University of Tripoli
Faculty of Sciences
Zoology Department / Developmental Biology

Mutation of 15 Autosomal STR Loci used in Paternity Testing in the Libyan Population

Amaal Rajab Beleid Mohammed

Prof. Taher Ahmed Shaibi
(Professor)

Dr. Othman A. Mohamed
(Associate Professor)

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التوقيع:

عميد الكلية
الاسم: أ.د. رمضان المبروك الجدي
التوقيع:

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Student name: Amaal Rajab Belied Mohammed.

Signature:

Date:

University of Trioli
Faculty of Science
Zoology Department

Mutation of 15 Autosomal STR Loci used in Paternity Testing in the Libyan Population

Amaal Rajab Beleid Mohammed

This thesis has been approved by the examination committee

Dr. Mustafa M. Drah (External examiner)
Faculty of Science, Misurata University

Dr. Fauzia M. Alrabty (Internal examiner)
Faculty of Science, University of Tripoli

Prof. Tahe A. Shaibi (Supervisor)
Faculty of Science, University of Tripoli

Dr. Fathi G. AL Sghair
Head of Graduate Studies and Training office

Prof. Ramadan E. Eljadi
Dean of the Faculty

22/09/2022

جـامعة طرابلس
كـلية الـعـلـوم
قسم علم الحيوان

الطفرات في 15 موقع من التتابعات المتكررة القصيرة الجسمية المستخدمة لاختبارات الأبوة لبعض السكان الليبيين

أمال رجب بلعيد محمد

الرسالة مجازة من قبل لجنة المناقشة

الاسم: د. مصطفى محمد دراه كلية العلوم، جامعة مصراتة	العضو الخارجي	.....
الاسم: د. فوزية محمد الرابطي كلية العلوم، جامعة طرابلس	العضو الداخلي	.....
الاسم: أ.د. الطاهرأحمد الشائبي كلية العلوم، جامعة طرابلس	المشرف	.....

الاعتماد

د. فتحي جمعة الصغير
مدير مكتب الدراسات العليا والتدريب

أ.د. رمضان المبروك الجدي
عميد الكلية

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منير مكتب الدراسات العليا بكلية العلوم

عميد الكلية جامعة طرابلس

Mutation of 15 Autosomal STR Loci used in Paternity Testing in the Libyan Population

Amaal Rajab Beleid Mohammed (Master degree).

University of Tripoli (2022).

Prof. Taher Ahmed Shaibi (Professor) and Dr. Othman A. Mohamed (Associate Professor).

ABSTRACT

Background: Short Tandem Repeats (STR), which are characterized by genetic polymorphism, are powerful genetic markers widely used in forensic genetic fields. Unfortunately, mutations in different STR loci may make interpreting STR data difficult. As a result, the mutation rate of STR loci is crucial for data interpretation in human identification and paternity testing. **Aim**: This study aims to determine locus-specific mutations of 15 autosomal STR markers used in paternity testing in the Libyan population and calculate the mutation rate of these 15 autosomal STR markers. **Materials and Method:** To confirm that the STR loci have been mutated in the Libyan population, we investigated the mutation of autosomal STRs in paternity tests, including 172 trios and duos sets, in a DNA fingerprint laboratory, over a period of four years (2012-2015) by using AmpFlSTR® Identifiler™, AmpFlSTR® IdentifilerPlus™ kits from applied biosystem. We analyzed 3285 parent-child meiotic allelic transfer, including 15 autosomal STRs, that corresponded to the expanded CODIS core of 15 STRs (D8S1179, D7S820, D5S818, D3S1358, CSF1PO, THO1, FGA, D21S11, D19S433, D2S1338, VWA, TPOX, D18S51, D16S539, and D13S317). **Results:** We detected nine mutation events in 7/15 loci in 219 meiosis numbers. Six cases out of the nine detected allelic changes were one-step mutations, two cases were two-step mutations, and one case was a three-step mutation. The proportion of paternal versus maternal mutations was 6:3. We observed that mutations caused by the deletion of a repetitive unit occurred more frequently than mutations caused by the insertion of a unit, where the ratio between them was 5:4. D7S820, D3S1358, TH01, D13S317, D19S433, D2S1338, and VWA were all found to be mutation-free. The overall germline mutation rate across the 15 STR loci was 2.74×10^{-3}. The observed mutation rates across the 15 STR loci ranged from 0.0000 to 0.00913. The highest mutation rates were observed at loci D8S1179 and D21S11, while the lowest mutation rate was observed in CSF1PO, D18S51, D16S539, FGA and TPOX. **Conclusion:** This work is the first attempt to generate a local database of autosomal STR mutations for the population of Libya to be used in forensic genetic analysis. The findings of this study are critical for determining the correct likelihood ratio in the Libyan population if mutation rates are to be used. Therefore, it has been determined that more research with a larger population is required for the interpretation of STR loci where no mutations have been identified.

Keywords: Autosomal STRs, Mutation rate, Paternity test, Libyan population.

الطفرات في 15 موقع من التتابعات المتكررة القصيرة الجسمية المستخدمة لاختبارات الأبوة لبعض السكان الليبيين

آمال رجب بلعيد محمد (ماجستير).

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أ.د الطاهر أحمد الشائبي (أستاذ) و د. عثمان عبد الجليل محمد (أستاذ مشارك).

