جامعة نايف العربية للعلوم الأمنية
Naif Arab University for Security Sciences

Naif Arab University for Security Sciences
Arab Journal of Forensic Sciences & Forensic Medicine
المجلة العربية لعلوم الأدلة الجنائية والطب الشرعي
https://journals.nauss.edu.sa/index.php/AJFSFM

الجمعية العربية لعلوم الأدلة الجنائية والطب الشرعي
Arab Society for Forensic Sciences and Forensic Medicine

Description of Rare Genetic Variants Discovered with Promega PowerPlex® Forensic Amplification Kits during STR analysis of Routine Paternity and Kinship Cases

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وصف المتغيرات الجينية النادرة التي تم اكتشافها باستخدام مجموعات تكثير الحمض النووي الجنائي من Promega® PowerPlex أثناء تحليل التكرارات المترادفة القصيرة STR لحالات الأبوة والقرابة الروتينية

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Received 02 Feb. 2020; Accepted 17 June. 2020; Available Online 07 Sep. 2020

Abstract

Short Tandem Repeats (STR) have been widely used to create a discriminating DNA profile during the forensic investigation of a crime. The Paternity and Kinship Division at Medico-legal Directorate (MLD)/ Baghdad, provides a DNA fingerprinting service for paternity and kinship requests from different courts of law and police departments from across the country. Several rare variants were observed during DNA analysis such as rare off-ladder alleles, tri-allelic pattern as well as allele drop-out. Variants that have been transmitted among family members were investigated in this study.

During the period between 2008 to 2019, 38309 samples have been analyzed in the Paternity and Kinship Laboratories for DNA profiling to resolve the referred cases. DNA profiles found to have unusual STR patterns (off-ladder, tri-allelic pattern, inter-loci variable were analyzed and documented).

A total of 17 variants were observed which were as shared among family members. Rare off-ladder alleles (9 cases + inter-loci variant 2 cases), as well as 6 cases of

Keywords: *Forensic Science, DNA Profile, Genetic Variant, Off-ladder Alleles, Tri-allelic Pattern, Inter-loci Variant.*

المستخلص

إن استخدام التتابعات القصيرة المتكررة (STR) يتم على نطاق واسع لغرض الحصول على بصمة وراثية مميزة ضمن مجال الفحوصات الجنائية. وتعنى شعبة العائدية والنسب في دائرة الطب العدلي ببغداد بفحوصات الحمض النووي لقضايا الأبوة والنسب من مختلف محاكم القانون وإدارات الشرطة من جميع أنحاء البلاد. وتم تسجيل عدة أنواع من المتغيرات أثناء تحليل الحمض النووي (مثل أليلات جديدة خارج السلم، ونمط ثلاثي الأليلات، ومواقع متعددة، بالإضافة إلى تسرب الأليل)، وتم فحص التغايرات المشتركة بين أفراد الأسرة ضمن هذه الدراسة.

وتم تحليل 38309 عينة في مختبرات العائدية والنسب للحصول على بصمة الحامض النووي لغرض حل القضايا المحالة للشعبة خلال الفترة بين 2008 إلى 2019. وتم توثيق وتحليل بصمات الحامض النووي التي وجدت أن لديها أنماط STR غير عادية (خارج السلم، أليلات بين المواقع ونمط ثلاثي الأليلات) ضمن هذه الدراسة.

كما تم رصد ما مجموعه 17 متغيرًا واكتشافها على أنها مشتركة بين أفراد الأسرة، والأليلات الجديدة المتغيرة خارج السلم (تسع

الكلمات المفتاحية: علوم الأدلة الجنائية، بصمة الحامض النووي، تغايرات وراثية، تغايرات خارج السلم، نمط ثلاثي الأليلات، أليلات بين المواقع.

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doi: 10.26735/PISP6332

tri-allelic patterns were recorded.

The presence of each type of these variants among family members proves that these variations are of genetic origin. They also represent rare genetic variants specific to the Iraqi populations that could be used for the establishment of a new Iraqi DNA database, which might be useful for genealogical studies as well as in terms of resolving familial, social, and moral disputes.

حالات إضافة إلى حالتين لأليلات بين المواقع)، وست حالات للنمط ثلاثي الأليلات .

كما أن وجود هذه التغيرات بين أفراد الأسرة هو دليل قوي على أن هذه الاختلافات ذات أصل وراثي، كما أنها توفر فكرة جيدة عن الطبيعة المتنوعة للسكان العراقيين، ويمكن استخدامها لإنشاء قاعدة بيانات للبصمة الوراثية التي قد تكون مفيدة لدراسات الأنساب وكذلك من حيث حل النزاعات الأسرية والاجتماعية والأخلاقية.

