Int J Legal Med (2005) 119: 164–166
DOI 10.1007/s00414-004-0508-6

SHORT COMMUNICATION

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Unusual variant alleles in commonly used short tandem repeat loci

Received: 15 July 2004 / Accepted: 2 November 2004 / Published online: 5 January 2005

Abstract Unusually large variant alleles were observed in the short tandem repeat (STR) systems D3S1358 and D21S11, both of which are included in the international standard set of loci (ISSOL) and routinely typed in National DNA intelligence databases worldwide. The observed alleles fell within the size range of the adjacent STR marker, which could easily cause problems with respect to correct allele assignments for both loci concerned. We compared the amplification and potential interpretation with three different commercially available kits, which are frequently used in forensic work. PCR products were cloned and sequenced in order to determine the structure of these unusual allele variants and confirm their size and designation (D3S1358 allele 26, D21S11 allele 46). In the locus D21S11 we observed an as yet undescribed partial duplication of the constant region.

Keywords D3S1358 · D21S11 · STR · International standard set of loci · Core loci · DNA intelligence databasing · Forensic

Introduction

Unexpected large STR alleles have been described for highly variable STRs such as SE33 [1], which included variants that fell outside the expected locus size range and therefore posed the problem of not being detected due to decreased amplification efficiency or misinterpretation of the signal. The large allele variants observed in this study, i.e. single events in the Austrian DNA intelligence database currently consisting of about 70,000 STR profiles, refer to markers with low (D3S1358) and medium (D21S11) sized fragment lengths. In order to compare the performance and discuss consequences for DNA intelligence databases, we typed these variants with commercial kits from three different companies (i.e. Applied Biosystems [2], Promega Corporation [3] and Serac [4]).

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Material and methods

The STR loci D3S1358 and D21S11 were amplified from buccal scrape samples from the Austrian DNA intelligence database (Chelex extraction [5]) using three different kits—the AmpFlSTR SGM Plus PCR amplification kit (Applied Biosystems), the GenePrint PowerplexTM 16 system (Promega Corporation) and the *genRES* MPX-2 multiplex-kit (Serac)—according to the manufacturers' instructions. PCR was performed on a Gene Amp PCR System 9600 (Perkin Elmer, Norwalk, CT, USA), electrophoresis on an ABI PRISM 310 and 3100 Genetic Analyzer using POP 4 and default conditions. All data were analysed using GeneScan Analysis (versions 2.1 and 3.7, AB) and Genotyper (version 3.6, AB).

Cloning and sequencing of the alleles

For the cloning experiments the variant alleles were amplified in single-plex reactions using unlabelled primers: D3S1358/f 5'-ACTGCAGTCCAATCTGGGT-3', D3S1358/r 5'-ATGA AATCAACAGAGGCTTG-3', D21S11/f 5'-ATATGTGAG TCAATTCCCCAAG-3' and D21S11/R 5'-GGTAGATAG ACTGGATAGATAGACGA-3' [6]. The cloning and sequencing of the cloned amplicons (D3S1358 n=8, D21S11 n=16) was performed as detailed in [7].

Results and discussion

In the AmpF*l*STR SGM Plus PCR amplification kit the marker D3S1358 is located in a category size range 114–142 base pairs (bp). Sample A displayed one allele 15

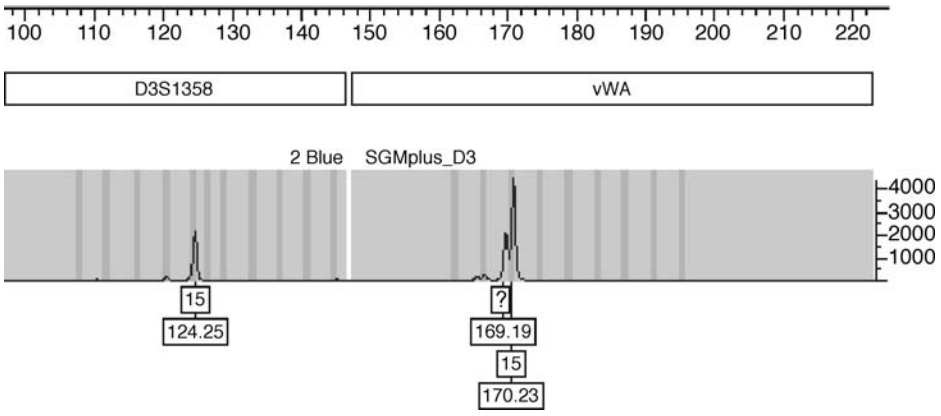

Fig. 1 AmpF*l*STR SGM Plus (Applied Biosystems) electropherogram of sample A (5-FAM/blue panel, only categories D3S1358 and vWA)