المستخلص

التتابعات المتكررة القصيرة (STR)، التي تتميز بتعدد الأشكال الوراثية، هي علامات وراثية قوية تستخدم على نطاق واسع في المجالات المختلفة للطب الشرعي. قد تجعل الطفرات التي تظهر في مواقع STR المختلفة تفسير بيانات STR أمرًا صعبًا. نتيجة لذلك، فإن حساب معدل الطفرات في مواضع STR أمر بالغ الأهمية لتفسير بيانات STR المستخدمة في تحديد هوية الإنسان واختبار الأبوة. **الهدف:** تهدف هذه الدراسة إلى تحديد الطفرات الخاصة بالمواقع الـخمسة عشر على الصبغيات الجسدية المستخدمة في اختبار الأبوة في المجتمع الليبي وحساب معدل الطفرات لهذه المواقع. **مواد وطرق العمل:** للتأكد من حدوث تطفر في مواقع STR في المجتمع الليبي، تم فحص طفرة المتتابعات المتكررة القصيرة الجسدية STR المستخدمة في اختبارات الأبوة في مختبر تحاليل البصمة الوراثية، لعدد 172عائلة، حيث كانت هذه العائلات في مجموعات ثلاثية وثنائية، والتي جُمعت على مدى أربع سنوات (2012-2015)، تم التحليل باستخدام كواشف AmpFlSTR® Identifiler ™، AmpFlSTR® IdentifilerPlus ™ من شركة Applied biosystem. قمنا بتحليل 3285 أليل منقول من الوالدين إلى الأبناء لخمسة عشر موقعاً للتتابعات المتكررة القصيرة الجسمية، والتي تتمثل في CODIS الموسع المكون من 15 موقع STRs وهي كالتالي: (D8S1179، D7S820، D5S818، D3S1358، CSF1PO، THO1، FGA، D21S11، D19S433، D2S1338، VWA، TPOX، D18S51، D16S539 وD13S317). **النتائج:** وُجدت تسع حالات من الطفرات في سبع مواقع STR من أصل الخمسة عشر موقعا التي تم فحصها حيث كان العدد الميتوزي 219. ست حالات من حالات التغيرات الأليلية (الطفرات) التسعة التي تم اكتشافها كانت طفرة من خطوة واحدة، وحالتان لطفرة من خطوتين وحالة واحدة من ثلاث خطوات. كما كانت نسبة الطفرات الأبوية مقابل الطفرات الأمومية (6: 3). لاحظنا أن الطفرات الناتجة عن حذف وحدة من المتتابعات المتكررة القصيرة حدثت بشكل متكرر أكثر من الطفرات الناتجة عن إدخال وحدة من المتتابعات المتكررة القصيرة، حيث كانت النسبة بينهما (5: 4). وُجد أن المواقع الآتية: TH01 D3S1358, D7S820 D19S433،D13S317 ، D2S1338 وVWA جميعها خالية من الطفرات. كان إجمالي معدل الطفرات للخمسة عشر موقعًا هو 2.74×10^{-3}. تراوحت معدلات الطفرات الملحوظة عبر المواقع المدروسة من 0.0000 إلى 0.00913. كما لوحظ أن أعلى معدل تطفر كان في الموقعين D8S1179 وD21S11. لوحظ أدنى معدل تطفر في المواقع CSF1PO، D18S51، D16S539، FGA وTPOX. **الخلاصة:** هذا العمل هو المحاولة الأولى لإنشاء قاعدة بيانات محلية لطفرات مواقع التتابعات المتكررة القصيرة الجسمية لسكان ليبيا لاستخدامها في اختبارات الطب الشرعي. نتائج هذه الدراسة ستكون مهمة لتحديد نسبة الاحتمالية الصحيحة لاختبارات النسب في السكان الليبيين إذا كان سيتم استخدام معدلات الطفرات عند حساب مؤشرات القرابة. مع ذلك، هناك حاجة إلى مزيد من البحث مع عدد أكبر من السكان لفحص مواقع STR التي لم يتم الكشف عن طفرات فيها.

الكلمات الدالة: المتتابعات المتكرر القصيرة الجسمية، معدل الطفرات، اختبار الأبوة، السكان الليبيين.

DEDICATION

To all members of my family

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Amaal Mohammed

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AB	Applied Biosystem
AABB	American Association of Blood Banks
AF	Alleged Father
AM	Alleged Mother
AL	Allelic Ladder
AM	Alleged Mother
BP	base pair
BU	Buccal swab
BL	Blood
C**H**	Child
CKI	Combined Kinship
CMP	Combined Matching Probability
CODIS	Combined DNA Index System
CPI	Combined Paternity Index
CSF1	PO Human c-fms Proto-Oncogene for CSF-1 Receptor Gene
DNA	Deoxyribonucleic Acid
DNTPS	Deoxy Nucleotide Tri Phosphates
DNA SEQ	Deoxyribonucleic Acid sequence
DTT	Ditheothreitol
EDTA	Ethylene Diamine Tetra Acetic Acid
GD	Gene Diversity
FBI	Federal Bureau of Investigations
FGA	Human Fibrinogen Alpha Chain Gene
HE	Expected Heterozygosity
HO	Observed Heterozygosity

OL	Off Ladder
H	Heterozygosity
HUM	Human
MgCL2	Magnesium Chloride
MDG	Multi-dimensional Scaling
mt-DNA	Mitochondrial DNA
MI	Maternity index
MINI STR	Minisatellite DNA
NA	Not Available
nt	Nucleotide
Pr	Probability
PCR	Polymerase Chain Reaction
PD	Power of Discrimination
PE	Probability of Exclusion
PIC	Polymorphism Information Content
POP	Probability of Paternity
POP-4	Performance Optimized Polymer 4
PI	Paternity Index
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
S	Single Copy Sequence
SSR	Simple Sequence Repeats
SMM	Stepwise mutation model
SSDNA	Single strand DNA
STR	Short-tandem Repeats
T	Thymine
TH01	Human Tyrosine Hydroxylase Gene
TPM	Two-phase model
TPOX	Human Thyroid Peroxidase Gene
UK	United Kingdom
USA	United States of America

VNTR	Variable Number of Tandem Repeats
VWA	Human von Willebrand Factor Gene

1. INTRODUCTION

Short-tandem repeats (STRs), also known as simple sequence repeats (SSRs) or microsatellites, are short tandemly repeated DNA sequences with 1–6 bp repetitive motifs that form series of up to 100 nucleotides (nt) in length (Fan and Chu, 2007).

STR-markers that are located on autosomal and sex chromosomes are used mainly in forensic examination to solve identification problems and, most importantly, disputed paternity/maternity and kinship tests (Mustafayev *et al.*, 2019), because selected microsatellite loci show a high degree of polymorphism and their analysis is feasible and reproducible even on low amounts of DNA or degraded human material (Dauber *et al.*, 2012).

In contrast to other genetic markers, STR mutations are typically higher than other genetic markers ($10^{-6} – 10^{-2}$) nt per generation (Gaviria *et al.*, 2017). A mutation event in DNA markers must be taken into careful consideration in genetic paternity determination (Jacewicz *et al.*, 2004). The principal mutation takes place in the slippage of the polymerase during DNA replication (Gaviria *et al.*, 2017), also known as slipped strand mispairing, DNA slippage, or polymerase slippage (Fan and Chu, 2007). When a repetitive region is in replication, DNA strands are associated incorrectly, giving as a result the insertion or deletion of repetitive units (Gaviria *et al.*, 2017).

The entire concept of paternity testing is predicated on comparing DNA profiles. In the case of mismatching at STR loci between the potential father and a child, the relationship between them can be assigned as non-biological paternity, which leads to the exclusion of biological paternity (Zametica *et al.*, 2018).

Most parentage cases in paternity investigations can be solved using the 13–15 STR markers found in commercially available kits. However, when the results show an inconsistency between the genotype of the child and the suspected parents in a specific forensic marker, an exclusion case may be mistakenly assigned in a true paternity case. If it is determined that the inconsistency is caused by a mutation, the mutation rate must be included in the reported results (Mardini *et al.*, 2013).

Parentage testing can be used in duo cases, which are cases in which genetic information about the mother or father is missing. However, in the absence of genetic information about the mother or father, a mismatch in the STR loci in parentage testing of duo cases may make determining parentage difficult (Zhang *et al.*, 2014). Mutations at

STR loci are recognized as alleles that are not inherited in the Mendelian pattern. When a mutation is discovered, the number of analyzed loci must be increased in order to increase and validate the paternity index. As a result, much attention must be paid to analyzing spontaneous mutations that may result in an incorrect exclusion (Zametica *et al.*, 2018).

1.1. The aim of Study

This study aims to:

1) Determine locus-specific mutations of 15 autosomal STR markers used in paternity testing in the Libyan population.
2) Calculate the mutation rate of these 15 autosomal STR markers.

2. LITERATURE REVIEW

2.1. STR and DNA typing

The human genome is composed of about 3 billion base pairs, and while it is large, only about 5% of it contains genetically relevant information, also known as gene-coding DNA. The remaining 95% is made up of non-coding DNA and is made up of repeated sequences in some places. Although the human genome is largely similar between individuals, there are still enough differences to allow people to be distinguished (Buadu, 2018).

