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Tri-Allelic Autosomal STR Patterns Observed in Pakistani Population during Forensic Case Work

أنماط التتابعات القصيرة المتكررة الجسدية ثلاثية الأليل التي لوحظت في السكان الباكستانيين أثناء العمل على القضايا الجنائية

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Abstract

The tri-allelic pattern is a genotyping abnormality that can be observed during routine short tandem repeat (STR) profiling in the field of forensic science. Fourteen tri-allelic patterns have been observed in nine different loci during routine forensic casework consisting of 20,000 STR profiles. All these 20,000 STR unrelated profiles were profiled using AmpFlSTR Identifiler Plus® and Global Filer® kits. Tri-allelic patterns can be divided into two types based on RFUs (relative fluorescent units) in peaks of three component alleles. Unequal RFUs of all three peaks are observed in the Type-I pattern, whereas in the Type-II pattern, RFUs of all three peaks are nearly equal. A total of nine novel tri-allelic genotypes were observed out of 20,000 unrelated individuals in the Pakistani population. All of these forensic cases belonged to the Type-I pattern which means that the sum of the height of two smaller peaks is nearly equal to the height of the third larger peak. The frequency of occurrence for all these pat-

Keywords: Forensic science; Tri-allelic pattern; Pakistani population; Punjab Forensic Science Agency (PFSA).

المستخلص

النمط الثلاثي الأليل هو نمط غير معتاد في التنميط الجيني يمكن ملاحظته أثناء الفحص الروتيني للتتابعات القصيرة المتكررة (STR) في المجال الجنائي. تمت ملاحظة أربعة عشر نمطًا ثلاثي الأليل في تسعة مواقع مختلفة أثناء إجراءات الروتينية للقضايا الجنائية وعددها عشرون ألف ملف (20000) تعريف لأنماط التتابعات القصيرة المتكررة. تم تحديد جميع ملفات تعريف ال STR لأشخاص غير أقارب والبالغ عددها 20000 ملف تعريف باستخدام مجموعات أطقم تكثير تحديد ملفات التعريف الوراثية (AmpFlSTR Identifiler Plus®) وأطقم تكثير فايلر جلوبال (Global Filer®. يمكن تقسيم الأنماط الثلاثية الأليل إلى نوعين بناءً على وحدات (RFU) (وحدة تقدير الأشعة الفلوريسية) في مكونات قمم الأليلات الثلاثية. تم ملاحظة أن وحدات RFU غير المتكافئة لجميع القمم الثلاثة في نمط النوع الأول، بينما في نمط النوع الثاني، تكون وحدات RFU لجميع القمم الثلاثة متساوية تقريبًا. تمت ملاحظة ما مجموعه تسعة أنماط وراثية ثلاثية الأليلات من بين 20.000 ملف لأفراد لا تربطهم علاقة قرابة في عينة من السكان الباكستانيين. وقد كانت كل ملفات التعريف الوراثية لهذه القضايا تنضوي تحت النمط الأول مما يعني أن مجموع ارتفاع القمتين الصغريين يساوي تقريبًا

الكلمات المفتاحية: علوم الأدلة الجنائية، النمط ثلاثي الأليل، السكان الباكستانيون، هيئة علوم الأدلة الجنائية في البنجاب.

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terns was compared with already reported data. In this study, eight novel tri-allelic patterns have been reported which are not listed in the National Institute of Standards and Technology (NIST) database as well as in any published article.

ارتفاع القمة الثالثة الأكبر. تمت مقارنة معدل تكرار حدوث جميع هذه الأنماط مع بيانات الملفات التي تمت تسجيلها بالفعل سابقاً. في هذه الدراسة، تم التعرف على ثمانية أنماط جديدة ثلاثية الأليل وغير مدرجة في قاعدة بيانات المعهد الوطني الأمريكي للمعايير والتكنولوجيا (NIST) وكذلك لم يتم ذكره في أي مقالة سابقة منشورة.

