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TECHNICAL NOTE

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Characterization of the Variant Allele 9.2 of Penta D Locus*

ABSTRACT: DNA profiles of forensic cases of Córdoba Province, Argentina, typed by PowerPlex® 16 kit (Promega), have shown in the Penta D locus few samples with a variant allele migrating as an off ladder between alleles 9 and 10. In order to determine the molecular basis of the new variant allele, three samples were subject to polymerase chain reaction amplification of the Penta D locus by monoplex, and were further purified and sequenced. The sequence analysis revealed that the off ladder allele has ten repeats motifs AAAGA as allele 10, with three nucleotides (TAA) deletion in the 3′ flanking region, 128 nucleotides after the last repeat. Therefore, the variant allele could be explained by a deletion of allele 10, and was designated 9.2. Mse I digestion assay allows to corroborate allele 9.2 without sequencing. A population study in Córdoba Province indicates that allele 9.2 of Penta D locus has a frequency of 0.0063.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, Powerplex 16, Penta D, variant allele

The short tandem repeats (STRs) polymorphisms, generated by repetitions of 2–7 bp units (1), are extensively used for linkage testing and human identification in forensic cases. There are commercial kits that by a multiplex polymerase chain reaction (PCR) and capillary electrophoresis analysis, allow the simultaneous detection of several STRs loci. The PowerPlex® 16 System (Promega) kit amplified 15 STRs, included Penta D locus. Penta D is a pentanucleotide tandem repeat marker located on chromosome 21, containing an AAAGA repeat motif. The known alleles of this locus are 2.2, 3.2, 5, and 7 to 17 according to the number of repeats, with a size range from 376 to 449 bp. These alleles have a frequency >0.001 (2).

Several reports have shown and characterized variant alleles in the STR systems, such as one base shorter alleles in the Penta E locus, caused by a partial repeat motif (3), interalleles in the HumD21S11 locus with a TA insertion in the variable region 3 (4), and dinucleotide insertion within the 3′ flanking region of D18S51 locus (5).

In the present study, a variant allele of Penta D locus, typed with the PowerPlex® 16 kit, that migrates by capillary electrophoresis as an off ladder between alleles 9 and 10, was detected in few individuals of Córdoba Province of Argentina. Even though there are other Penta D variants reported in the NIST Standard Reference Database 130, STR DNA Internet database (http://www.cstl.nist.gov/div831/strbase), at present, no sequence data for the Penta D variants allele are available. Therefore, the goal of the current study was to characterize at molecular level the new variant allele besides determining the frequency in Córdoba population of Argentina.

Materials and Methods

Samples

Ethylene diamine tetracetic acid (EDTA) whole blood samples were collected from 631 unrelated individuals from Córdoba Province of Argentina. This population includes 250 individuals from a previous study done in the laboratory (6). These individuals were randomly selected from paternity and criminal cases, including both urban and countryside population.

DNA Extraction

Genomic DNA was purified from blood leukocytes using CTAB (*N*-cetyl-*N*,*N*,*N*-trimethyl-ammonium bromide) procedure as was previously described by Corach et al. (7).

PCR STR Amplification

The sixteen DNA loci of the kit GenePrint PowerPlex® 16 System (Promega, Madison, WI) including Penta D locus, were simultaneously amplified according to the manufacturer's instructions (2). PCR amplifications were performed in a GeneAmp PCR System 9600 or 9700 (Perkin Elmer Corporation, Norwalk, CT).

STR Typing

Samples amplified by PowerPlex® 16 kit were analyzed using the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Perkin-Elmer, Foster City, CA) according to the manufacturer's recommendations (8) using the separation medium Performance Optimized Polymer (POP) 4 (PE Applied Biosystems). The allele assignment was made using the GeneScan® analysis software (Ver. 3.1) and

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the Genotyper® software (Ver. 2.5) (Applied Biosystems) by size comparison with allelic ladders from Promegas kits.

