



A compromising position for a variant: A new allele for D1S1656 that invades its neighbors and can lead to misinterpretations

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ABSTRACT

Short tandem repeats (STRs) are the markers of choice for purposes of human forensic identification because of their considerable degree of polymorphism. This variability may occasionally become a challenge for the analyst when a new variant invades the allele distribution range of the neighboring locus. We present here a novel variant at locus D1S1656 showing a molecular length of 211.32 bp corresponding to 21.3 repeating units. This variant is superimposed on the D12S391 locus when the Global Filer™ (Thermo Fisher Scientific Inc., USA) is used, invading the shortest allele range and being assigned as Off-ladder (OL). In contrast, typing with PowerPlex® Fusion (Promega Corp., Madison, USA) overlaps the D2S441 locus, generating an allele that is recognized as 8. In both cases the invasion was observed as a tri-allele pattern. The results were confirmed by DNA re-extraction and typing with the two independent commercial megaplexes described above. The variant was detected during analysis of a reference sample throughout a paternity exclusion case. This is the first time this variant has been described and reported to the NIST STR Base. Since D1S1656 is included in widely-used multiplex kits from several vendors - Biotype (Dresden, Germany) Qiagen (Venlo, The Netherlands), Promega (Madison, USA) and Thermo Fisher (Foster City, CA, USA) - it would then be recommendable that other forensic labs be aware of this new micro-variant in dealing with similar interpretation challenges and that the kit producers take this fact into account in designing new multiplex kits.

1. Introduction

STRs (Short Tandem Repeats) are useful diagnostic elements in human identification, genetic mapping and population studies due to their high polymorphism (great variability among people), their relatively low mutation rate and their amplification efficiency due to their small size [1].

The specific variability of the STRs used in human identification may occasionally become a challenge for the analyst when a previously undescribed variant invades the allele distribution range on the neighboring locus.

Santa Cruz province is the southernmost Patagonian province (46°00'–52°23' de Latitude South and 65°43'–73°35' de Longitude West).

The origin of the current population of Santa Cruz is based on successive internal and external migration events, constituting an interesting model from a molecular demography point of view [2].

The aim of this work was to describe a new D1S1656 allele that is responsible for an apparent homozygous condition for that locus and for a tri-allelic profile in neighboring markers when using different

amplification kits.

2. Methodology

2.1. Case description

The tri-allele pattern was observed in a paternity exclusion case, where blood samples were collected on absorbent paper from the legal father, the biological mother and a daughter.

2.2. DNA extraction and typing

DNA extraction was performed by osmotic lysis followed by a direct amplification with

GlobalFiler (Thermo Fisher Scientific Inc., USA) and PowerPlex Fusion (Promega, Madison, USA) for autosomal STRs and Argus X 12QS (Qiagen, Venlo, The Netherlands) for X-chromosome STRs.

A complete control region of mitochondrial DNA was assessed following EMPOP guidelines using ten primer pairs and Big-Dye v.3.1

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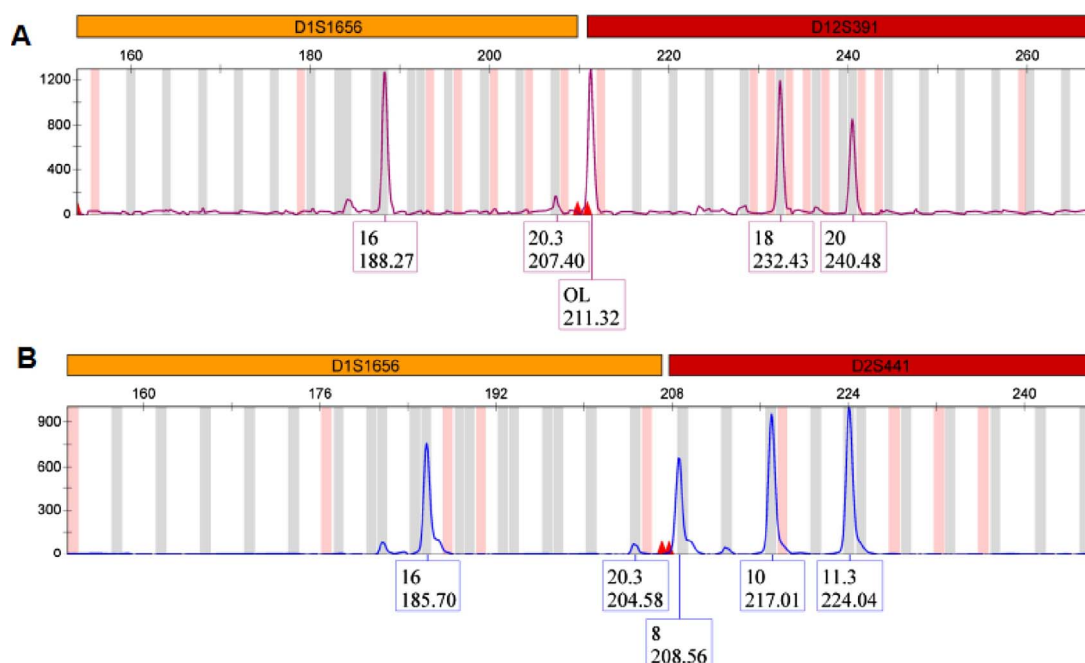