1. Introduction

STRs base identification of humans has been widely applied all over the world to solve criminal/civil cases in the field of forensic DNA analysis. In forensic DNA analysis, Short Tandem Repeat (STR) profiling is routinely used for rape, murder and paternity cases. Amplification products of evidence and reference samples are subjected to run on capillary electrophoresis for separation of dye-labeled fragments produced during Polymerase Chain Reaction (PCR). These dye-labelled fragments are then separated and detected through capillary electrophoresis on genetic analyzers.

During routine casework analysis, unusual genotype patterns may be observed in the form of OMR, OL, Null allele and tri-allele (Type-I, Type-II). Three-banded pattern genotyping irregularity is named as tri-allelic pattern. The tri-allelic pattern is an uncommon category of genotyping irregularities that can be observed during forensic casework STR profiling [1,2]. Unusual peak patterns are sporadically observed in STR typing due to mutations, genetic variations and other abnormalities (environmental factors).

Tri-allelic patterns are mainly of two types. In the Type-I pattern, three peaks would be observed which may have unequal heights. The sum of the heights of two smaller peaks is nearly equivalent to the height of one larger peak. This Type-I pattern is usually the result of somatic mutation having no effect on duplication of chromosome number and is non-transferable to the next generation during gamete formation.

In the Type-II pattern, two or three peaks would be observed with peak heights of 2:1 and 1:1:1 respectively. This type of genotype may occur due to chromosomal duplication and rearrangement events in the germ line. Commonly observed Type-II pattern has three alleles with equal heights. Rare events observed for two peaks with a peak height ratio of 2:1 show homozygosity of one allele with a larger height. Very rare and unusual genotypes with only one allele may occur which may not be distinguishable in height from the homozygous peak. It was revealed in recent studies that the Type-I tri-allelic patterns have more frequency of occurrence than the Type-II pattern [3,4].

The occurrence of both types of tri-allelic patterns was searched from routine forensic samples processed in Punjab Forensic Science Agency (PFSA), Lahore, Pakistan. A comparison was made with the NIST database and the latest reported tri-allelic patterns from different populations. NIST database has the largest number of published tri-allele genotypes.

2. Material and methods

Buccal swab standards from suspects, victims, alleged biological fathers, mothers, siblings and children were collected during the routine forensic casework analysis. DNA was extracted using the organic method and was processed for STR typing including autosomal and sex STR (Amelogenin) loci using Amp-FISTR Identifiler Plus® and Global Filer® kits. Capillary electrophoresis was performed