Sequence Analysis

Penta D locus was amplified by monoplex PCR using the primers published by Krenke B. E. et al. (9), in a total volume of 30 μL containing 1× GoTaq™ Reaction Buffer (Promega Corporation), 200 μM each dNTP (Promega Corporation), 0.8 μM each of the unlabeled Penta D primers (Invitrogen Life Technologies, Carlsbad, CA), 1.5 mM MgCl and 1.5 U GoTaq™ DNA Polymerase (Promega Corporation). The amplification cycling conditions were as described in the PowerPlex® 16 System technical manual (2). The PCR products of alleles 9 and 10, from homozygous individuals, were directly purified with the Wizard® PCR preps DNA purification system (Promega Corporation), following the manufacturer's recommendations (10). For the off ladder allele, the PCR products from three different heterozygous individuals (two of them with the variant allele and allele 12, and the third one with the variant allele and allele 16), were run in a nondenaturing 5% polyacrylamide gel electrophoresis in TAE Buffer (0.04 M Tris-Acetate, 0.001 M EDTA, pH 8.5). The bands corresponding to the off ladder allele, were cut from the gel, electro eluted into dialysis bags (11), and further purified with the Wizard® purification system using the corresponding protocol (10). The purified fragments were sequenced using the BigDye™ terminator cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions (12). Sequencing products were purified by ethanol precipitation and the capillary electrophoresis was carried out on an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems, Perkin-Elmer) using the separation medium Performance Optimized Polymer (POP) 6 (PE Applied Biosystems), according to the manufacturer's recommendations (8). The data analysis was made using the Sequencing Analysis (Ver. 3.3) software (Applied Biosystems).

Statistical Analysis

Allele frequencies for Penta D locus were calculated from the observed genotypes in the population sample, by standard counting procedure and using the Cervus 2.0 program (13). The observed and expected heterozygosities and the Polymorphic Information Content were determined by the Cervus 2.0 program (13). Possible divergence from HWE was tested either by chi-square or Exact test with the TFPGA program (Tools for Population Genetic Analyses version 1.3, Miller MP, 1997).

Endonuclease MseI Digestion of the PCR Products

PCR products of the Penta D locus were directly digested with endonuclease Mse I (BioLabs New England, Beverly, MA) according to the manufacturer's recommendations. The digestion products were analyzed by nondenaturating 15% polyacrylamide gel electrophoresis in TBE Buffer (0.09 M Tris-borate, 0.002 M EDTA pH 8) and compared with the 10 bp DNA Step Ladder (Promega Corporation). The fragments were visualized by silver staining. Briefly, the gel was soaked in the following conditions: 5 min of the fix/stop solution (10% ethanol, 0.5% acetic acid), 10 min of the staining solution (0.2% silver nitrate, 10% ethanol, 0.5% acetic acid), 10 sec of deionized water, developing solution (0.074% formaldehyde, 3% sodium hydroxide) until bands are visible, 5 min of fix/stop solution and 15 min of distillated water.

Results and Discussion

DNA profiles typed by the PowerPlex® 16 kit, both from linkage testing and forensic human identification cases in Córdoba Province of Argentina, have shown in Penta D locus few samples with an off ladder allele migrating between alleles 9 and 10 (Fig. 1). This off ladder allele migrates in the ABI 310 genetic analyzer as a fragment of *c.* 401.65 bp. To rule out that this migration was due to a technical artifact, DNA proceeding from blood and semen of the same person carrying the off ladder allele were purified by classical extraction method (7) or with Microcon-100 (14), respectively. The DNAs were further submitted to dissimilar PCR conditions (template amount and nucleotides concentrations), and electrophoresis settings (voltage and injection time). The same capillary electrophoretic pattern was obtained in all settings (data not shown), confirming the presence of a new variant allele in the Penta D locus.

