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A Unique Allele Variant at STR Locus D2S1338 in a Paternity Testing Case

متغير أليلي فريد في الموقع الوراثي D2S1338 في قضية اختبار أبوة

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Abstract

Relationship testing through DNA profiling may undesirably be affected by the rare allele variants, tri-allelic patterns and null alleles. Therefore, it is vital to report such anomalies. We report a paternity testing instance in a sexual assault case studied at Punjab Forensic Science Agency (PFSA), Lahore, Pakistan showing a unique allele variant in mother and child.

DNA was extracted from the buccal swabs of reference samples using organic extraction method. DNA profiling was done for 15 autosomal STRs and amelogenin using Identifiler Plus kit.

A novel out of marker range (OMR) allele variant between STR Loci D16S539 and D2S1338 was observed in the DNA profiles of victim and her child. At STR locus D2S1338, twenty-one different allele variants are listed at STRBase ranging from 11 to 28. The allele variant observed in this case study appeared at less than the marker range (< D2S1338) with a size of 297.50 bp. The novel

المستخلص

قد يتأثر اختبار النسب من خلال تحديد سمات الحمض النووي بشكل غير مرغوب فيه في حال وجود متغيرات الأليل النادرة والأنماط الثلاثية الأليلات والأليلات الصفرية. لذلك، من الضروري الإبلاغ عن مثل هذه الحالات الشاذة. وتستعرض هذه الورقة حالة اختبار الأبوة في حالة اعتداء جنسي تمت دراستها في وكالة علوم الأدلة الجنائية بالبنجاب (PFSA) في لاهور بباكستان، والتي تُظهر متغيرًا فريدًا من الأليل في الأم والطفل.

تم استخلاص الحمض النووي من المسحات الشدقية للعينات المرجعية باستخدام طريقة الاستخلاص العضوي وتم تحديد سمات الحمض النووي لـ 15 موقعاً للتكرارات المترادفة القصيرة (STRs) الجسدية والأميلوجينين باستخدام مجموعة Identifiler Plus.

تمت ملاحظة متغيّر أليلي جديد خارج نطاق العلامة (OMR) بين الموقع الوراثي D16S539 والموقع D2S1338 في سمات الحمض النووي للضحية وطفلها. وفي الموقع D2S1338 تم إدراج واحدٍ وعشرين نوعًا مختلفًا من الأليل في قواعد بيانات التكرارات المترادفة القصيرة (STR-Base) تتراوح من 11 إلى 28. وظهر متغير الأليل الذي لوحظ في دراسة الحالة هذه في أقل من نطاق العلامة (<D2S1338) بحجم 297.50 نقطة

Keywords: Forensic sciences, DNA Profiling, Allele Variants, D2S1338, OMR, Paternity Testing, STR Rare Allele

الكلمات المفتاحية: علوم الأدلة الجنائية، السمات الوراثية، المتغيرات الأليلية، موقع D2S1338، خارج نطاق العلامة OMR، اختبار الأبوة، مواقع التكرارات المترادفة القصيرة النادرة.

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variant OMR allele at D2S1338 was calculated as allele 13 with the help of allelic ladder. Moreover, this allele variant (allele 13 at locus D2S1338) was present in only two other distinct DNA profiles in PFSA DNA database, out of a total 10,125 profiles.

The overall frequency of this unique allele variant was 3 in 10,125 unrelated individuals with occurrence frequency of 0.000296. According to our limited knowledge, it is the first report of a novel OMR allele variant at D2S1338 within the Pakistani population.

أساس.

وتم حساب المتغير الأليلي الجديد (OMR) في الموقع D2S1338 على أنه أليل 13 بمساعدة السلّم الأليلي. علاوة على ذلك، كان هذا المتغير الأليلي (الأليل 13 في الموقع D2S1338) موجودًا فقط في ملفي سمات وراثية لشخصين مميزين آخرين في قاعدة بيانات PFSA DNA، من إجمالي 10125 ملفًا شخصيًا.

وبلغ التكرار الإجمالي للمتغير الأليلي الفريد المحدد 3 في كل 10125 فردًا لا يربطهم أي صلات فيما بينهم، مع تكرار حدوث 0.000296. ووفقًا لمعرفتنا المحدودة، هذا هو التقرير الأول لمتغير أليل OMR جديد في D2S1338 داخل السكان الباكستانيين.