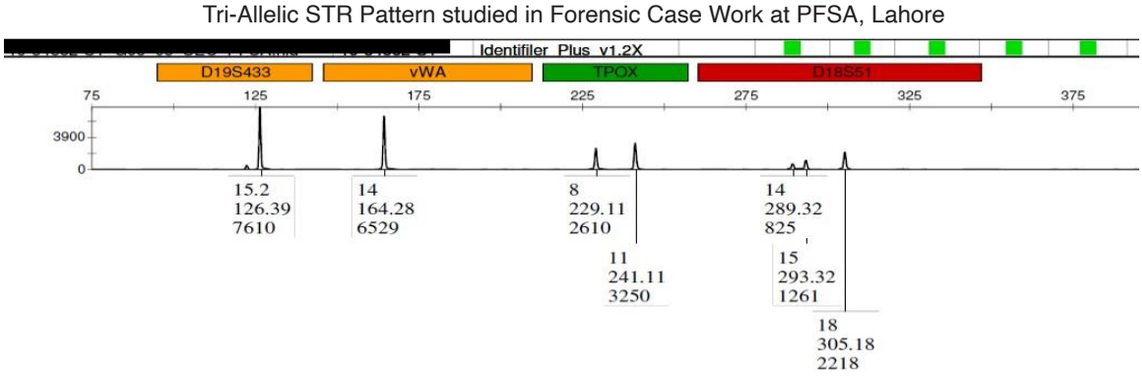

Figure 1- *Tri-allelic genotype 14; 15; 18 at the genetic locus D18S51*

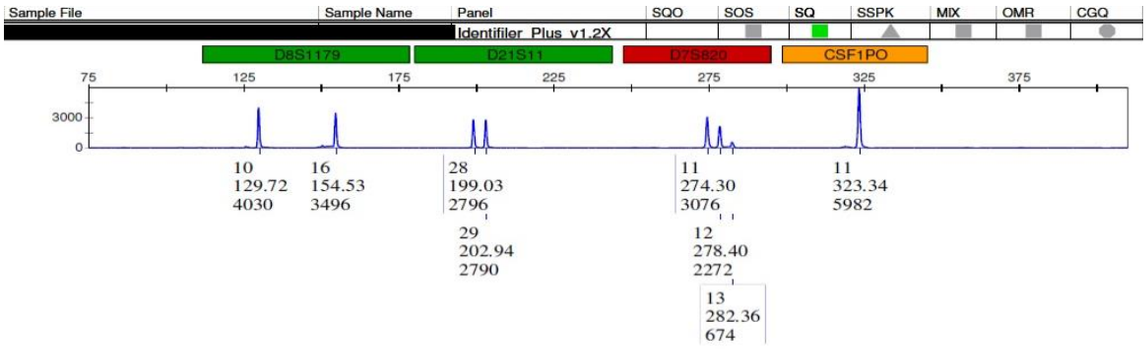

Figure 2- *Tri-allelic genotype 11; 12; 13 at the genetic locus D7S820*

on ABI 3500 Genetic Analyzers for the separation of PCR products and alleles were identified using Gene-Mapper ID-X Software v1.4. All the unrelated casework samples containing tri-allelic patterns were reconfirmed at extraction and amplification stages employing the same method.

3. Results

The first tri-allelic pattern with genotypes of 14;15;18 was observed at the genetic locus D18S51. Fig. 1 The heights of all three peaks were unequal whereas the sum of the RFUs of two smaller peaks was almost equal to the RFU of the largest peak at the same locus. This was an example of the Type-I tri-allelic pattern according to the nomenclature of Clayton et al. [3]. The frequency of this tri-allelic pattern at this locus was 0.00005% which was lower than 0.0056% for CODIS loci in the Chinese population [4]. The second tri-allelic pattern was observed at the same locus with genotype observed 16; 17; 18 with the same peak height pattern which is already reported in NIST database. Third tri-allelic genotype 14;15;16 was observed for the same locus also reported by NIST database [6].

A NIST reported tri-allelic pattern with genotype 23; 24; 25 at genetic locus FGA was observed and the frequency of occurrence was 0.00005% which was lower than the already published 0.0050% for the Chinese population [4]. The heights of alleles 24 and 25 almost sum up to the height of allele 23. In another case, the same genotype was observed. Again this was the example of a Type-I tri-allelic pattern in which peaks 24 and 25 summed up to the peak height of allele 23. A second genotype 23; 24; 26 was observed for the same locus. Fig. 9 The peak heights for alleles 24 and 26 are almost equal