In order to investigate the molecular basis of the new variant, alleles 9, 10 and the off ladder allele were amplified by monoplex PCR with the same primers used in the Powerplex 16 kit, and the PCR products were purified and sequenced as described in Material and Methods. The nucleotide sequences of the variant allele, obtained from three nonrelated persons, showed ten AAAGA repeats motif as allele 10 (data not shown), with a deletion of the nucleotides TAA at 128 nucleotides downstream of the repeat region (Fig. 2). This result could be interpreted as the off ladder allele was generated by a deletion of allele 10. Following ISFG (International Society of Forensic Genetic) recommendations (15) the variant allele of 411 bp was designated as 9.2. The discrepancy with the *c.* 401.65 bp assigned in the capillary electrophoresis (Fig. 1), could be due to dissimilar migration resulting from sequence differences between the alleles fragments and those of the internal size standard used in the electrophoresis. Dissimilar migration is also observed for all alleles of the Penta D locus (2). The 9.2 variant is different at molecular level than alleles 2.2 and 3.2, as these ones have deletions of 13 and 8 bp respectively, upstream of the five AAAGA repeats (16).

With the purpose of having a simple and fast technique to check if an off ladder allele migrating between allele 9 and 10 is effectively the variant 9.2, an enzymatic digestion with endonuclease

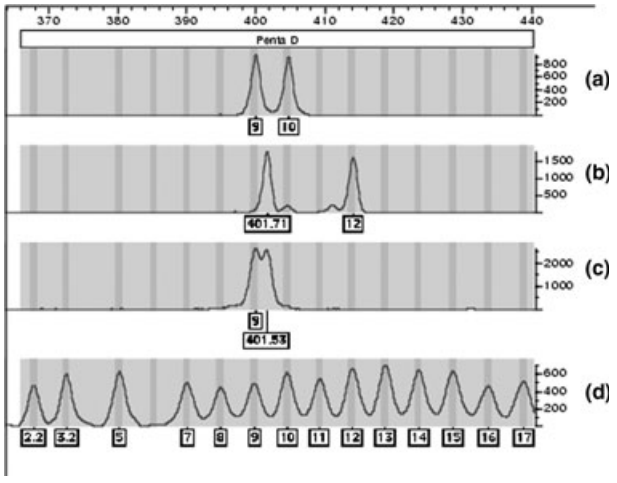

FIG. 1—*PowerPlex® 16 electropherogram of the Penta D locus. (a) Sample with alleles 9 and 10. (b) Sample with the off ladder allele and allele 12. (c) Sample with allele 9 and the off ladder allele. (d) Allelic ladder. The value showed in each box is either the allele name or the base pairs size.*

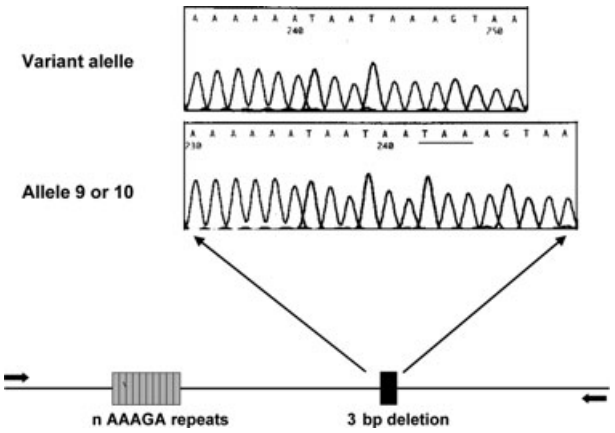

FIG. 2—*Sequence of the PCR products of Penta D locus. Upper panel: sequence electrophenograms of alleles 9 or 10, and the variant allele, in the region embracing the deletion. Lower panel: schematic representation of Penta D locus indicating the region with the deletion.*