1. Introduction

Forensic and parentage testing using Short Tandem Repeats (STR) have been widely used to generate highly discriminating DNA profiles. STR typing is based on the comparison of amplified alleles to standardized allelic ladders that possess the most common alleles sequenced to detect the true number of DNA repeats, and assign allele designations accurately [1].

As more samples are run for STR analysis, new alleles are constantly being discovered that do not fit with the ladder alleles. These ‘‘off-ladder’’ alleles can be variants with more or less of the core repeat unit that is present in common alleles found in the commercially available allelic ladder. These variant alleles may contain partial repeats or insertions/deletions in the flanking region close to the repeat. In many instances, these alleles are simply classified as greater than the largest allele or as smaller than the smallest allele in the ladder [2]. An inter-loci allele is an off-ladder allele in which the variant allele occurs between two adjacent loci in a multiplex STR electropherogram. When one of the loci contains two alleles (heterozygous) and the other one has only one allele (homozygous) within the common allele range, the inter-locus allele is easily attributed to the apparent homozygous locus [2].

Tri-allelic patterns are categorized as a genotyping irregularity that can be found in STR profiling. It may have several causes, such as duplication of the locus, chromosomal mutations, or chimerism [3,4]. Although tri-allelic genotypes are generally rare (compared to the normal di-allelic state), data indicate that tri-allelic STR genotypes can be more frequent, as it has been registered on the STRBase website (http://www.cstl.nist.gov/biotech/strbase). Two types of the tri-allelic patterns have been distinguished: in the Type I pattern, the sum of the peak heights for two of the alleles is approximately equivalent to the third allele, while in the Type II pattern all three peaks have approximately the same height [5].

Type I indicates somatic mutation of an allele at a heterozygous locus during the development of the individual, resulting in chimerism. Tri-allelic patterns of type II indicate an event of duplication located on the same chromosome or translocated or chromosomal aneuploidy (trisomy). When it comes to localized duplication, the two alleles are likely to be inherited together because they will be strongly linked [2,5]. The TPOX locus (which occurs closest to the tip of a chromosome) has the highest number of observed tri-allelic patterns [6,7].

Allele dropout as a result of primer mismatch is a consequence of sequence variation that occurs in the flanking regions (primer binding site) of the STR loci. Different assays or commercial STR kits have primers that anneal to different flanking region sequences around a particular STR locus. Some PCR primers are affected by a primer binding site mutation, which can lead to allele drop-out at a certain locus [2]. Mutations under primer-binding sites have an impact on the detection of different loci when using various PCR primer sets [8,9] which might lead to a primer mismatch.

In Iraq, the Paternity and Kinship Division at Medico-legal Directorate (MLD)/ Baghdad, is concerned with DNA fingerprinting to resolve all requests that had been sent for paternity and kinship testing from different courts of law and police departments from across the country (with exception of Kurdistan) to authenticate identity cards and/or to settle kinship and paternity problems. DNA fingerprinting with multiplex –STR DNA analysis has been the method of choice to resolve family disputes. Different multiplex DNA amplification kits were used according to each case including PowerPlex® 16, PowerPlex® 18D System, PowerPlex® Fusion System and PowerPlex® Y23 System DNA amplification kits (Promega Corporation, USA) as well as AmpFISTR® Identifiler, Profiler® and AmpFISTR® Yfiler™ DNA amplification kits (Applied Biosystems®) that are commonly used by forensic laboratories for paternity, kinship, and genealogical testing.

From 2008 to July 2019, 38,309 samples have been analyzed in the Paternity and Kinship Laboratories for DNA profiling to resolve the referred cases. Several types of variants have been registered during DNA analysis which included: rare variant off-ladder alleles, tri-allelic patterns, inter-loci variants as well as allele dropout. Here we describe variants that have been transmitted among family members like parent/child or siblings, which have been observed during routine casework investigations in the laboratory of the Paternity and Kinship division.

2. Materials and Methods

During the routine workflow of the Paternity and Kinship division, cases are divided into two types Direct (father, mother, and children) and Indirect (a family with one or more missing parents, child, or extended family member (uncle/aunt/grandparent…). In complicated indirect cases, the use of Y-STR (for male subjects) is required. For each referred case, a bloodstain sample was obtained from each subject, samples were labeled and the proper DNA extraction method was applied (for Promega PowerPlex amplification kits the direct method was applied, while DNA extraction using Chelex® 100 Resin was used for the other amplification kits).