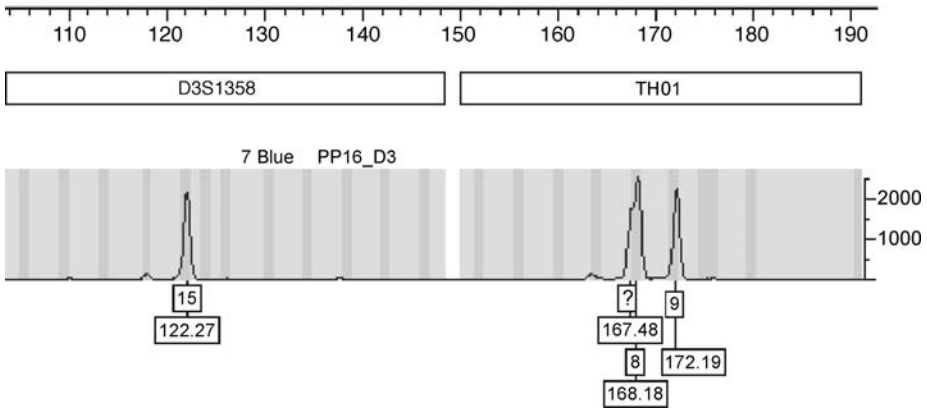

Fig. 2 Powerplex 16 System (Promega Corporation) electropherogram of sample A (FL/blue panel, only categories D3S1358 and TH01)

(124.25 bp) within the category, whereas the long heterozygote (169.19 bp) was shifted into the neighbouring category vWA (Fig. 1). vWA displayed a homozygote allele 15 at 170.23 bp, almost masking the long D3 allele, which could potentially be misinterpreted as a n-variant of the 15 allele, in which non-template dNTP addition was not completed for all labelled strands by the polymerase [8]. Another wrong, albeit less likely, interpretation of the 169 bp peak could be the designation as a heterozygote 14.3 variant of vWA allele 15. In the Powerplex 16 system the locus D3S1358 nearly displays the same size range (115–147 bp) as described above, here located next to TH01, (Fig. 2). The long allele of D3S1358 (167.48 bp) was partially masked by TH01 allele 8 (168.18 bp). In this constellation, the long D3S1358 allele is likely to be overseen, especially if the analyst lacks experience and/or performs superficial analyses. Only by careful individual locus inspection can the slightly broadened TH01 peak come to the attention of the analyst. The *genRES* MPX-2 multiplex-kit displays the locus D3S1358 in the yellow panel (NED-labelled) within a size range 105–142 bp. The marker next to D3S1358 is FGA with a size range of 152–205 bp. The long D3S1358 allele (168.39 bp) produced an off-ladder allele approximately 5 bp next to the homozygote FGA 21 allele (173.73 bp). In this case the balanced heights of the 2 D3S1358 alleles simplified their identification, but a mis-assignment as a FGA off-ladder variant is still possible.

Sequence analysis from cloned single-plex amplicons revealed the typical sequence structure at the locus D3S1358 [9] (Table 1). The large allele variant consisted of $(AGAT)_{22}$ and $(AGAC)_4$ repeat units confirming allele 26.