1. Introduction

Forensic DNA analysis for human identification and kinship studies rely on DNA profiles generated through amplification of 12-24 STR markers [1-4]. STR allele variants are observed in various forensic samples resulting in the DNA profile anomalies [5]. Commercial kits offer the PCR amplification of various STR regions and provide allelic ladders with all the known alleles for a given amplified STR system and correct sizing. Mostly, STR alleles have sizes that match sizing bins in allelic ladder still few allele variants are also noticed. These variants are labelled as “off-ladder (OL)” if present within the marker range of some STR loci while “out of marker range (OMR)” if they do not fall within the vicinity of marker [6-10]. It is vital for forensic scientists to ensure the updated information of variant alleles in population to evade misinterpretation. These variants may probably increase the power of discrimination in DNA analysis [11]. Allele variant information is being updated on the NIST STRBase website on a regular basis for the past two decades [12-14] and 808 variants have been catalogued. STR anomalies and variant from diverse populations have been reported previously [15-23].

The STR marker D2S1338 is a compound tetra nucleotide repeat present on chromosome 2 long arm (Embank; GRCh38, Chromosome 2, location 218014859-218014950, reverse strand, allele designation 23) with two primary repeat motifs having significant sequence variation. One motif with tetra nucleotide TGCC has 4-9 repeats and TTCC has 6-19 repeats, collectively accounting for repeat count ranging from 10 to 26: [TGCC]n [TTCC]n. The second motif is analogue of first with interruption of single repeat GTCC: [TGCC]n [TTCC]n [GTCC] [TTCC]2 having 19 to 27 repeats. Other motifs are seen more rarely, resulting from SNPs or Indel in the repeat region [24, 25]. D2S1338 was found to have approximately two-fold more allele variants when sequenced as compared to length-based alleles. A number of these alleles may be rare and population studies should be done to evaluate the increase in alleles that would be anticipated in forensic samples [26]. In this report, we found a unique variant [13] at D2S1338 in a sexual assault case, it was present in the DNA profile of victim, as well as in the profile of baby. This rare allele has not been reported before within the Pakistani population or other populations internationally.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

The Buccal swab standard references of victim (mother), Suspect (father) and Child (offspring) were collected at Reference Receiving Unit, Pun-

jab Forensic Science Agency (PFSA), Lahore, Pakistan. DNA extraction was done using organic extraction method following the in-house standard operating procedures. The phenol-chloroform method was used for the isolation of DNA from samples [27, 28] followed by concentration and purification by Amicon® Ultra Centrifugal Filters following manufacturer's instructions [29].

2.2. DNA Quantification and Amplification

Quantification of human and male DNA was performed using QuantifilerTM Duo kit (ThermoFisher Scientific, USA). The samples were run and analysed on the ABI 7500 Real-Time PCR System (Applied Biosystems, USA) using HID Real-Time PCR Software as recommended by the manufacturers.

Amplifications of 15 autosomal STR loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and Amelogenin were achieved by Identifiler Plus kit [30]. Thermal cycler (Applied Biosystems Model 9700) was used for the amplification of STR loci, and the PCR conditions were set according to the instruction of kit manufacturer.

2.3. DNA Profiling and Analysis

Using the ABI 3500 Genetic Analyzer, 8-capillary array system (Applied Biosystems, USA) following manufacturer's protocols, with POP-4™ Polymer, the genotyping analysis of the amplified STRs products was done [31]. Data was collected and analyzed using GeneMapper® IDX V3.2 software (Applied Biosystems, USA) [32]. The comparison of the size of a sample's alleles to the size of alleles in allelic ladders for the same loci being tested in the sample was done [33]. The Data was analyzed by three forensic analysts. The DNA profiling of samples containing allele variant was done twice using the same standard procedures.

3. Results and Discussion

Short tandem repeat (STR) loci are frequently analyzed for forensic purposes all over the world. Because of their small size, efficacious results can be often obtained from highly degraded DNA [34]. Various studies have demonstrated that STR analysis by multiplex PCR amplification is the potential application for forensic personal identification and paternity testing, thereby empowering its efficacy for forensic practice [35-39]. The STRBase website, kept running by the U.S. National Institute of Standards and Technology (NIST), comprises useful information on STR markers used in human identity testing [40]. STRBase regularly updates the allele variants on its website and numerous laboratories are involved in this process.

