# Rare Allele 29 at Locus D2S1338 Observed during Routine Casework in Bulgarian Population

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***Abstract:*** *In this work, we report a rare allele 29 at locus D2S1338, established during routine forensic practice in a case of first-degree kinship (parenthood). This rare allele variant 29 at locus D2S1338, to the best of our knowledge, is reported for the first time in the Bulgarian population. So far it has not been registered in studies of allele frequencies in the same locus for 20 population groups in Europe and Asia. The presentation of similar genotyping findings relating to rare/unexpected population genetic variation is very important for the examination and documentation of such anomalies. The analysis has been performed for 16 STR loci: D2S1338, SE33, D16S539, D18S51, TH01, D12S391, D3S1358, FGA, vWA, D21S11, D1S1656, D2S441, D8S1179, D19S433, D22S1045, D10S1248 and 2 sex determination systems – Amelogenin and Y indel, set in NGM Detect™ PCR Amplification Kit (Applied Biosystems). The use of allelic witnesses in the diagnostic practice is mandatory in the standard fragment DNA analysis. The allelic witness contains well-known preset alleles for the examined locus. Establishing alleles that are outside the factory preset is important for broadening the scope of the witness and heightening the accuracy of the analysis. Rare allelic variants significantly increase the strength of discrimination when DNA profiles are compared. In this regard, it is important to report any*

*new information about the emergence of rare allele variants detected in a particular population group.*

***Keywords:*** *DNA analysis, STR, Rare allele, Locus D2S1338, Bulgarian population.*

## Introduction

Genotyping of non-coding, highly polymorphic, and conservative (low-mutation) loci, by visualizing the Short Tandem Repeats (STR) contained in them, which are widely used in Forensic Medicine and Forensics. Commercial kits for STR-DNA analysis have allele witnesses, including all allele variants found so far, through which the user determines the alleles in each DNA profile [7]. Allele witnesses are based on so far observed allele variations, but there are constantly new allele variants that are not presented in the allele witness. When the tested populations differ from those that had been scanned during the development of the respective allele set, new allele variants will likely appear that are not included in the commercial kit. Practitioners of forensic STR profiling should be aware of the possibility of rare allelic variants so that they can be identified and processed correctly when interpreting the obtained results. Rare allelic variants significantly increase discrimination strength when comparing DNA profiles [1]. In this regard, for Forensic Medicine and Forensics, it is important to report any new information about the emergence of new allele variants, detected in a particular population group. The National Institute of Standards and Technology (NIST) maintains a DNA Internet database (http://www.cstl.nist.gov/biotech/strbase/) since 1997 – STRBase, which registers all new allele variations: rare alleles, microvariants, or alleles outside the bins of the allelic witness, triallelic states and mutations. Unexpected or other genetic variations that can complicate STR typing take several forms: rare alleles, microvariants, or alleles outside the allele witness bins, triallelic states, and mutations [3-5, 12]. New allelic variants that are not presented in the allele witness may be due to the insertion/deletion of complete repeats or the single base insertion/deletion, or partial repeats (microvariants) [1, 8, 9].

Here we report a rare allele with a nomenclature value of 29, at locus D2S1338, detected in our forensic practice. The autosomal locus D2S1338 is located in chromosome 2 with localization: 2q35, with the exact position in the chromosome – Chr 2, 219.082 Mb – NCBI Build 34 and Chr 2 218.705 Mb – NCBI Build 35 [3]. The rare allele 29 in locus D2S1338 is reported for the first time for the Bulgarian population and has not been registered so far in the studies of allele frequency in the D2S1338 locus in 20 different population groups from the populations of Europe and Asia.

## Materials and methods

### *Case presentation*

In the DNA Laboratory at the Department of Forensic Medicine and Deontology at the University Multiprofile Hospital for Active Treatment (UMHAT) Alexandrovska, Sofia, Bulgaria, tests are performed on various biological samples – cellular material from buccal mucosa, blood, semen, tissues, and other physical evidence, obtained during a criminal investigation, as well as comparative materials in cases of kinship determination, materials for the identification of corpses with an unknown identity, etc.

This is an expert case for establishing the parental origin of a female child. Comparative materials of buccal swabs seized from two women and a man identified as the father of the child have been examined.

### *Isolation and DNA amplification*

DNA has been isolated from the cell nuclei and has been prepared for amplification with the AutoMate Express™ Forensic DNA Extraction System using the PrepFiler Express Forensic Extraction Kit (Thermo Scientific LSG, USA).