DNA amplification was done using a GeneAmp® PCR System 9700 thermal cycler according to the amplification parameters recommended by the manufacturer for each kit. PCR products were run with 3130xl Genetic Analyzer® (Applied Biosystems, USA) according to the technical manual and the data were analyzed with Gene-Mapper ID® Analysis Software V.3.2 software (Applied Biosystems, USA).

The DNA profiles were analyzed and direct comparison was made between samples to determine the relationship between individuals involved in order to send the results to competent authorities. DNA profiles that were found to have unusual STR patterns (off-ladder, tri-allelic pattern, inter-loci variable, allele-dropout) were registered. Each registered sample was re-examined with another amplification kit and re-processed to rule out human and technical errors. The variants that were shared between family members were used in this paper.

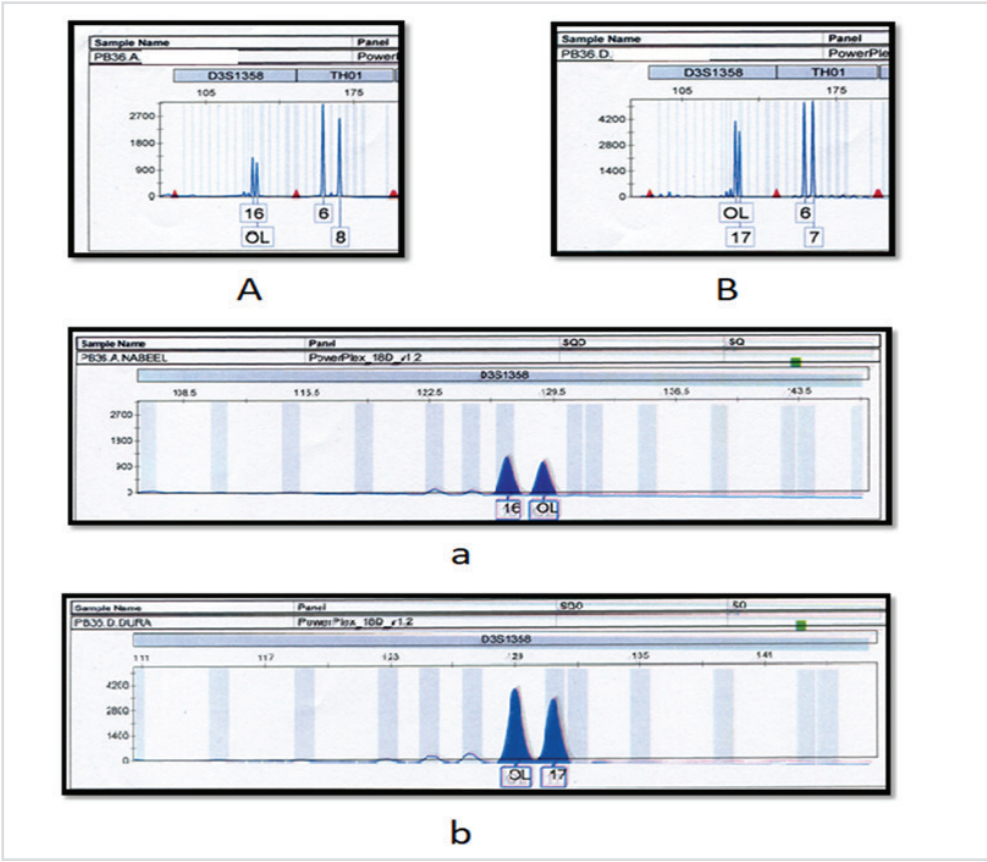

Figure 1- *Off-ladder variants shared among family members (A, a: Father, B, b: Daughter).*