There are numerous loci in the human genome that can be used for identification. Single nucleotide polymorphisms (SNPs), Short-tandem repeats (STRs (Goodwin *et al.*, 2020). Short-tandem repeats are simple blocks of DNA that are highly polymorphic in non-coding DNA regions. STRs are short DNA-motives of 2-7 base pairs in length that are typically repeated 5-50 times and are found near the centromere of the chromosomes (Buadu, 2018), in other reference mentioned that short tandemly repeated DNA sequences with 1–6 bp repetitive motifs that form series of up to 100 nucleotides (nt) in length(Fan and Chu, 2007) . To identify STR repeat sequences, the length of the repeat unit is used. Dinucleotide repeats are composed of two nucleotides that are repeated repeatedly next to each other. In the core repeat, there are three nucleotides in trinucleotides, four in tetranucleotides, five in pentanucleotides, and six in hexanucleotides (Abu Halima *et al.*, 2008).

Tetranucleotides, which are sequence motives of four base pairs, are the most common STRs used in forensic genetics. The STR-markers chosen are distributed across as many chromosomes as possible to ensure that they are inherited independently (Abu Halima *et al.*, 2008). There are some benefits for using tetranucleotide STR loci in DNA typing over other markers (di- and trinucleotide repeat STRs) (Abu Halima *et al.*, 2008; Buadu, 2018):

- The ability to generate small PCR product sizes where forensic DNA evidence is frequently degraded, making PCR amplification products difficult to obtain. The STRs, on the other hand, are small in size, making them ideal targets for use in forensic genetics, which aids in information recovery from degraded DNA specimens;

- A small allele size range, which reduces allelic drop-out due to preferential amplification of smaller alleles;
- Decreased stuttering product formation compared to dinucleotide repeats, which aids in the interpretation of PCR results.

2.2. STR locus nomenclature

The terminology for DNA markers is fairly simple. The gene name is used in the designation if a marker is part of or falls within a gene. The short tandem repeat (STR) marker TH01, for example, is derived from the human tyrosine hydroxylase gene on chromosome 11. The '01' in TH01 refers to the repeat region in question, which is located within intron 1 of the tyrosine hydroxylase gene. To indicate that a locus is from the human genome, the prefix HUM- is sometimes added to the beginning of its name. As a result, the STR locus TH01 would be listed correctly as HUMTH01 (Butler, 2005).

Outside of gene regions, DNA markers can be identified by their chromosomal position. Markers that are not found within gene regions include the STR loci D5S818 and DYS19. The 'D' in these cases stands for DNA. The following character denotes a chromosome number, 5 for chromosome 5 and Y for the Y chromosome. The 'S' indicates that the DNA marker is a single copy sequence. The final number represents the order in which the marker was discovered and classified for a specific chromosome. Each identified DNA marker is given a unique number using sequential numbers. Thus, for the DNA marker D3S1358:

D DNA

3 Chromosome 3

S Single Copy Sequence

1358 1358th locus described on chromosome 3 (Abu Halima *et al.*, 2008).

2.3. Applications of STR-based systems

Short-tandem repeat (STR) analysis is an informative approach to genetic identification that is commonly associated with:

1. Criminal investigation (forensic examination), such as rape cases.
2. Family Relationship Testing (kinship testing) such as;

- Paternity testing: To seek the biological father of a child, or to seek the biological child of father.
- Maternity testing: To seek the biological mother of a child, or to seek the biological child of a mother.

3. Identity testing.
4. Population genetics studies (Ancestry testing) (Alsafiah, 2019).

2.4. Using STRs kinship testing for human identification

STRs have a variety of characteristics that make them suitable for human identification. Individuals have different STR alleles, and some are more common than others. Because allele frequencies differ between populations, STRs are very effective at separating individuals within populations (Buadu, 2018).

Forensic DNA testing was first used in the mid-1980s, when Dr. Alec Jeffreys of the University of Leicester used restriction fragment length polymorphism (RFLP) and variable number of tandem repeats (VNTR) analysis of DNA to show genetic linkages between related individuals (McKiernan and Danielson, 2017). They were quickly used in forensic genetics after they were discovered and characterized (Goodwin *et al.*, 2020). The Federal Bureau of Investigation (FBI) Laboratory in the United States evaluated and selected 13 STR loci (CSF1PO, FGA, TPOX, TH01, VWA, D5S818, D3S1358, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11) as the core STR loci for forensic purposes in 1997 (McKiernan and Danielson, 2017).

At present time, STR loci are the most commenly used genetic markers in paternity test, forensic investigation and human identifecation (Xiao *et al.*, 2018). Through genetic testing, remains from missing people and mass disasters have been identified, and questions about parentage and kinship have been answered (McKiernan and Danielson, 2017).

The following characteristics are among the selection criteria for candidate STR loci in human identification applications (Abu Halima *et al.*, 2008):

- High discriminating power, typically greater than 0.9, with observed heterozygozygosity greater than 70%.
- Separate chromosomal locations to ensure that closely linked loci are not chosen.
- When combined with other markers, the results are robust and reproducible.

- The stuttering characteristics are minimal.
- Low rate of mutation.
- Allele lengths are predicted to be in the 90–500 bp range with smaller sizes.

Because each individual inherits one STR copy from each parent, the locus will display two possible alleles. The individual will be homozygote for that marker if the two copies have the same repeat numbers. If the two inherited copies have different repeat numbers, the individual is heterozygote for that marker (Buadu, 2018). By examining a sufficient number of STR markers, each individual will obtain a unique DNA profile that will make them different from others (Buadu, 2018).

Identifying distant relatives such as a sibling, half-sibling, grandparent/grandchildren, uncle/nephew, aunt/niece, or first cousin may also be part of kinship testing. Parentage testing is the most basic type of kinship testing, which typically seeks to identify the true father (paternal testing) by looking for shared alleles between the alleged father and the disputed child (Alsafiah, 2019).

2.5. STR Mutation

STR markers are highly polymorphic, owing to their relatively high mutation rate. This is due to their molecular architecture, which makes them particularly vulnerable to a particular type of mutation (Sun *et al.*, 2014).

Replication errors, known as replication slippage, can result in STR mutations. During replication, the DNA polymerase separates (slips) from the DNA template and anneals to nearby homologous sequences. This error is more likely to occur in repetitive DNA regions, resulting in repeated deletions or insertions in the sequence. As a result, STRs typically have length polymorphism, but point mutations that change the sequence within one of the repeats or the flanking region may also occur. Any changes that do not affect the length of the fragment cannot be detected by electrophoresis, but can be detected by sequencing instead (Buadu, 2018).

Strand-slippage replication is widely accepted as the primary mechanism of STR mutation. Several factors, including sex, age, and the number of repeat units, are thought to play a role in STR locus mutation (Shao *et al.*, 2016). It is well known that mutations on the STR markers under consideration almost always consist of a gain or loss of a specific number of STR repeat motif units (Slooten and Ricciardi, 2013).

If (d) such units are lost or gained, we refer to the mutation as a “d-step mutation”, where d is the distance of the mutation. It is also known that 1-step mutations are by far the most common, and that mutations become less likely as the mutational distance increases. However, if a d-step mutation has been passed down from father to child, the genotypes of the father, mother, and child can be determined (Slooten and Ricciardi, 2013).