The amplification of the DNA has been applied to STR markers in 18 chromosome loci embedded in NGM Detect™ PCR Amplification Kit (Applied Biosystems, USA) – D2S1338, SE33, D16S539, D18S51, TH01, D12S391, D3S1358, FGA, Y indel, vWA, D21S11, D1S1656, D2S441, D8S1179, D19S433, D22S1045, D10S1248 and sex determination system Amelogenin [11].

The polymerase chain reaction has been performed by PCR SimpliAmp™ Thermal Cycler (Applied Biosystems, USA).

The DNA present in the samples has been quantified using the Real-time PCR system 7500 (Applied Biosystems, USA), with Quantifiler™ Trio DNA Quantification Kit and HID Real-time PCR Analysis Software v1.2.

### *Fragment DNA analysis*

The fragment DNA analysis has been performed on an automatic sequencer 3500 Genetic Analyzer for Human Identification (Applied Biosystems, USA) by capillary electrophoresis (with 3500 POP-4™ Polymer), with fragment laser detection and computer analysis using Gene Mapper™ v1.2 Full Software (Applied Biosystems, USA) for HID analysis.

The control and standardization of the results of the analysis have been performed by: positive control – DNA control 007; negative control – HC; internal standard – GeneScan™ 600 LIZ™ Size Standart v2.0; internal quality control markers – IQCS and IQCL; allele witness (NGM Detect™ Allelic Ladder) for the respective STR markers, validated and embedded in the NGM Detect™ Kit (Applied Biosystems).

The results of the analysis are presented considering the alleles with their nomenclature values of the analyzed STR markers, contained in the NGM Detect™ PCR Amplification Kit, with a confirmed genotype for the control DNA and the absence of an amplification product in the control blank.

## Results and discussion

The analysis has been performed for 16 STR loci: D2S1338, SE33, D16S539, D18S51, TH01, D12S391, D3S1358, FGA, vWA, D21S11, D1S1656, D2S441, D8S1179, D19S433, D22S1045, D10S1248 and 2 sex determination systems Amelogenin and Y indel, embedded in the NGM Detect™ PCR Amplification Kit.

During the comparative analysis it has been determined that the DNA profile of the child can be derived from the DNA profile of the examined man, i.e., he is not excluded as the biological father of the child. The probability of paternity is calculated and gives a PP value in the range of 99.9999999999999951 – 99.9999999999999964%. As a result of the comparative analysis, it has been found that the two women have been excluded as possible biological mothers of the child.

During the analysis of the D2S1338 locus, a rare allele has been registered in the genetic profile of one of the women. The allele variant detected by us falls within the bin for allele

29 in the NGM Detect™ PCR Amplification Kit and has a base size of 164.26 bp. Fig. 1 shows the allele witness (NGM Detect™ Allelic Ladder) for locus D2S1338. The locus contains 18 established alleles (grey stripes) that are present in the allele witness and at the beginning and end of the locus, and two virtual bins (pink stripes) that are not present in the allele witness.