Fig. 1. Details about false tri-allelic pattern and a false homozygous condition with A) Global Filer™ and B) PowerPlex® Fusion.

chemistry (Thermo Fisher Scientific Inc., USA).

3. Results

A tri-allele pattern was observed in the child. That pattern was composed by an Off-Ladder (OL) allele and alleles 18 and 20 at the D12S391 locus with Global Filer™. Typing with PowerPlex® Fusion, detected a tri-allele pattern, but in this case it was composed by alleles 8, 10 and 11.3 at the D2S441 locus (Fig. 1a and b).

To corroborate these results we re-extracted the DNA and re-amplified it with the two kits.

In both cases D1S1656 was located preceding the tri-allele pattern in the electropherogram. Analyzing the context and size of the extra allele present in both systems, it was observed that a new allele for marker D1S1656 invaded the reading range of its neighbor markers.

We here by present a novel allele for locus D1S1656 with a molecular length of 211.3 bp in Global Filer™ and 208.6 bp in PowerPlex® Fusion, corresponding to an allele of 21.3 repeats.

Since this variant is present only in the child, it is presumed that it was inherited from her unknown father.

In order to identify the ancestry of this variant, we tried to reconstruct the haplotype of the X chromosome of the biological father based on the available data from the mother and child. The results were analyzed with the help of the database available at www.chrx-str.org [3].

The control region sequence of mtDNA demonstrated that the mother and the daughter belonged to C2, one of the four major and characteristic Native American haplogroups.

4. Discussion

To our knowledge this is the first instance that the allele 21.3 for locus D1S1656 is described and reported to the NIST STR Base [4]. This new allele for D1S1656 invades neighboring loci, (D12S391 in GlobalFiler and D2S441 in PowerPlex Fusion), while simultaneously brings about a false homozygous condition at locus D1S1656. Particular care

should then be taken when analyzing this locus that belongs to the expanded CODIS markers.

Kit manufacturers may also take this fact into account when designing new multiplex kits by increasing the reading range for marker D1S1656 so that the 21.3 variant could be detected within the corresponding marker range.

The results of the analysis of the X-STRs haplotype frequencies are not quite precise. None of the populations could be ruled out as ancestral, since the corresponding databases are currently incomplete. A better knowledge could be achieved by analyzing the Y chromosome of the biological father in the event it became available.

As stated above, it is likely that the new variant had been inherited through the paternal route. While mtDNA results do not yield relevant data on the ancestry of the father, they would provide a more complete description of the carrier in the event the new variant had been produced by a mutation.

Databases such as the NIST STR Base, strongly depend on what researchers report worldwide. It is of great importance to delve into cases of unusual allele positions – such as those mentioned here – to prevent the misinterpretation of results leading to erroneous conclusions. It is probable that the variant that we present here had previously appeared, but this invasive allele might have been assigned, then going unnoticed.

We show the importance of using different commercial kits to confirm observations, of consulting the available databases and of reporting the observed variants for any autosomal, X and Y STR system. Having complete databases is a responsibility that concerns to all of us.

References

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