	1	30.2 / 31.2	9	32.2 / 33.2	11
30 / 30	43	28 / 33.2	7	30.3 / 32	1	33.2 / 33.2	2
30 / 31	35	28 / 34.2	1	30.2 / 32.2	8	33.2 / 34.2	1

The most frequent alleles in the sample tested were 30 (22.2%), 29 (21.5%) and 28 (17.3%) (Table II). The rarest of the observed alleles were the alleles 26, 32 and 33 and interalleles 29.2, and 34.2, which appear very rarely in Europe. The allele 34 was not observed in other populations. During population studies one new allele with an electrophoretic mobility between 33 a 33.2 alleles was observed.

Analysed locus meets the Hardy-Weinberg equilibrium (HWE) using likelihood ratio, LR test (0.980) and the exact test (0.977). D21S11 is characterized by high heterozygosity ($H_{obs.}$ = 0.860) in accordance with the value of the calculated expected heterozygosity. The high power of discrimination (PD = 0.958) and PIC value (0.831) for D21S11 locus in the Polish population sample was observed. All these parameters indicate the very high usefulness of this system in identification tests.

TABLE II. OBSERVED FREQUENCIES AND NUMBERS OF D21S11 SYSTEM ALLELES IN POLISH POPULATION

Allele	Frequency observed / Number observed
26	0.0022 / 4
27	0.0192 / 35
28	0.1729 / 315
29	0.2151 / 392
29.2	0.0006 / 1
30	0.2223 / 405
30.2	0.051 / 93
31	0.0735 / 134
31.2	0.0922 / 168
32	0.0126 / 23
32.2	0.0955 / 174
33	0.0016 / 3
33.1	0.0006 / 1
33.2	0.03732 / 68
34	Not observed
34.2	0.0033 / 6

DNA sequencing and designation of a new allele D21S11*33.1

During routine testing of references samples a new variant of allele 33 was found. GeneScan™ analysis showed the presence off-ladder allele

Fig. 3. Electropherogram presenting the separarion of D21S11 locus products run under denaturating conditions using ABI Prism 310 Genetic Analyzer.

(218,53 bp) exactly one nucleotide longer than 33 allele (217,62 bp) and one nucleotide shorter than allele 33.2 (219,60 bp) (Figure3).

This allele was sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit. As result of the sequencing the presence of the following sequence was confirmed: $(TCTA)_5$ $(TCTG)_6$ CR $(TCTA)_{13}$ A TCTA, when CR (Control Region): $(TCTA)_3$ TA $(TCTA)_3$ TCA $(TCTA)_2$ TCCA TA.

The new allele of 218 bp was found by sequence analysis to have one extra base, A, inserted in front of the last TCTA unit of type 33. According to the Moeller nomenclature the allele was named D21S11* 33.1. Till now this allele was not described in any population. The sequence analysis data are shown in Figure 4.

Fig. 4. Sequence analysis of D21S11 * 33.1 allele.

CONCLUSIONS

1. The multiplex STR system (Profiler Plus) provides a rapid and precise tool for forensic identity, paternity and population studies.
2. The D21S11 locus is very polymorphic in the Polish population.
3. The presence of the new allele not observed till now D21S11*33.1 was proved by sequencing analysis.

References:

1. Brinkmann B., Meyer E., Junge A., Complex mutational events at the Hum D21S11 locus, *Human Genetics* 1996, vol. 98, pp. 60–64.
2. Buscemi L, Tagliabracci A., Sassaroli C. [et al.], Polymerase chain reaction typing of D21S11 short tandem repeat polymorphism by capillary electrophoresis. Allele frequencies and sequencing data in a population sample from central Italy, *Forensic Science International* 1998, vol. 92, pp. 251–258.
3. Entrala C., Lorente M., Lorente J. [et al.], Fluorescent multiplex analysis of nine STR loci: Spanish population data, *Forensic Science International* 1998, vol. 98, pp. 179–183.
4. Klintschar M., Al-Hammadi N., Reichenpfader B., Population genetic studies on the tetrameric short tandem repeat loci D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818 and D7S820 in Egypt, *Forensic Science International* 1999, vol. 104, pp. 23–31.
5. Möller A., Meyer E., Brinkmann B., Different types of structural variation in STRs: HumFES/FPS, HumVWA and Hum D21S11, *International Journal of Legal Medicine* 1994, vol. 106, pp. 319–323.
6. Zhou H., Sato K., Nishimaki Y., [et al.], The HumD21S11 system of short tandem repeat DNA polymorphisms in Japanese and Chinese, *Forensic Science International* 1997, vol. 86, pp. 109–118.
7. Xiao F., Gilissen A., Cassiman J. [et al.], Quadruplex fluorescent STR typing system (HUMVWA, HUMTH01, D21S11 and HPRT) with sequence-defined allelic ladders. Identification of a new allele at D21S11, *Forensic Science International* 1998, vol. 94, pp. 39–46.