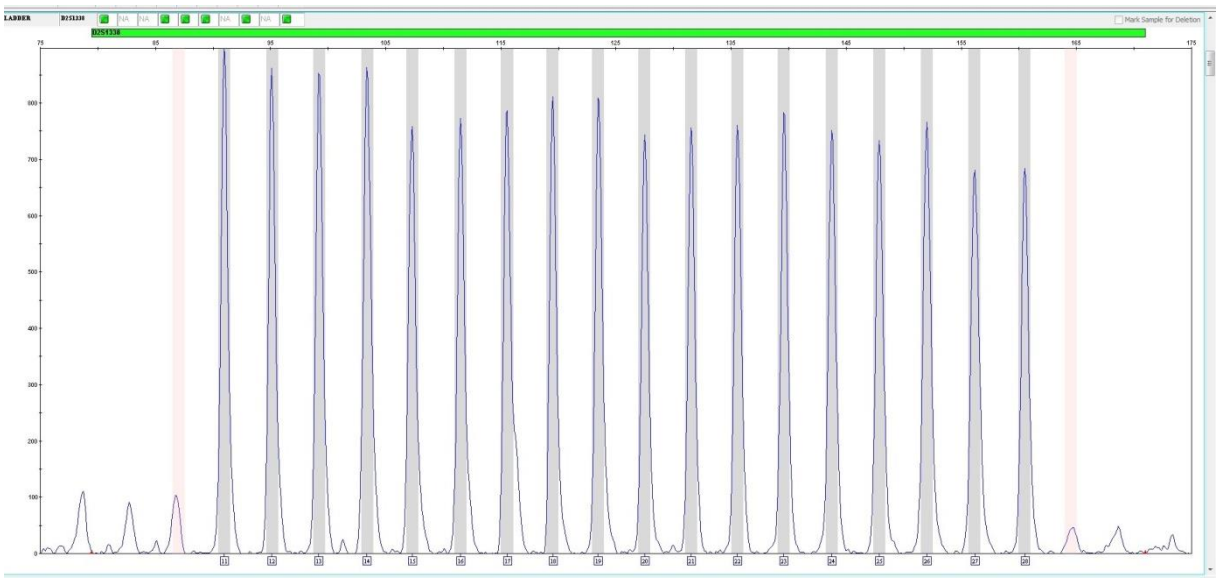

Fig. 1 Allele witness (NGM Detect™ Allelic Ladder) for locus D2S1338

In general, allelic witnesses in the multiplex STR analysis are divided into three coloured longitudinal stripes: grey, pink, and white (Fig. 1). Gray indicates an established bin, pink for a virtual bin, and the white stripe is out of the range of the allele witness. Peaks within grey or pink stripes are automatically marked by STR analysis software (Gene Mapper™ v1.2 Full Software). The set of bins in the allele witness provides reference allele sizes for: alleles physically present in it (physical bins – grey stripes) and alleles that are not present in the witness (virtual bins – pink stripes), which are either reported in the STR base (www.cstl.nist.gov/div831/strbase) or detected during validation. To compensate for the virtual bins, the software uses the offset from the nearest physical bin or virtual bin to the left of the bin [6].

Virtual bins have been created using the offset value from a neighbouring allele and the reference (sequence length) size of the virtual allele. In addition to the substantial expansion of nearly 300 configured markers, support for novel microvariants has been included for all loci with expanded 'virtual bin sets' comprising each potential base call within the allelic range rather than only observed nominal allele bins [10].

Fig. 2 presents electropherograms of the analyzed subjects in locus D2S1338 as follows: No. 1 – female No. 1, No. 2 – female No. 2, No. 3 – female child, No. 4 – male indicated as the child's father.

From the provided comparative material by female No. 2, a rare allele has been observed with nomenclature value 29, outside the physical bins of the allele witness in locus D2S1338, but within the virtual bin (Fig. 3). It is registered for the first time for the Bulgarian population. The rare allele has been re-confirmed by re-amplification of the sample.

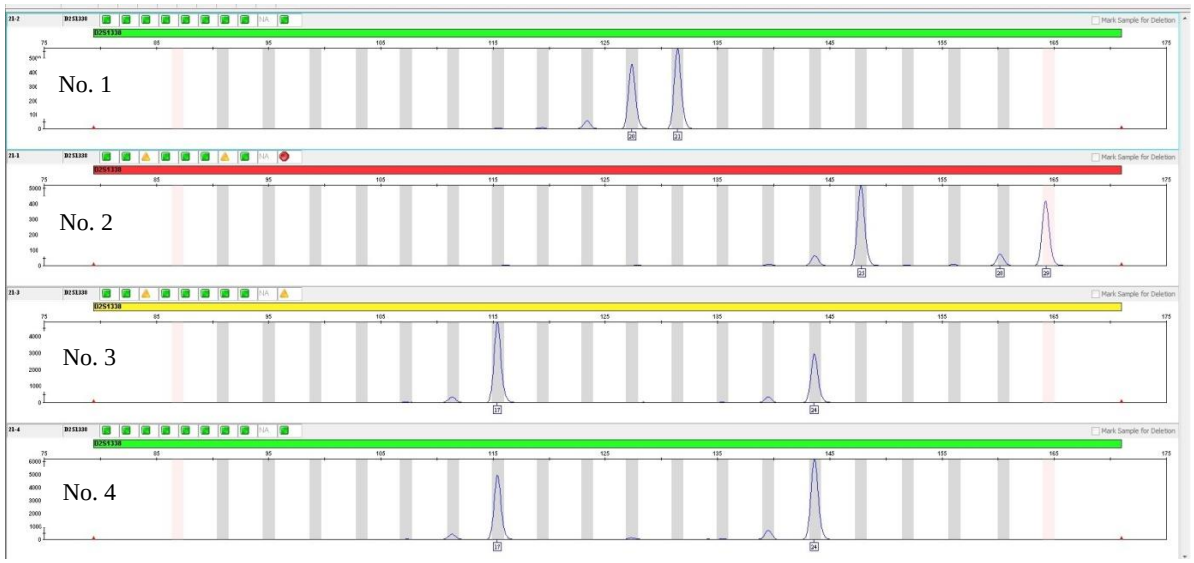


Fig. 2 Electrophoregrams No. 1 – female; No. 2 – female; No. 3 – female child and No. 4 – male, indicated as the child's father. A rare allele (29) is visualized at locus D2S1338 in comparative material from female No. 2 – electrophoregram No. 2.