Mse I was assayed as described in Materials and Methods. The restriction pattern for the known Penta D alleles, should have DNA fragments of 35, 110, and 14 bp for alleles 2.2, 3.2, 5, and 7 to 17, beside a fragment ranged from 217 to 290 bp according to the number of repeats (Fig. 3, upper panel), this was confirmed with alleles 9, 10, 11, 12, and 16 (data not shown). Allele 9.2 should have the same DNA fragments than allele 10, except in the deletion region where should have a 32 bp fragment instead the 35 bp fragment. This was confirmed by polyacryalmide gel electrophoresis of the restriction fragments from the Mse I digestion corresponding to the deletion region (Fig. 3). Sample with allele 9 (lane e), shows the band that corresponds to the 35 bp fragment. The faster migration of this fragment could be due to dissimilar migration resulting from sequence difference between the size ladder and the restriction fragment as it was described for the capillary electrophoresis. The same result is obtained with allele 10 (not shown). On the other hand, eight heterozygous samples carrying the off ladder allele showed two bands, one corresponding to the nonvariant allele and the other with a lower molecular size corresponds to the 32 bp fragment of allele 9.2. Four of those heterozygous samples are showed in lanes a, b, c, and d of Fig. 3. Consequently Mse I digestion assay can be an easy and fast method to corroborate allele 9.2.

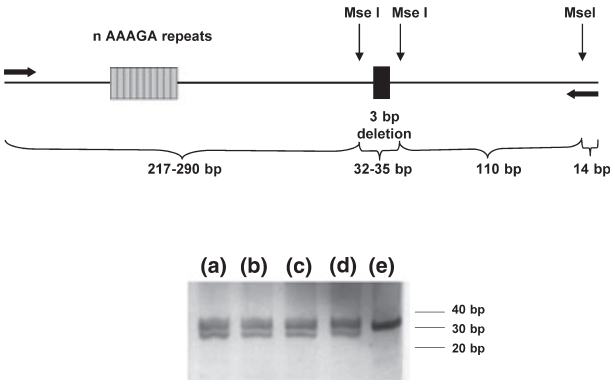

FIG. 3—*Digestion of the PCR products of Penta D locus with Mse I restriction enzyme. Upper panel: scheme of Mse I restriction sites in the locus Penta D. Lower panel: electrophoresis in polyacrylamide gels after Mse I digestion, showing the fragments originated in the region embracing the deletion in individuals with different genotypes: (a) 9,9.2; (b) 9.2,11; (c) 9.2,12; (d) 9.2,16; and (e) 9. The arrows indicate the position in the gel of the 10 bp DNA Step Ladder fragments.*

TABLE 1—*STR allele frequencies data and statistical parameters of Penta D locus for Córdoba (Argentina) population.*

Allele	Frequency
2.2	0.0040
5	0.0016
7	0.0040
8	0.0111
9	0.2076
9.2	0.0063
10	0.1957
11	0.1704
12	0.1632
13	0.1513
14	0.0626
15	0.0174
16	0.0047
Hob	81.14
Hex	83.62
PIC	0.814
P*	0.0328
P†	0.0190

Hob, observed heterozigosity; Hex, expected heterozigosity; PIC, polymorphic information content.
*Hardy–Weinberg equilibrium, chi-square test.
†Hardy–Weinberg equilibrium, exact test based on 10,000 permutations.

A population study of Penta D locus was previously done in the laboratory with 250 nonrelated individuals of Córdoba Province (6). Afterwards the detection of the variant allele 9.2, a new study of this locus was performed rising the population to 631 individuals of Córdoba Province (Table 1). Eight out of those individuals presented the allele 9.2, indicating a frequency of 0.0063. The statistical data showed a deviation from Hardy–Weinberg equilibrium determined by chi-square and Exact test. After employing the Bonferroni correction for the 19 loci analyzed in Córdoba, Argentina, population in previous studies (6,17,18), the departure observed at this locus was not significant (0.05/19 = 0.00263).

The deletion in the 3′ flanking region of the variant allele 9.2 has to be considered to design primers, particularly for mini STRs as they are usually complementary to a more internal sequence. For instance, the mini STR for Penta D locus developed mainly to analyze degraded DNA (19), will type the variant allele 9.2 as allele 10, due to the region between the primers do not include the nucleotides deletion. This limitation has to be taken into consideration to avoid mistaken conclusions.

Because of this is the first population where allele 9.2 was described, to look for a founder effect, it will be necessary to study the presence of this new variant allele in others populations of Argentina, Latin America, and the European countries that have contributed to the genetic pool of Cordoba, Argentina.

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