In the present case study of sexual assault, the DNA profiling of Mother, Father and Child was done using Identifiler Plus kit, which includes 15 STR loci and sex determining amelogenin. The DNA profiles of both mother and child showed an out-of-marker range peak between STR Loci D16S539 and D2S1338. The STR locus D16S539 in both mother and child was heterozygous with similar peak height suggesting that the OMR belongs to the D2S1338, which is heterozygous with normal allele and rare allele having comparable RFU values in both (Table-1). Figure 1 illustrates that this rare allele does not fall within the range of commercially available allelic ladder for D2S1388, so it was marked as an OMR. The DNA profile obtained from the child has the same rare allele 13 (475 RFU) at D2S1338, with allele 24 (913 RFU) from father while it was heterozygous on D16S539 with parental shared alleles 10 (915 RFU) and allele 11 (870RFU) (Figure-1). Furthermore, it was also confirmed by another com-

Table 1- *D16S539and D2S1338 Peaks Height, Size and Label for Mother, Child and Father.*

DNA Profile	Genetic Locus	Peak Size	Peak Height (RFU)	Allele Call (Repeats)	Comment
Mother	D16S539	271.47	3601	10	Heterozygous
		275.56	3521	11	
	D2S1338	297.49	2910	OMR (13)	Heterozygous
		321.56	3107	19	
Child	D16S539	271.59	915	10	Heterozygous
		255.56	870	11	
	D2S1338	297.50	683	OMR (13)	Heterozygous
		341.69	837	24	
Father	D16S539	271.47	1014	10	Heterozygous
		175.56	1424	11	
	D2S1338	337.63	870	23	Heterozygous
		341.57	717	24	