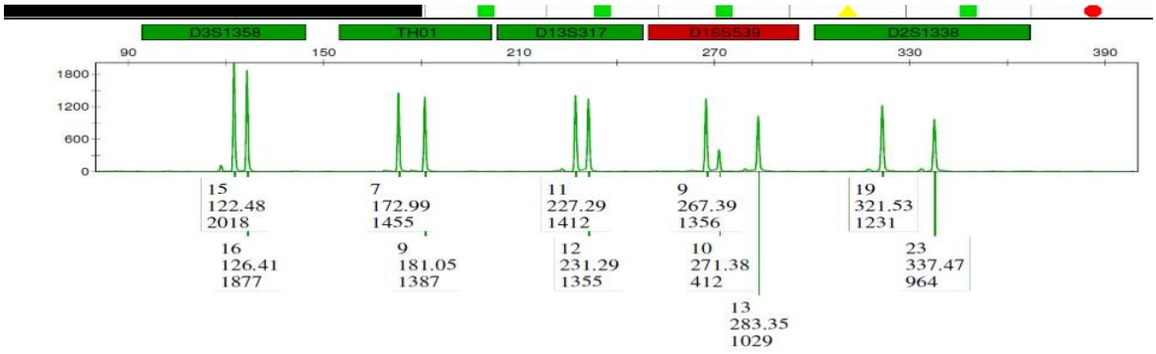

Figure 3- *Tri-allelic genotype 9; 10; 13 at the genetic locus D16S539*

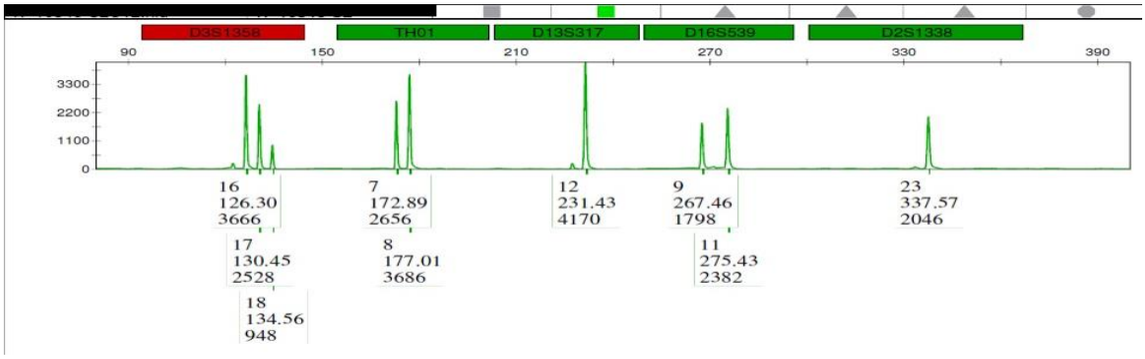

Figure 4- *Tri-allelic genotype 16; 17; 18 at the genetic locus D3S1358*

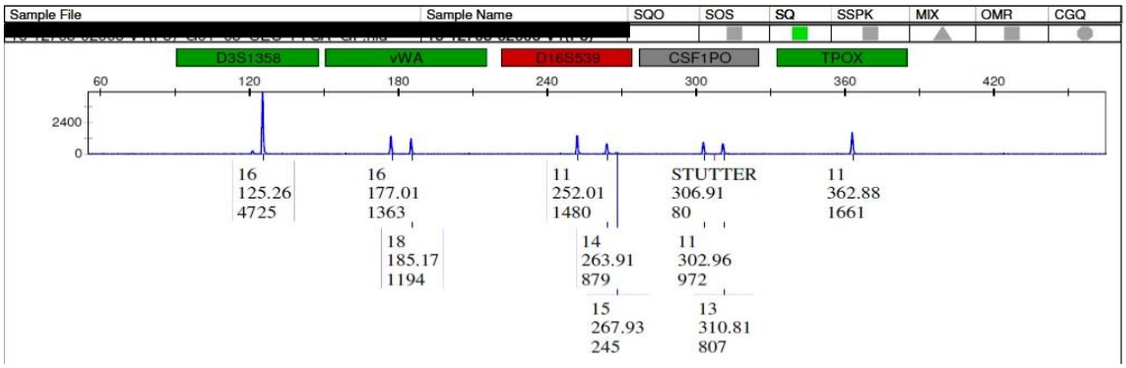

Figure 5- *T ri-allelic genotype 11; 14;15 at the genetic locus D16S539*

but the peak height for 23 is almost 1/3 of the single peak present at this locus.

Another genotype of 11; 12; 13 for Type-I pattern. Fig. 2 was observed at genetic locus D7S820 with a frequency of occurrence 0.000050% which has not been reported for the type-1 pattern [4]. Tri-allele pattern at locus vWA having genotype 16; 17; 18 was also observed as Type-I pattern which is already reported in NIST database. The frequency of occurrence of this pattern at the same locus was 0.000050% in comparison with the already published frequency of 0.0022% for Chinese population [4]. Another pattern with genotype 15;16;18 was also observed.