In paternity testing, the presence of germline mutations at STR loci makes determining non-fatherhood more difficult. Because STRs are more likely to be mutated than conventional genetic markers, the likelihood of detecting mutations rises. While STR systems have been the most frequently employed systems in paternity tests and in human identification (HID) for more than two decades, the discovery of genetic inconsistencies during paternity investigation research can complicate the analysis and resolution of these cases (Geada *et al.*, 2003). Because of their susceptibility to slippage events during DNA replication, these loci are highly prone to mutations, resulting in high polymorphism and multiple alleles at each of them (Pilli and Berti, 2021). The susceptibility of STR to mutation results in sporadic mismatches among the father/mother and child when paternity is tested. Although the rate of single-step mutations is likely overestimated, reports based on a large number of meiosis show that one-step mutations account for over 90% of STR mutations, with two-step mutations coming in second and multiple-step mutations occurring infrequently. As a result, multistep STR mutations are much less common in paternity testing than single-step mutations (González-Herrera *et al.*, 2020).

Paternity testing is based on allele matches at the 15 STR loci between the alleged father, child and mother (trio cases). In duo cases, motherless or fatherless, alleles of the STR loci are matched between the child and the alleged father or between the child and the alleged mother, respectively. If the child's and alleged father's DNA profiles matched, the test was considered a non-excluded paternity case. However, if the alleged father's and child's DNA profiles did not match, the case was classified as an excluded paternity case, likewise in the case of the child and the alleged mother (El-Alfy and Abd El-Hafez, 2012). The calculation of the paternity index (PI)/maternity index (MI) in parentage testing is dependent on the genotypes of the tested individuals (homozygote or heterozygote), and specific equations are used to calculate each of the probabilities (i.e., X and Y) (Alsafiah, 2019). Prior to conclude that a putative father is not the biological father of a child, in cases of disputed paternity exanimated by DNA analysis, it is widely

accepted that at least two independent exclusions must be observed (Gunn *et al.*, 1997). But sometimes a faulty interpretation can occur. If even one or two mismatches occur between children and parents resulting from mutations, the mutation must be considered when calculating paternity probability (Xiao *et al.*, 2018).

The likelihoods of paternity are computed as Bayesian á posteriori probabilities (assuming equal á priori probabilities of 0.5 for the two alternative paternity hypotheses) (Poetsch *et al.*, 2013). The exclusion of paternity is when more than two mismatches are found. The following criteria are applied to cases with one or two isolated exclusions:

- It eliminated genotyping mistakes.
- The American Association of Blood Banks (AABB) recommended mutation rate (μ) and power of exclusion (PE) were used for paternity index (PI) computation in the presence of isolated mutations (PI = μ /PE) (García-Aceves *et al.*, 2018).
- A combined paternity index (CPI) is calculated by multiplying all of the individual PIs (Anone, 2021).

In the testing of parentage cases, single inconsistencies are common. If a laboratory concludes that the inconsistency is the result of a mutation, the mutation result must be included in the reported results. The parentage index (PI) for these loci, which is calculated by dividing the mutation frequency by the average probability of exclusion, is the most commonly used formula in laboratories (AABB.org, 2020).

The rates at which new mutations appear are critical in a wide range of genetic applications, including population events, evolutionary disease studies, genetic and ancestry dynamics studies and forensic evidence evaluation. As a result, accurate estimates of mutation rates can be used to quantify the exclusion or inclusion of an alleged parent during parentage testing, as well as convict felons in forensic cases. Thus, when STR markers are used for human identification in both civil and forensic scenarios, the use of advanced tools and a consistent understanding of the STR mutation pathways will ensure reliable results (Ge *et al.*, 2009; Jin *et al.*, 2016).

2.6. Microsatellite Mutation Models

There are two mutation models for the evolutionary dynamics of microsatellites (Sainudiin *et al.*, 2004)

1. The classical stepwise mutation model (SMM): It is the most fundamental and widely used model of microsatellite evolution, in which one repeat unit is either lost, resulting in a contraction, or gained, resulting in an expansion, when a mutation occurs. The stepwise mutation model is excluded if there are more than two mutation steps.
2. The two-phase model (TPM): this is addressed by allowing mutations of one repeat unit (one-phase) with probability p, and mutations of more than or equal one unit(s) (two-phase) with probability $1 - p$, on the other hand, the distribution of multiunit mutation lengths is geometric.

A microsatellite in the SMM and TPM is assumed to mutate at a steady rate, regardless of repeat length (Sainudiin *et al.*, 2004; García-Aceves *et al.*, 2018).

STR mutation rates are critical in forensic and population genetics for evaluating and interpreting genetic evidence (Xu *et al.*, 2019); a high mutation rate of STRs may lead to incorrect interpretation of paternity examinations. As a result, understanding the mutation rate of locus-specific STRs is critical for determining paternity or kinship (Jochens *et al.*, 2011).

Previous studies have investigated the mutation of STR loci in populations from several geographical regions in the world. In Libya there are three studies of autosomal STR markers, but not for their mutation. Two of these studies was conducted to describe allele frequencies for 15 autosomal STR markers, one of them performed in Tunisia on some of Libyan patients (Khodjet-el-Khil *et al.*, 2012), another one performed in Libya by Almallah (2017). The third study was performed by Aboaleid (2016), that focused on using of STRs to identification of human that found in mass graves in some of Libyan cities.

In many of Arabic countries, studies on mutations of autosomal STR markers are not available so far except in Syria whereas, a study of mutation of STRs in Paternity Testing was performed during years from 2007 to 2010 in Laboratory for Research and Genetic Consultation at the Faculty of Medicine at Damascus University, which were 44 cases, the study found two STRs mutations discoverers using STRs Systems in

investigating cases at D3S1358 and D13S317 and in both cases the mutations were from the paternal origin (Elias *et al.*, 2014).

In Egypt, there was a study of paternity testing and forensic DNA typing by multiplex STR analysis in 2012 (El-Alfy and Abd El-Hafez, 2012), but it did not discuss the presence of mutation or mutation rate in STR. In Saudi Arabia, DNA polymorphism for kinship testing in the population of Saudi Arabia was evaluated (Alsafiah, 2019); the study suggested using (The ForenSeq™ DNA Signature Prep Kit) when it was suspected that two or three mismatches were mutations, but did not discuss the mutation rate in STR.

There are several studies describing mutations in some STR loci in Asian, European and Latin American countries, which are used in performing paternity tests. Zametica *et al.* (2018) profiled 1253 people within 583 parenthood testing cases, and 14 mutations were discovered at 11 loci in 13 of them. Mustafayev *et al.* (2019) surveyed 250 family cases of disputed paternity, they discovered mutated alleles in several cases on FGA, D19S433, D13S317 and the D5S818 locus. The mutations most likely affected the paternal alleles, while one affected the maternal allele.

Three genetic incompatibilities were discovered between the child and the putative father, two in STR markers D8S1179, D18S51 and one in RFLP marker D7S21 (Jacewicz *et al.*, 2004),. they suggested that the best solution is to compute the most convenient statistical estimates in each case.