A similar situation was observed in D21S11, which is located in the size range 187–243 bp in the AmpF*l*STR SGM Plus kit. Sample B showed one allele 29 (205.08 bp) in category D21, the adjacent category D18S51 displayed a 3-peak pattern including the alleles 18 (307.66 bp), 20 (315.93 bp), and an off-ladder peak (272.67 bp), which could be misinterpreted as a 9.1 allele. In the Powerplex 16 system the locus D21S11 is located similarly as described above, and the same situation applies. In the *genRES* MPX-2 multiplex-kit the locus D21S11 ranges from 203 to 245 bp. Here, D21S11 is adjacent to the locus D8S1179, which displayed the 3-peak pattern. In this case, the long D21S11 allele (283.53 bp) fell between the 2 authentic D8S1179 alleles 11 (269.12 bp) and 16 (289.31 bp, Fig. 3).

Table 1 Allele designation, repeat structure andlength of the two sequenced D3S1358 alleles (sample A)

Allele designation	Repeat region	Length (bp)
15	$(AGAT)_{11}$ $(AGAC)_2$ $(AGAT)_2$	127
26	$(AGAT)_{11}$ $(AGAC)_2$ $(AGAT)_9$ $(AGAC)_2$ $(AGAT)_2$	171

Flankingregions not shown.

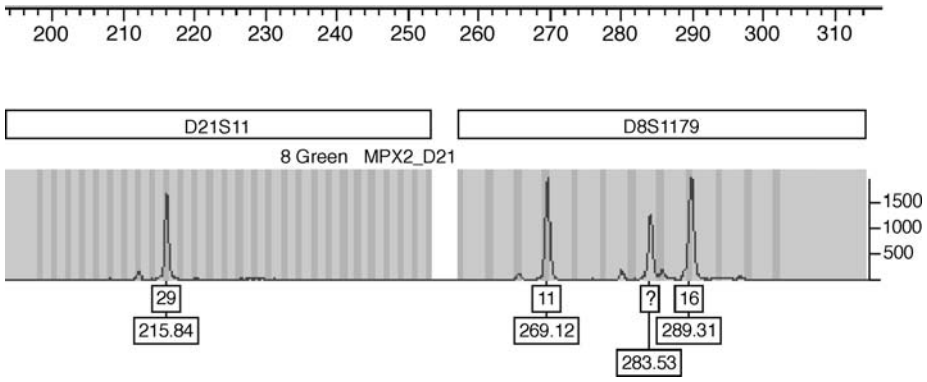

Fig. 3 *genRES* MPX-2 (Serac) electropherogram of sample B (HEX/green panel, only categories D21S11 and D8S1159)

Table 2 Allele designation, repeat structureand length of the two sequenced D21S11 alleles (sample B)

Allele designation	Repeat region	Length (bp)
29	$(TCTA)_4$ $(TCTG)_6$**$(TCTA)_3$ TA $(TCTA)_3$ TCA $(TCTA)_2$ TCCA TA $(TCTA)_8$** $(TCTA)_3$	185
46	$(TCTA)_5$ $(TCTG)_6$**$(TCTA)_3$TA $(TCTA)_3$ TCA $(TCTA)_2$TCCA TA $(TCTA)_8$** *TCA $(TCTA)_2$ TCCA TA* $(TCTA)_{13}$ TA TCTA	252

Bold Constantregion.
Bold Duplicated part of constant region.
Flankingregions not shown.

Sequence analysis of the cloned single-plex amplicons from sample B revealed that a part of the constant region (17 bp) was duplicated in the long allele (Table 2). Due to this duplication event a repeat-based nomenclature would assign this allele as 43.2 [10], whereas it is displayed as allele 46 with respect to the electrophoretic separation. Similarly, variation in the constant region of D21S11 was described by Walsh et al. [11] and Brinkmann et al. [12, 13], where a 14 bp deletion was observed.

Conclusions

It is important to be aware of unusual variants of alleles, especially if samples are typed for DNA intelligence databases. It should be stressed that a careful analysis of data is fundamental to avoid misinterpretation of unusual and therefore unexpected alleles. If such an allele is suspected, alternative multiplex kits with different adjoining markers or a single-plex PCR amplification for the particular marker may help to determine its specific fragment length and consequently assign the alleles in a correct way.

Acknowledgements This study was partially supported by a grant from the Österreichische Nationalbank, Jubiläumsfondprojekt Nr.: S97/4 (Austrian National Bank).

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