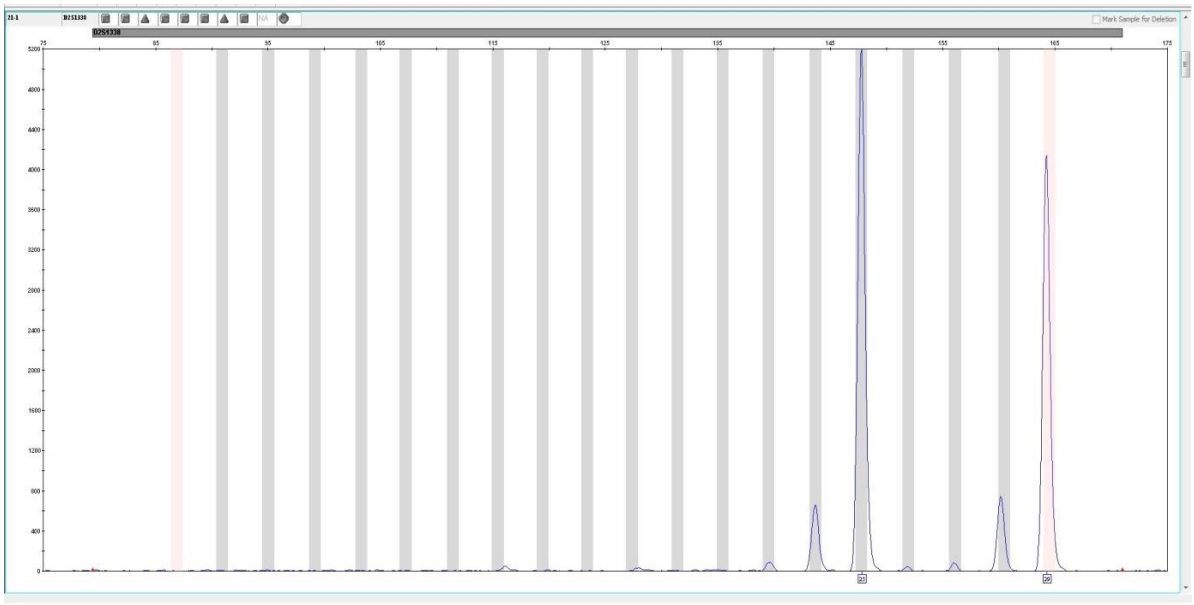

Fig. 3 Electrophoregram of locus D2S1338 from the DNA profile of comparative material from female No. 2. A rare allele is found within a virtual bin, automatically labelled 29, when analyzed with Gene Mapper™ v1.2 Full Software.

Locus D2S1338 has chromosome localization 2q35 and repeats of a four-nucleotide tandem repeat motif: [TGCC] *m* [TTCC] *n*. No such allele of 40 sequential variants of alleles from this locus has been reported [5]. According to the user guide of NGM Detect™ PCR Amplification Kit allele 29 from locus D2S1338 has not been detected in population groups: African American ($n$ = 330), U.S. Pat. Caucasian ($n$ = 343) and U.S. Hispanic ($n$ = 368). It was detected in the Asian population ($n$ = 153) with an incidence rate: 0.33 [11]. After referring to the website https://strbase.nist.gov/ of the NIST it has been found that this allele variant has been reported only once by Malin Sanga from the Swedish National Laboratory of Forensic Science (SKL). The allele has a base size of 267.22. The analysis has been performed with an ESX 16 fragment DNA analysis kit and an ABI 3130xl sequencer.

The rare allele has been obtained for a reference sample and confirmed by reamplification and re-electrophoresis. After referring to the website: http://strider.online, for D2S1338, it has been found that such an allele variant (29) for this locus has not been detected in routine analysis of the alleles in 20 population groups (a total of 10073 DNA profiles of individuals: 7073 from Europe and 3000 from Asia).

## Conclusion

As a unique allele variant, allele 29 at locus D2S1338 is reported for the first time for the Bulgarian population. To our best knowledge, this is a very specific detail, because it is the first report of such an allele, reported in studies of the allele frequencies for 19 other population groups in Europe.

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