The previous studies focused solely on the presence of mutation at some autosomal STRs, whereas many other studies expanded the research to estimate the mutation rate of some autosomal STRs for specific populations around the world, as well as the gender origin of STR mutation (paternal/maternal mutation) and its rates. One of those studies was performed by Xu *et al.* (2019), who aimed to investigate the mutation rates of 21 autosomal STRs in a population from central South China; they analyzed 3,420 paternity cases from different regions in south China and found the mutation rate of STR loci is 0.9 $x10^{-3}$. The researchers concluded that inter-population comparisons of locus-specific mutation rates of several STRs revealed significant differences. In another population study of 19,037 trios from the population of Mainland China by Zhao *et al.* (2015), they found that the paternal mutation rate was higher than the maternal mutation rate at 15 autosomal STR loci. Furthermore, the average mutation rate in coastal areas was higher

than in inland areas. In another study in a Chinese population, performed by Qian *et al.* (2015), an overall mutation rate of 1.20×10^{-3} was estimated in the populations across 18 out of 19 STR loci, except for the THO1 locus, which had no mutation. The majority of STR mutations (97.7%) were single step mutations, with only a few (2.30%) involving two or more steps. The researchers concluded that the data from their study would be useful in parentage testing, kinship analysis, and population genetics.

In South America, Mardini *et al.* (2013) expanded the study of STR mutation to include mutation rate and types of mutation; they studied 13 STR loci in 10,959 paternity investigation cases in Rio Grande do Sul, Brazil's southernmost state. A total of 355 mutations were found; 348 were one-step gains or losses, three were two-step gains or losses, and four were non-stepwise gains or losses.

As can be seen from the analysis of the above cited literature data, the study of mutations at the practical application of STR-markers has a great importance in forensic individual discrimination and paternal testing. Because of there are no STR mutation studies done before at autosomal STR loci in the Libyan population therefore, this study aims to perform such a study for some of the Libyan population.

3. MATERIALS AND METHODS

3.1. Sample collection

In this study, the samples were obtained from paternity cases that were analyzed in a Libyan DNA fingerprint laboratory of the National Commission for DNA fingerprint Research and Analysis (www.ncra.org.ly) from March 2012 to December 2015.

A total of 172 families were included. 47 of them were trios (father/mother/child), the other 101 were duos (mother/child) and 24 were duos (father/child) confirmed paternity or maternity testing cases.

- **Buccal swabs**

Buccal swabs were used (whatman swab) to take samples from live people in disputed paternity/maternity tests by passing them on the buccal cavity in a circular fashion.

- **Tissue samples**

Samples were taken from unidentified (dead) persons. They consist of blood, muscle tissue, bone or tooth. All samples were kept at -80° C until the start analysis procedures.

3.2. Materials

3.2.1 Chemicals and reagents

Chemicals and reagents used in this study are shown in Table (1):

Table (1) Chemicals and reagents used in this study

Chemicals and reagents	Supplier
Prep filer Express standard:	Applied Bio systems, Foster City, USA
Lysis Buffer	Applied Bio systems, Foster City, USA
DTT	Applied Bio systems, Foster City, USA
Isopropyl Alcohol	Sigma, USA
Alcohol (absolute)	Sigma, USA
Prep filer Express™ cartridges	Applied Bio systems, Foster City, USA

Absolute Ethanol Alcohol 99%	Sigma, USA
Ethylene -Diamine -Tetera -Acetic acid (EDTA)	Sigma, USA
Sodium dodecyl sulfate (SDS) 20%	Sigma, USA
Ampf/STR® YFiler™ Kit	Applied Bio systems, Foster City, USA
Quantifiler® Duo DNA Quantification: PCR reaction Mix Primer set DNA Standard DNA dilution Buffer Standard 200ng/µl	Applied Bio systems, Foster City, USA
Ampf/STR® YFiler ™ Kit: PCR reagents Mix Primer set Control DNA 9947A (10ng/µl) Ampli Taq DNA polymerase Nuclease -Free Water	Applied Bio systems, Foster City, USA
Taq man® RNase plate 96 well	Applied Bio systems, Foster City, USA
Instrument verification plate	Applied Bio systems, Foster City, USA
Water mol Biograde (DNas ، RNase) and protease free (50µl)	Applied Bio systems, Foster City, USA
Spectral calibration kit (cy3™, Dye, cys™, Dye, Texes Red® Dye)	Applied Bio systems, Foster City, USA
Spectral calibration plates with texas7500 Red® Dys	Applied Bio systems, Foster City, USA
Genetic Analyzer Kit	Applied Bio systems, Foster City, USA
Gene scan ™ - 600 Liz® size standard V2.0 (Internal Lane Standard)	Applied Bio systems, Foster City, USA
HiDi™ formamide	Applied Bio systems, Foster City, USA
Ampf/STR® Identifiler® plus Kit	Applied Bio systems, Foster City, USA

Allelic Ladder Identifiler or Identifiler plus	Applied Bio systems, Foster City, USA
Cathode Buffer container 3500 series	Applied Bio systems, Foster City, USA
Anode Buffer container 3500 series	Applied Bio systems, Foster City, USA
performance Optimized polymer POP4™ (960) 3500	Applied Bio systems, Foster City, USA
Capillary Array 30cm	Applied Bio systems, Foster City, USA
10X Genetic Analyzer Buffer with EDTA	Applied Bio systems, Foster City, USA
Condation reagent 3500 series	Applied Bio systems, Foster City, USA
HID5.dye Installation standard Multi-capillary D5-33(Dye set G5) Matrix std	Applied Bio systems, Foster City, USA
Kit (6FAm™, VIC®, NED™, PET®, LIZ®, Dyes)	Applied Bio systems, Foster City, USA
Medical Ethanol 96% v/v	

3.2.2. Disposables

The major disposables used in this study are listed in Table (2)

Table (2) The major disposables used in this study

Item	Supplier
(Buccal swab)	Applied Bio systems, Foster City, USA
What man (FTA)	Applied Bio systems, Foster City, USA
Vacutainer 5ml ks – EDTA TUBE	Weihai Sun Genius – China
Micro Amp® Optical 96-Well Plate and Septa for the 3500 Genetic Analyzer	Applied Bio systems, Foster City, USA
Optical Adhesive Cover film	Applied Bio systems, Foster City, USA
Plate Septa, Retainer and Base	Applied Bio systems, Foster City, USA
Aerosol-Resistant Pipette Filter Tips	Labcon, USA
Eppendorf Tubes	Labcon, USA
Micro Tubes, 1.5ml Capacity	Labcon, USA
Micro Tubes, 0.2ml Capacity	Labcon, USA
Disposables Gloves Free Powder	Weihai Sun Genius – China
(spin tube) sample tube	Applied Bio systems, Foster City, USA
Prepfiler lySep™ columns	Applied Bio systems, Foster City, USA
Prepfiler Elution™ tube	Applied Bio systems, Foster City, USA
Tips and Tip Holders	Applied Bio systems, Foster City, USA
Auto Mate Express™	Applied Bio systems, Foster City, USA
Prepfiler Express™ BTA Plastics	Applied Bio systems, Foster City, USA
Blue rack	Applied Bio systems, Foster City, USA

3.2.3. Equipment

All experiments for this study were done at the Libyan DNA fingerprint laboratory of the National Commission for DNA Research and Analysis. The major equipment that was used is listed in Table (3):