Tri-allele pattern at locus D16S539 with Type-I genotype 9;10;13. Fig. 3 was observed with a fre-

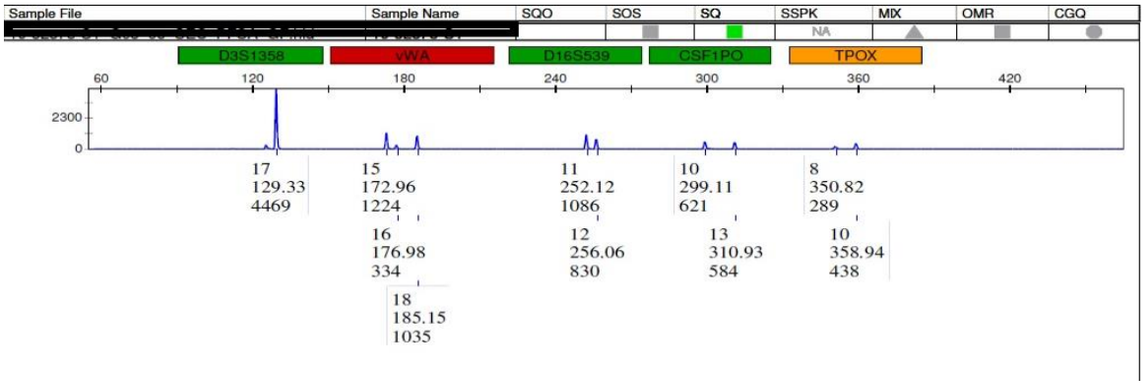

Figure 6- *Tri-allelic genotype 15;1 6; 18 at the genetic locus vWA*

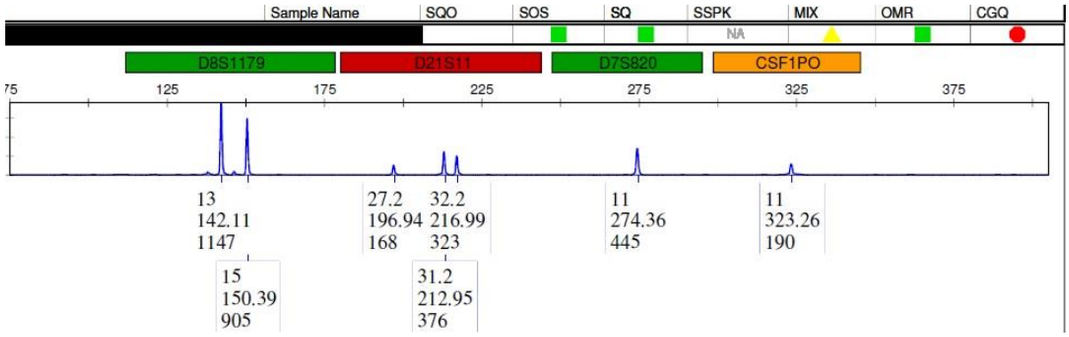

Figure 7- *tri-allelic genotype 27.2;31.2;32.2 at the genetic locus D21S11*

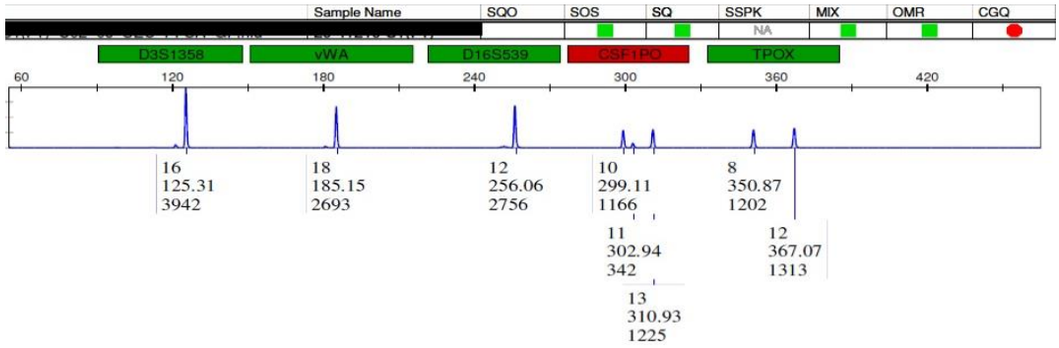

Figure 8- *tri-allelic genotype 10;11;13 at the genetic locus CSF1PO*

quency of occurrence of 0.000050% whereas no such pattern of Type-I at same locus was observed in the Chinese population [4]. 2nd genotype 11; 14; 15 was observed at the same locus and was confirmed through two rounds of extraction and profiling. Another tri-allele pattern with the Type-I genotype of 16; 17; 18 was observed at locus D3S1358 with a frequency of occurrence of 0.000050%. This had been reported in the Chinese population where the frequency was 0.0011% [4], Fig. 4. This genotype has already been published at NIST website but with Type-II pattern.