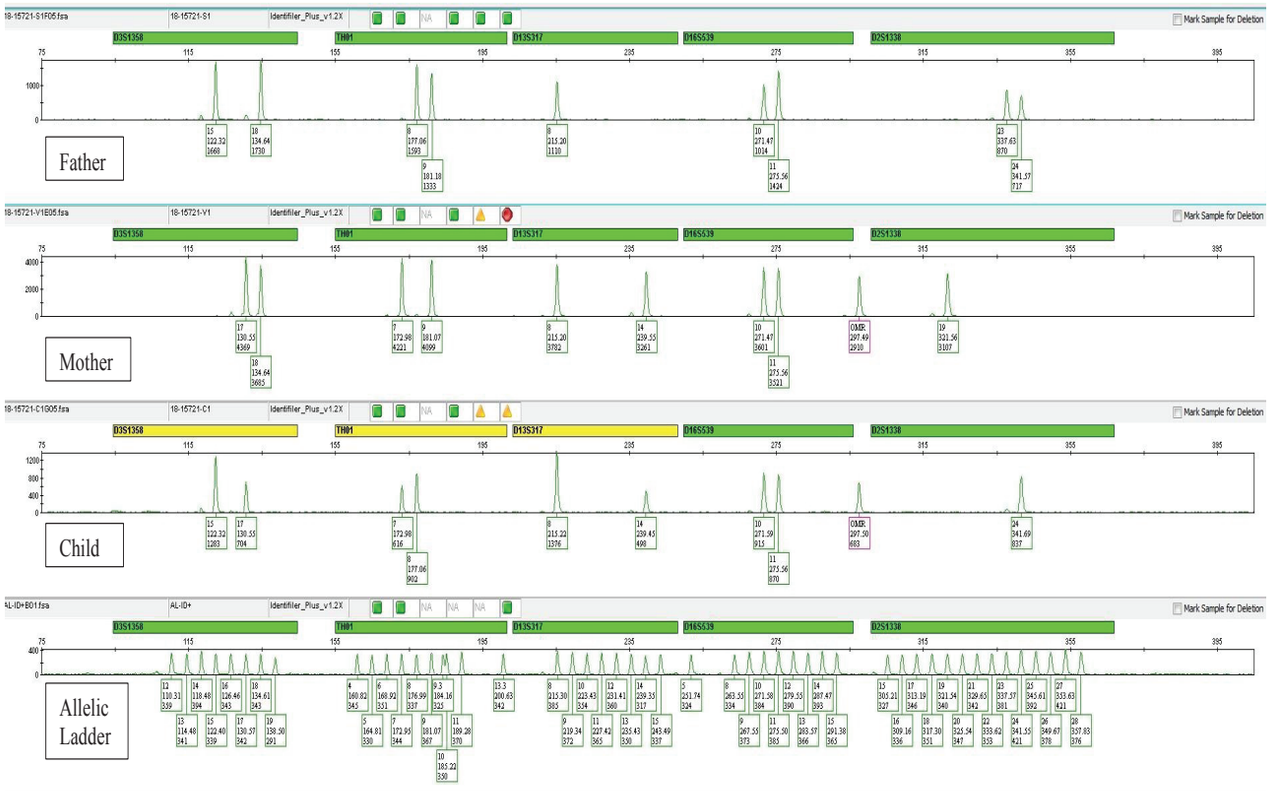

Figure 1- *The STR profile of five genetic loci showing DNA profiles of Father (18-15721-S1), Mother (18-15721-V1) and Child (18-15721-C1). Last panel shows the allelic ladder with all alleles at these specific genetic loci.*