Table (3) The major equipment used in this study

Instruments	Manufacture
DNA Extraction system (Magne sphere ®Technology magnetic separation stand)	Applied Bio systems, Foster City, USA
AB PRISM Genetic analyzer 3500	Applied Bio systems, Foster City, USA
Real time PCR system 7500	Applied Bio systems, Foster City, USA
AB Gene AMP PCR system 9700 Thermal cycler	Applied Bio systems, Foster City, USA
Digital reading Micropipette	Socorex – Switzerland
Thermo Electron corporation Centrifuge (4i) microcentrifuge	Centurion Scientific LTD, UK
Safety Cabinet (class 2)	Heraeus, Germany
Holten Lamin Air, Holten PCR (PCR Hood)	Heraeus, Germany
Shaker (Thermo Mixer comfort)	LW Scientific – USA
Vortex Mixer	LW Scientific – USA
Electrical balance	LW Scientific – USA
Tabletop refrigerated centrifuge	Centurion Scientific LTD, UK
Freezer -80C°	Napco, Czech Republic
Freezer -20C°	LG, Korea

3.3. Methods

3.3.1 DNA extraction

The DNA was extracted from buccal swabs, blood, muscle tissues, bone and tooth by using principle of the magnetic method or (Magnesphere® Technology magnetic separation stand). After transferring the samples to the extraction unit, they were coded by a special code that determine the number of experiment, type of sample and number of sample.

- After transferring the samples to extraction unit, they were coded by special code which determine the number of experiment, type of sample, number of sample as following: L1-07-BU1, BL1, MU1, BO1, TO1.
- The hood was sterilzed by UV for 10 minutes then put filter paper with large size inside the hood.
- DDT solution was prepared as following:
    - Prepared a sterile plastic tube with 10µl volume.
    - Weighted 1.45g from DDT powder then put in the sterile tube.
    - Added 10 µl from nuclease-free water to the sterile tube.
    - Mixed the sterile tube to dissolve DDT powder and forming DDT solution.

        5 µl * 8 samples = 40 µl

- Calculated the volume of lysis buffer which needed to make a mixture with DDT solution according to number of samples in the experiment and was taken into consideration adding of an excess sample over total number of samples to compensate any loss in the volumes of reagents occurring during the preparation of mixtures.

    Lysis buffer: 500 µl * 8 samples = 4000 µl

- The mixture of DDT and lysis buffer was prepared by taking 4000 µl of lysis buffer from the suitable extraction kit and adding it to a sterile tube then adding 40 µl DDT to the sterile tube also, after that closed the sterile tube tightly and finally, mixed the mixture well.

All of experimental procedures of DNA extraction were carried out in accordance with the manufacturer's guidelines.

3.3.2 DNA Quantification

Quantifiler® Duo DNA Quantification kit was used which contains reagent mentioned in Table (4):

Table (4) reagents of Quantifiler® Duo DNA Quantification kit

Reagents	Contents	Quantity
Reaction Mix	One tube contains: dNTPS, BsA, Mgcl2 in %0.05 Azide sodium buffer solution	1.1 ml in one tube
Primer Mix	One tube contains of forward and backward primers connected with fluorescent dyes	0.55 ml in one tube
Taq gold DNA Polymerase	Two tubes contain active enzyme (5 unit / microliter.	100 µl in two tubes
Standard sample Control DNA	One tube contains 0.01nanogram/ microliter of DNA from autosomal cell in 0.05% Azide sodium buffer solution	0.3 ml in one tube

Preparation of DNA standard solutions:

- The standard solutions series were prepared for performing DNA quantitative analysis by using 200ng/µl DNA standard solution from Quantifiler® Duo DNA Quantification kit as a following in the Table (5).

Table (5) Standard solutions from Quantifiler® Duo DNA Quantification kit

Standard solutions	Concentration (ng/µl)	Dilution factor
Standard solution 1	50.00	4
Standard solution 2	16.700	3
Standard solution 3	5.560	3
Standard solution 4	1.850	3
Standard solution 5	0.620	3
Standard solution 6	0.210	3
Standard solution 7	0.068	3
Standard solution 8	0.023	3

- Preparation of the standard solution was performed inside the Hood (Holten Lomin Air).
- Before opening the kit package, keep it at -25° C, and afterward, keep it at 2-8° C.
- Calculation is done by applying the following formula:

C1 * V1 = C2 * V2

200g * 10 =? * 40

Where:

200 g: concentration of DNA standard solution of the kit.

10 µl: volume which taken from DNA standard solution.

C2: required concentration.

40 µl: dilution factor (10 standard + 30 dilution).

e.g.:

Std1:

$$\text{Std.}1 = \frac{200\ \text{ng}/\ \mu\text{l} \ * \ 10}{40}$$

Std.1 = 50 ng/ µl

Std2:

10 µl was taken from first tube of (50 ng/ µl) standard solution to prepare second standard solution (16.7 ng/ µl)

$$\text{Std. } 2 = \frac{60\text{ng/ µl} * 10}{30}$$

Std.2 = 16.7 ng/ µl

30 µl: dilution factor (10 standard + 20 dilution).

Continued of calculation of rest serial standard solution by the same way until 8th standard solution (0.023 ng/ µl).

Preparation of PCR reaction:

- To quantify the amount of extracted DNA from samples, the exact number of samples was determined plus double number of standard solutions and adding extra sample to compensate any loss occur during preparation.
- For each sample the quantities of reagents should be as following:

  Reaction mix: 12.5 µl.

  Primer mix: 8.5 µl.

  DNA polymerase: 2 µl.

- Master mix reagent was prepared by Quantifiler® Duo DNA Quantification kit as following steps:
  - The total number of samples was determined including 16 standard samples.
  - Calculation is done by applying the following formula:

    Reaction mix = 12.5 µl * total number of samples.

    Primer mix = 8.5 µl * total number of samples.

    DNA polymerase = 2 µl * total number of samples.

All of experimental procedures of DNA quantification were carried out in accordance with the manufacturer's guidelines.

3.3.3. PCR – amplification of STR loci

The amplification of the DNA loci of all the study samples was carried out for (15) autosomal STR loci (D8S1179, D7S820, D5S818, D3S1358, CSF1PO, THO1, FGA, D21S11, D19S433, D2S1338, VWA, TPOX, D18S51, D16S539, and D13S317), in addition to the specific sex STR locus (Amelogenin) by AmpFlSTR® Identifiler™, AmpFlSTR® IdentifilerPlus™ kits from (AB) and using AB Gene AMP PCR system 9700 Thermal cycler according to the manufacturer's instructions.

Identifiler plus kit has a system containing five fluorescent dyes (6-FAM™, VIC™, NED™, PET™) that are suitable for automatic analysis of the genetic fingerprint loci in the automatic analysis instruments (Burke, 1996). The reagents listed in table (6) were used in the AmpFlSTR® IdentifilerTM and AmpFlSTR® Identifiler PlusTM kits from (AB).