One tri-allele pattern with genotype 27.2; 31.2; 32.2 was observed on locus D21S11 Fig. 7 with the frequency of occurrence of 0.000050% in the Pakistani population which was far below than already

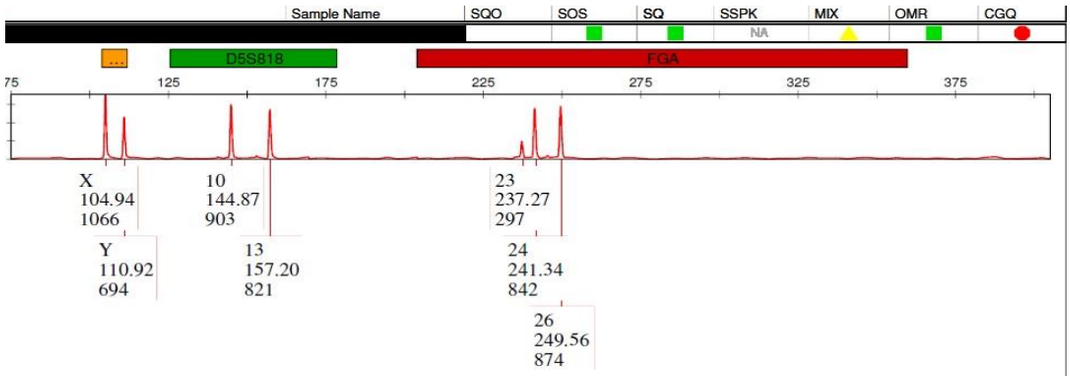

Figure 9- *tri-allelic genotype 23;24;26 at the genetic locus FGA*

published 0.0017% for Chinese population [4]. One tri-allele genotype 14;15;16 was observed at locus D8S1179 and was confirmed through a second round of extraction and profiling. Other tri-alleles were also reported at the same locus in 2020 [4, 11]. Tri-allelic pattern with genotype 10; 11; 13 at locus CSF1PO had a frequency of occurrence of 0.000050% as compared to CODIS reported for the Chinese population at 0.0022%.

4. Discussion and conclusion

Huel and co-workers [8] reported 15 different tri-allelic cases from which only 3 patterns were of Type-II whereas the rest were of Type I. It indicated that the frequency of Type-I pattern (unequal peak heights) is much higher than Type-II which has also been confirmed by our observation during casework. Type-I genotypic pattern can be explained by the somatic mutation of an allele during the normal growth of an individual which may result in chimers with some cells containing the mutant alleles and some having the original allele which may further cause the unequal peak height of all three alleles at the same locus [9, 10].

We report here tri-allelic variants at nine different loci including D18S51, D21S11, D7S820, CSF1PO, D3S1358, D16S539, vWA, D18S51 and FGA with fourteen different genotypes in fifteen unrelated individuals from the profile pool of 20,000 unrelated Pakistani individuals at PFSA. Out of fourteen, 08 genotypes are novel which are not published in the NIST tri-allele database and are being reported for the first time. 401 different patterns of tri-allele variants of autosomal STRs including Type-I and Type-II have been reported on the NIST database website.

Type-II genotypic pattern is caused by the localized duplication of the chromosomes during the meiosis stage before gamete formation which is the reason for all three alleles having equal peak heights. Type-II tri-allelic inheritance case was reported at genetic locus D3S1358 by Vidal and Cassar [5]. At the time of article writing, 401 tri-allelic patterns have been observed so far at different loci with the highest reported frequency at locus D18S51 which was 44 in 401 with the highest percentage of 10.97 as reported in NIST STR base [6].