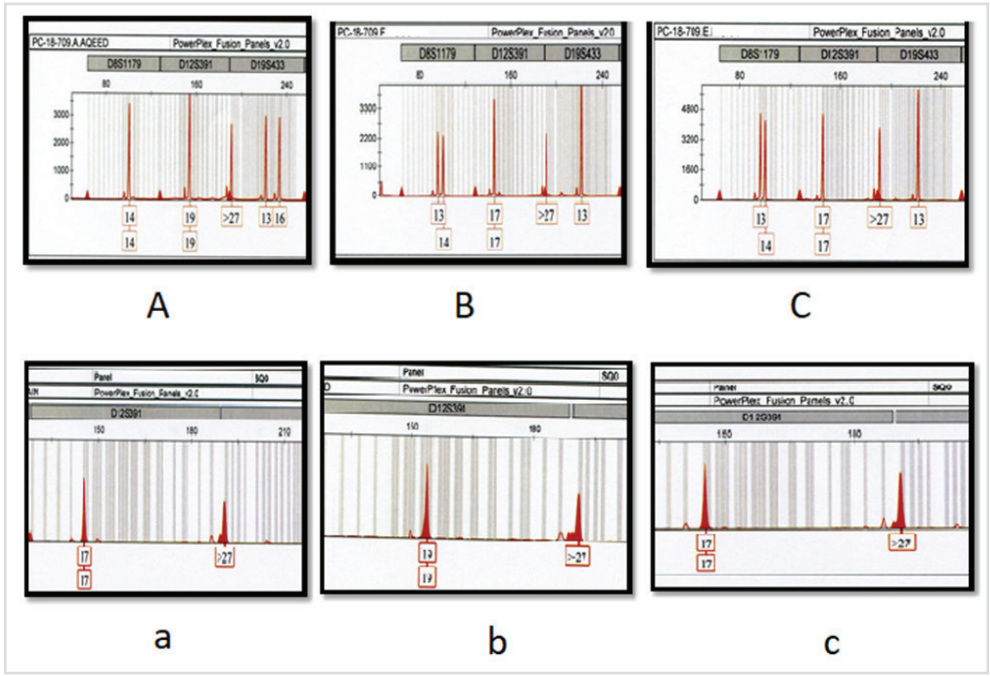

Figure 2- *Inter-loci variants shared among three family members (A, a: Father, B, b: Son, C, c: Daughter).*

3. Results and Discussion

Out of 38,309 DNA profiles, a total of 17 variants (unregistered genetic polymorphisms) were observed and detected as shared among family members; unshared variants were not included in this study. Each sample was handled carefully and re-processed for confirmation to exclude any technical error that might interfere with the results. Four types of variants were detected which included out of allelic ladder variants, inter-loci variants, and Tri-allelic patterns.

Most off-ladder variants were either greater than the largest allele or smaller than the smallest allele, while some variants were detected within the previously identified bin's range. These variants might be a result of the insertion of a partial or one core repeat than the previously registered alleles [2]. All detected off-ladder variants were greater than the registered allele for that position. This might be because insertion has a higher frequency rate than deletion in STR loci [10,11] (Table-1, Figure-1). Also, there were two cases of inter-loci off-ladder variants that were confirmed with the use of another kit for sample amplification which is very valuable for result accuracy (PowerPlex®18D Kit was used because it lacks locus D12S391 (Table-2, Figure-2):

Tri-allelic patterns are another irregularity that was detected within some DNA profiles. Both types of the tri-allelic pattern have been distinguished and recorded, with an increase in Type II pattern (three peaks almost the same height) compared to Type I pattern (the sum of the two peak heights is equivalent to the third allele) [3]. Tri-allelic patterns that were detected in both D21S11and Penta D were not included in this study because they are related to the medical condition of chromosome 21 trisomy (Down syndrome) [12]. Tri-allelic patterns of type II indicate an event of duplication located on the same chromosome or translocated or chromosomal aneuploidy (trisomy). When it comes to localized duplication, the two alleles are likely inherited together because they are strongly linked [3, 13]. Even though TPOX locus is reported to have the highest frequency of tri-allelic pattern [6], no tri-allelic patterns of TPOX were reported in the study. This anomaly corresponds to the results of Poiares et al., who found several tri-allelic patterns in different loci but not at TPOX [14]. These results might be due to genetic variation in the populations (Table-3, Figure-3):

A previous local study mentioned the detection of a tri-allelic pattern at the D16S539 and Off-ladder variants at D12S391, D19S433, and D1S1656 loci [15], which is in agreement with our results. This is the first report about STR variation that included off-ladder variants, tri-allelic patterns, as well as primer mismatch and inter-loci variants that were detected during routine multiplex PCR-based forensic testing using Promega Power-