Table (6) AmpFlSTR® Identifiler™, AmpFlSTR® Identifiler Plus™ kits reagents

Reagents	Contents	Quantity
Reaction Mix	Two tubes contain: dNTPS, BsA, Mgcl2 in %0.04 Azide sodium buffer solution	2 ml divided in two tubes
Primer Mix	One tube contains of forward and backward primers connected with fluorescent dyes	1 ml in one tube
Standard sample Control DNA	One tube contains 0.01 nanogram/ microliter of DNA from autosomal cell in 0.05% Azide sodium buffer solution	0.3 ml in one tube
Allelic Ladder	One tube contains standard sample has all alleles of STR loci	50µl in one tube

Preparation of master mix:

At this stage, the primers and the polymerase enzyme were mixed together in a tube with a total volume of 15µl and DNA of 0.1 ng/µl according to the following steps:

- the total number of samples including standard samples (positive and negative control) was determined.
- The required quantity of the components was calculated with taking into account the calculation of an excess sample for every ten samples to compensate any loss in the volumes of the reagents that occur during preparation as following:
    - The reaction mixture: number of Sample * 10 µl
    - Primer mix: number of samples x 2.5 µl
    - DNA polymerase: number of samples x 2.5 µl
- The reaction master mix was mixed with a vortex mixer for five seconds at 1300 rpm.

All of experimental procedures of DNA amplification were carried out in accordance with the manufacturer's guidelines.

3.3.4 Capillary electrophoresis by Genetic Analyzer

Amplified products were analyzed by capillary electrophoresis by the ABI Prism™ 3500 Genetic Analyzers (AB) the Genemapper® lD software (version v2.1x.v1.1x), and the corresponding allelic ladders included in the kits were employed for allele calling.

the mixture of the reaction was prepared inside the sterilized hood of the Genetic analyzer unit by using Ampf/STR® Identifiler, Identifilerplus from (AB) according to the following steps:

- The total number of samples including standard samples (positive, negative control and allelic ladder) was determined.
- The following reagents were mixed by using vortex mixer for five seconds:

  HiDi™ formamide, Internal Size Standard 600 Liz, Allelic Ladder
- The required volumes of reagents were calculated as follows: (taking into account adding an excess sample to compensate any loss that occurs during preparation):

  Number of samples * 8.5 µl of formamide

Number of samples * 0.5 µl of the 600 Liz

All of experimental procedures of DNA genotyping were carried out according to the manufacturer's instructions.

Genotype determinations:

Immediately after finishing the electrophoresis process, the sample files were transferred from the data collection software data program to Genemapper® lD software (version v2.1x.v1.1x) from (AB).

The ABI 3500 Genetic Analyzer sample data were represented as peaks that corresponded to the various STR alleles amplified from the DNA sample. These detected peaks represent different alleles that appear in multiple colors and are plotted as fluorescent signal intensity, where various fluorescent dyes fuse with the DNA fragments of the samples by means of a laser beam.

The various dye colors were separated and the peaks representing DNA fragments were identified and associated with the appropriate color. The DNA fragments are then sized by comparison to an internal sizing standard. Fragment sizes were checked manually to avoid any false calling of alleles.

Control samples

- **Allelic ladder:**

The allelic ladder is the standard to which STR alleles are compared to obtain the sample genotype. STR genotyping was performed by comparison of the sizes of a sample's alleles to sizes of alleles in allelic ladders for the same loci being tested in the sample.

Where the allele ladder is designed by collecting DNA samples or PCR products from different people with alleles, where each allele represents the differences between the alleles of each gene, the sum or total of these samples is re-amplified to obtain a sample with the most common alleles among people. Alleles were balanced by adjusting the amount used for each allele so that the alleles appear in balanced quantities on the allele scale. For example, to obtain a scale that contains five alleles with 6, 7, 8, 9, and 10 repetition units, samples of people should be included with the following genotypes (7,

10) and (9, 9), also the following genotypes (6, 9), (8, 7), (10, 10), (6, 6), (7, 7), (9, 9) and (10,10) (Sajantila *et al.*, 1992; Baechtel *et al.*, 1993).

- Positive control sample:

Positive control sample 9947 is a female DNA sample extracted by (AB) company that is known for its genotype and DNA concentration (10 ng/µl).

- Negative control:

Nuclease-free water instead of the template DNA was used as a negative control to check for any DNA contamination.

3.4. Mutation analysis

After obtaining all samples' genotypes, a Microsoft Excel spreadsheet was used to arrange the family sets as (trios and duos), and then check all alleles of both parents and children at 15 autosomal STR markers, which were used in the paternity test, to detect if there were any mismatches among them.

Germ line mutations of 15 STRs were investigated for 172 parenthood testing cases: 47 father–mother–child trios, 24 father/child duos, and 101 mother/child duos. This provided a total of 3285 parent/child allele transfers at 15 loci (involving 219 parent–child meiosis numbers) for this study, when one or two mismatches are observed, the possibility of mutations must be considered.

- Meiosis number is calculated as following:

Meiosis is a type of cell division that results in the formation of haploid gametes (1 N) from diploid parental cells (2 N). During meiosis, the number of chromosomes decreases (Roeder, 1997). That is mean the cases which consist of father, mother and child meiosis occurs two time in trios families and one time in duos families, then by a simple arithmetic:

Meiosis number = 2 * number of trios cases + 1 * number of duos cases

= (47*2) + (101+24)

= 94 + 125

= 219.

- Meiotic allelic transfer is computed by using simple addition as following:

Meiotic allelic transfer = (2 * (trios cases) * number of STR studied) + ((duos cases) * number of STR studied)

Meiotic allelic transfer = (2*47*15) + ((101+24) *15)

= 1410 + 1875

= 3285.

- The mutation rate for each STR locus was calculated by dividing the total number of meiosis by the number of mutation events, and 95 % confidence intervals (CIs) were obtained for both the single STR mutation rates and the overall using a website tool (https://statpages.info/confint.html) based on a binomial distribution (Lu *et al.*, 2012).
- The combined paternity index (CPI) was calculated for each case by a kinship calculator (Kin Calc 4.0) with and without mutation rate.
- Expected heterozygosity (Hexp) for each locus and polymorphism information content (PIC value) were calculated by using Excel-microsatellite toolkit version 3.1 (Park, 2001), where genotype data was obtained from all family members.
- Correlation analysis between mutation rate and heterozygosity was carried out by IBM SPSS statistics 20, whereas Spearman's test was used to assess this relationship between H exp and the mutation rate at the 15 STRs (Lu *et al.*, 2012).

4. RESULTS

This study was based on the analysis of 15 autosomal STR loci using the Ampf/STR PCR reagents Identifiler, Identifiler plus for 317 samples from 172 families. The samples were obtained from paternity cases and analyzed in a Libyan DNA fingerprint laboratory of the National Commission for DNA Research and Analysis.

4.1. Overview of mutation events detected in 15 STR loci in the Libyan population

Germ line mutations of 15 STRs were studied for 172 parenthood testing cases: 47 father–mother–child trios, 24 father/child duos, and 101 mother/child duos. This provided a total of 3285 parent/child allele transfers at 15 loci (involving 219 parent–child meiosis numbers) for this study. Mutations were observed in nine cases.

In the first case, deletion mutation at the D21S11 locus, assuming that the paternal allele is not mutated (i.e., child1 inherited allele 29 from the father), the more likely scheme of mutation formation can be represented as one-step mutation process: (child) Ch1 (27) = (Alleged mother) AM1 (28) – 1 repeat or (child) Ch1 (27) = (Alleged father) AF1(29) – 2 repeats if the paternal allele is mutated. The first scheme is more logical. (allele size reduction) (Table 7, appendix 1).