The overall highest frequency of tri-allelic pattern was observed at D18S51 with genotype pattern of 16; 17; 18, 14; 15; 18 and 14; 15; 16, with a frequency of occurrence of 0.00015 % in the Pakistani population which is also the highest frequency reported by NIST 1(0.97%) for the same locus. We observed genotype pattern 14;15;18 as a novel finding that has not been published in the NIST database nor in any other published data. In a review article by J. Fan et al. 13; 14; 16 genotype at locus D18S51 and 22; 24; 25 geno-

Table 1- *Details of 9 observed novel tri-allelic patterns in the Pakistani population. NIST tri-allelic patterns are also shown for comparison*

Number of Novel Tri-allele pattern(s) Observed	Novel Type-1 Tri-allelic pattern(s)	Frequency from PFSA database	from PFSA % database	Tri-alleles Reported NIST	from NIST % database	Locus
1	32.2 ,31.2 ,27.2	0.00005	0.005	27	6.733	D21S11
1	13 ,12 ,11	0.00005	0.005	22	5.486	D7S820
1	13 ,11 ,10	0.00005	0.005	9	2.244	CSF1PO
1	type-2)18 ,17 ,16 (already reported	0.00005	0.005	11	2.743	D3S1358
2	/13 ,10 ,9 15 ,14 ,11	/0.00005 0.00005	0.001	13	3.242	D16S539
1	18 ,16 ,15	0.00005	0.005	26	6.484	vWA
1	18 ,15 ,14	0.00005	0.005	44	10.973	D18S51
1	26 ,24 ,23	0.00005	0.005	40	9.975	FGA

type for locus FGA was reported [8]. The genotypes 14; 15; 18 for D18S51 and 23; 24; 26 for FGA are also novel findings as these have not been reported in the NIST database nor in any publication [6].

The second novel genotype 23; 24; 26 was observed in the FGA locus which showed the two tri-allelic patterns in three samples having genotypes 23; 24; 25 and 23; 24; 26 with 0.00015 % in the Pakistani population and 13.3% among observed tri-allele patterns whereas NIST reported 9.97% of this pattern for the same locus [6].

Genetic locus D16S539 showed two novel genetic tri-allelic patterns 9;10;13 and 11;14;15 with frequency of occurrence 0.0001% in the Pakistani population and 13.3% among observed tri-allele patterns. The frequency of occurrence in NIST database is 3.42% among all reported tri-alleles patterns [6]. Two patterns 15; 16; 18 and 16; 17; 18 were observed on genetic locus vWA with a frequency of occurrence 0.0001% in the Pakistani population and 13.3% among observed tri-allele patterns whereas NIST reported 6.48% for the same locus among all tri-allelic patterns [6]. One of the genotypes 15; 16; 18 was novel.

D21S11, D7S820 and CSF1PO with genotypes 27.2; 31.2; 32.2; 11; 12; 13 and 10; 11; 13 respectively were also novel with the frequency of occurrence 0.000050% in the Pakistani population and comprised 6.6% among observed tri-allele patterns [6]. NIST reported frequencies for the loci D21S11, D7S820 and CSF1PO were 6.73%, 5.48% and 2.24% respectively [6].

One genotype pattern 16;17;18 was observed on locus D3S1358 with Type-I which has already been reported in the NIST database but with Type-II category [6]. The frequency of occurrence was 0.00005% in the Pakistani population and 6.6% among observed tri-allele patterns whereas NIST reported 2.74% for the same locus among all tri-allelic patterns. The last genotype pattern was observed at locus D8S1179 with a frequency of occurrence of 0.00005% in the Pakistani population and 6.6% among observed tri-allele patterns observed [6]. It was also published in NIST database with the same genotype having a frequency of occurrence of 5.48% among all reported tri-alleles.

We conclude that tri-allelic patterns in the Pakistani population reported here had a few novel pat-

terns and we need to continue to record these anomalies. The patterns reported help analysts during the interpretation stage and they can make an informed decision when such an anomaly is detected.

Conflict of interest

The authors declare no conflicts of interest.

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Punjab Forensic Science Agency, Lahore, Pakistan.

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