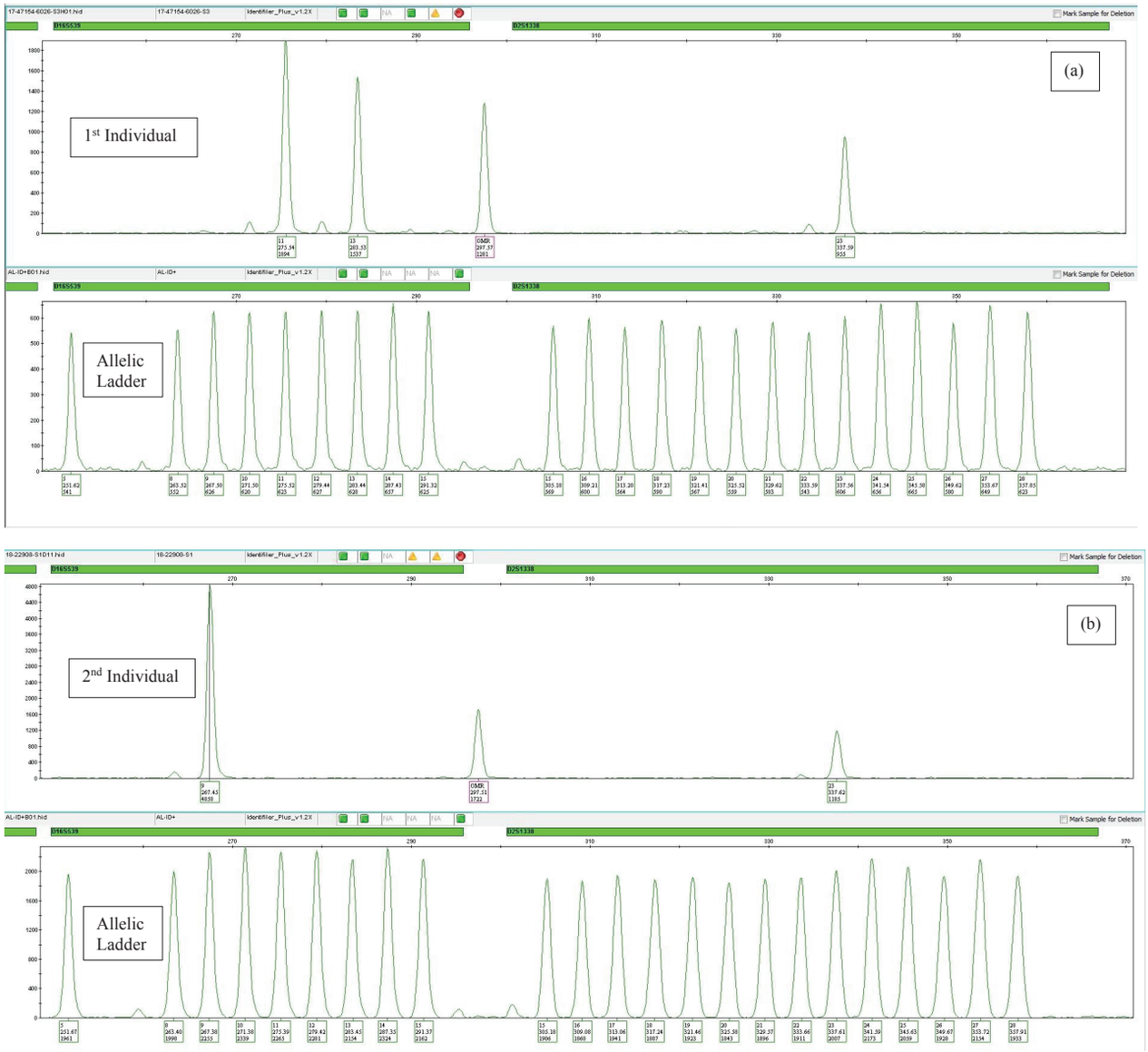

Figure 2- *The STR profile showing genetic loci D16S539 and D2S1338 profiles of two other individuals with allele variant 13 at D2S1338 (a) First Individual and (b) Second Individual. The lower panels shows the allelic ladder.*

mercially available kit, the GlobalFiler™ PCR Amplification Kit, which showed this allele variant as Allele 13 (Data not shown), as was calculated for OMR in Identifiler Plus kit. A similar mislabeling of an off-ladder variant allele was found on D2S1338 in DNA profile of a murdered woman within the Italian population (9) and once observed in a Portuguese Caucasian sample [41, 42]. Different studies of allele 13 at D2S1338 reported on NIST STRbase website are summarized in Table-2.

The peak which appears as < D2S1338 OMR was labeled as rare 13(<15) allele variant. It was in two other distinct cases in our in-house database. This DNA database comprises thousands of DNA profiles of Pakistani individuals acquired from forensic case work at PFSA, Lahore. When this database was searched for this unique out of marker range (OMR) allele variant it was observed that this

Table 2- *The allele variant 13 at genetic locus D2S1338 reported in different population at NIST STR Base website updated on January 15, 2021.*

Allele Size	Amplification Kit	Contributor	/Verification (Conformation Method(s	Frequency
282	SGM +	Institute of Forensic Medicine Medical University of Wroclaw, Poland	Re-amplified	1 in 600
282.31	SGM +	Faruk Asicioglu	Re-extracted and re-amplified	1
282	SGM+	Department of Forensic Medicine, Medical University of Bialystok, Poland	Re-extracted and re-amplified	1 in 200
298.15	ID	Laure Delpech, Police Forensic Laboratory of Marseilles	Re-amplification with SGM+	1 in 3000
298.32	ID	Genetica, DNA Laboratories, Inc., Cincinnati OH	Inheritance (found in Mother & Child)	-
282.3	SGM+	Gregor Kljucevsek, Ministry of the Interior, Forensic Science Centre,Slovenia	Re-extracted and re-amplified	6 in 16000
297.57	ID	Registro Nacional ADN, Servicio Medico Legal, Chile	Re-extracted and re-amplified	1
297.39	ID	Oscar García, Forensic Genetics Department, Autonomous Police of the Basque Country	Reamplified and reanalyzed	-
298.11	ID	Ashley Trausch, Saint Louis Metropolitan Police Department	Re-amplified	1
298.39	ID	Pamela Calogero, St. Louis Metropolitan Police Department Crime Lab	Re-amplified	1
232.41	PP21	Massimo Mangiola, Rhode Island Blood Center	Re-amplified & re-analyzed	4 in 2730
297.50	ID+	This Study	Re-extracted and re-amplified Reamplification with GlobalFiler	3 in 10125

was present only in 3 distinct profiles of unrelated individuals in a total of 10,125 DNA profiles at the time of search. The other two profiles with this allele variant were from the standard reference sample of suspects in two different cases as shown in Figure-2.

DNA profiles were finally designated as D16S539: 10, 11 and D2S1338: 13, 19 for mother and D16S539: 10, 11 and D2S1338: 13, 24 for child. According to our limited knowledge, this is the first report on this rare allele at genetic locus D2S1338 within the Pakistani population.

4. Conclusion

The present study demonstrates the presence of a rare allele variant at STR locus D2S1338 in a

sexual assault case. This rare allele variant 13 was not only found in DNA profile of mother but had also been transmitted to her child as well. This is the first report on the OMR allele variant at D2S1338 within the Pakistani population.

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Conflicts of interest

The authors declare no conflicts of interest.

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