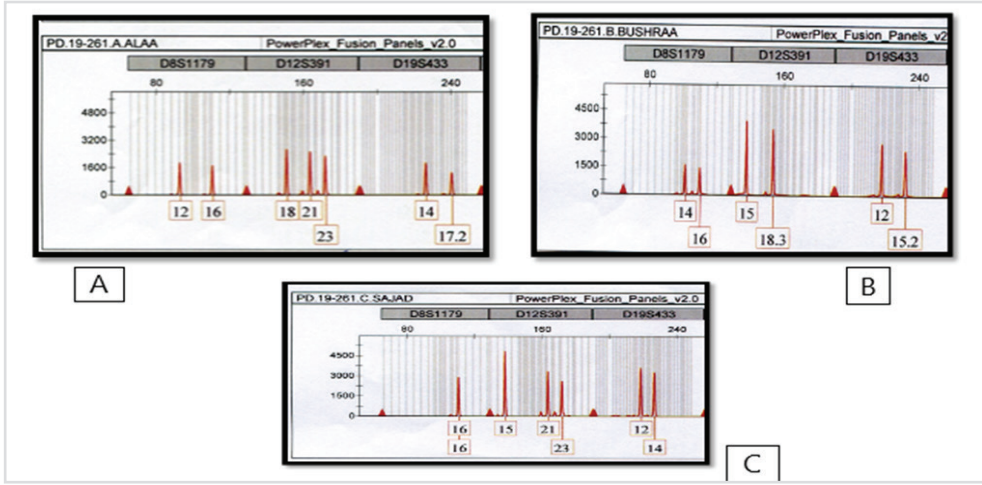

Figure 3- *Tri-allelic pattern shared between a father and his son (A: Father, B: Mother, C: Child).*

Table 1- *Variants detected out of allelic ladder range (out off and inside categories range).*

Locus	Variants	Cases
D7S820	>14	1-Brother and sister profiles 2- Father and daughter profiles
PENTA D	>17	- Father and son profiles
D3S1358	>16	- Father and daughter profiles
D21S11	>38	- Mmother and 2 daughter's profiles
PENTA E	>18	- Father and son profiles
D16S539	>15	- Father and son profiles
D1S1656	>15	1- Mother and daughter profiles 2- Mother and 2 daughter,s profiles

**Each sample was analyzed twice for result confirmation*

Table 2- *Inter-loci off-ladder variants that were detected with PowerPlex® Fusion vs. PowerPlex® 18D.*

Cases		PowerPlex®18D	PowerPlex® Fusion	
	Locus	D19S433	D12S391	D19S433
Case 1	Father	12/13	18/ off-ladder*	12/13
	Son	12/13	21/ off-ladder*	12/13
Case 2	Father	13/16	12/ off-ladder*	13/16
	Son	13/16	17/ off-ladder*	13/16
	Daughter	13/16	17/off-ladder*	13/16
*off -ladder variant was >27 in all samples				

**Each sample was analyzed twice for result confirmation*

Table 3- *Tri-allelic patterns that were detected within family members.*

Cases	Locus	Subjects	Alleles	Tri-allelic Pattern
Case 1	THO1	Mother and Daughter	7/9/9.3	I
Case 2	THO1	Brother	6/9/9.3	II
		Sister	6/9/9	II
Case 3	vWA	Two Brothers Profiles	16/17/20	I
Case 4	D13S317	Mother	8/11/12	II
		Daughter	8/12/12	II
Case 5	D3S1358	Grandfather and Granddaughter	16/17/18	II
Case 6	D12S391	Father	18/21/23	II
		Son	15/21/23	II

Each sample was analyzed twice for result confirmation

Plex amplification kits in Iraqi population. The presence of each type of these different variants among family members is solid proof that these variants are of genetic origin that gives more discrimination power in paternity disputes. The high genetic diversity of the Iraqi population is well-documented [16,17,18], which is in agreement with the polymorphic nature of STR worldwide and in the Iraqi population in particular.

4. Conclusion

The use of STRs in DNA fingerprinting for paternity and kinship testing is a valuable and very useful tool in familial disputes. The presence of rare genetic variants among family members increases the discrimination power of the given DNA profile and gives a good idea about the diverse nature of the Iraqi population. The current study indicates that rare genetic variants can be found in the Iraqi population and the establishment of a DNA database is necessary for their use in court as well as for genealogical study. Particular attention should be given when recording such variants, especially in cases of kinship or paternity because incorrect analysis of these variants could lead to a false interpretation of the results.

Acknowledgment

We would like to thank all the members of the Paternity and Kinship laboratory/ Medico-legal Directorate for their experienced scientific support.

Conflict of Interest

The authors declare no conflict of interest.

Source of funding

Nil

This research was conducted based on Article 2 of the Iraqi Forensic Medicine Law of 2013

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