The second mutational case at the D8S1179 locus represents a paternal allele mutation. This mutation can be represented as the insertion of 1 repeat (one-step mutation process): Ch2 (16) = AF2 (15) +1 repeat. (allele size extension) (Table 8, appendix 2).

Table (7) The mutation case at the D21S11 locus observed in family 1 of paternity test. (Ch = child, AF = alleged father, AM = alleged mother)

Locus	AF1		AM1		Ch1		Status
D8S1179	14	15	13	15	14	15	match
D21S11	29	29	28	29	27	29	mismatch
D7S820	8	11	8	12	8	8	match
CSF1PO	10	10	12	12	10	12	match
D3S1358	15	17	15	17	15	15	match
TH01	9	9	7	10	9	10	match
D13S317	12	13	10	11	10	13	match
D16S539	11	12	10	11	11	12	match
D2S1338	18	20	17	19	18	19	match
D19S433	12	14	12	13.2	12	13.2	match
VWA	16	18	17	19	16	19	match
TPOX	8	8	8	11	8	11	match
D18S51	15	18	12	22	15	22	match
D5S818	11	12	11	13	11	11	match
FGA	20.2	24	23	24	23	24	match
Amloginine	X	Y	X	X	X	X	

Table (8) The mutation case at the D8S1179 locus observed in family 2 of the paternity test. (Ch = child, AF = alleged father, AM = alleged mother).

Locus	AF2		AM2		Ch2		Status
D8S1179	15	15	12	13	13	16	mismatch
D21S11	29	31	32.2	34.2	31	32.2	match
D7S820	11	11	9	11	9	11	match
CSF1PO	13	13	11	12	12	13	match
D3S1358	18	18	16	16	16	18	match
TH01	8	8	6	9	6	8	match
D13S317	12	12	12	12	12	12	match
D16S539	9	12	11	13	9	11	match
D2S1338	22	23	17	19	17	23	match
D19S433	13	14	14	14.2	14	14.2	match
VWA	15	15	15	16	15	16	match
TPOX	9	11	8	8	8	9	match
D18S51	15	15	13	13	13	15	match
D5S818	12	13	12	13	13	13	match
FGA	19	20	21	26	19	26	match
amloginine	X	Y	X	X	X	Y	

The next mutation event is observed at the TPOX locus, representing a maternal allele mutation. This mutation can be represented as the insertion of 1 repeat (one-step mutation process): Ch3 (11) = AM3 (10) + 1 repeat. (i.e., Child3 inherited allele 11 from her father), or the scheme of mutation formation can be represented as allele dropout: mutations in the primer-binding region at the TPOX locus of Child3 cause null alleles which lead to false homozygosity (Table 9, appendix 3).

Table (9) The mutation case at the TPOX locus observed in family 3 of the paternity test. (Ch = child, AF = alleged father, AM = alleged mother).

Locus	AF3		AM3		Ch3		Status
D8S1179	13	14	13	15	13	15	match
D21S11	29	29	29	30	29	30	match
D7S820	9	12	8	11	8	9	match
CSF1PO	10	11	10	13	10	11	match
D3S1358	17	18	16	17	16	18	match
TH01	7	7	6	7	6	7	match
D13S317	12	12	11	11	11	12	match
D16S539	9	11	9	13	9	11	match
D2S1338	24	25	19	20	20	24	match
D19S433	13	13	12	13.2	13	13.2	match
VWA	16	17	15	16	16	16	match
TPOX	6	11	8	10	11	11	mismatch
D18S51	17	21.2	13	16	13	17	match
D5S818	12	12	12	12	12	12	match
FGA	25	25	23	25	25	25	match
amloginine	X	Y	X	X	X	X	

The fourth mutation case was detected at the CSF1PO locus represents a paternal allele mutation. This mutation can be represented as the deletion of 1 repeat (one-step mutation process): Ch4 (11) = AF4 (12) – 1 repeat (allele size reduction). Or the scheme of mutation formation can be represented as allele dropout: mutations in the primer-binding region at the CSF1PO locus of Child4 cause null alleles which lead to false homozygosity (Table 10, appendix 4).

Table (10) The mutation case at the CSF1PO locus observed in family 4 of the paternity test. (Ch = child, AF = alleged father, AM = alleged mother).

Locus	AF4		AM4		Ch4		Status
D8S1179	14	15	12	13	12	14	match
D21S11	28	30	29	32.2	29	30	match
D7S820	10	10	11	12	10	12	match
CSF1PO	12	12	10	11	11	11	mismatch
D3S1358	17	17	17	18	17	17	match
TH01	9	9	6	6	6	9	match
D13S317	10	11	12	12	10	12	match
D16S539	11	11	11	12	11	11	match
D2S1338	17	21	17	17	17	17	match
D19S433	13	14	12	14	12	14	match
VWA	16	16	14	14	14	16	match
TPOX	8	12	9	9	8	9	match
D18S51	14	18	12	15	14	15	match
D5S818	11	12	12	12	12	12	match
FGA	22	24	23	24	22	23	match
amloginine	X	Y	X	X	X	Y	

The fifth mutation event is observed at the D18S51 locus. It is a dropout allele caused by mutations in the primer-binding region for the paternal, resulting in non-amplification and the loss of the paternal allele, whereas the child appears to be homozygous (false homozygous) for different alleles at the D18S51 locus, or the scheme of mutation formation can be represented as the deletion of 3 repeats (three-step mutation process): Ch5 (14) = AF5 (17) – 3 repeat (allele size reduction) (Table 11, appendix 5).

Table (11) The mutation case at the D18S51 locus observed in family 5 of the paternity test. (Ch = child, AF = alleged father, AM = alleged mother).

Locus	AF5		AM5		Ch5		Status
D8S1179	12	13	13	14	13	13	match
D21S11	28	35	29	29	28	29	match
D7S820	8	10	9	12	8	9	match
CSF1PO	11	12	10	11	11	12	match
D3S1358	17	17	14	15	14	17	match
TH01	10	10	6	9	9	10	match
D13S317	10	11	11	12	10	11	match
D16S539	11	13	10	12	12	13	match
D2S1338	17	18	17	23	17	23	match
D19S433	13	14	12	15	12	13	match
VWA	17	18	16	19	16	18	match
TPOX	8	9	8	8	8	8	match
D18S51	17	17	14	14	14	14	mismatch
D5S818	10	11	12	13	11	13	match
FGA	21	26	22	23	22	26	match
amloginine	X	Y	X	X	X	Y	

The sixth mutation event occurred at the D16S539 locus. It is another case of dropout allele caused by mutations in the primer-binding region for the paternal allele, resulting in non-amplification and the loss of the paternal allele, whereas the child appears to be homozygous for different alleles at the D18S51 locus.

OR the scheme of mutation formation can be represented as insertion of 2 repeat (two-step mutation process): Ch6 (14) =AF6 (17) + 2 repeats. (allele size extension) (Table 12, appendix 6)

Table (12) The mutation case at the D16S539 locus observed in family 6 of the paternity test. (Ch = child, AF = alleged father, AM = alleged mother).

Locus	AF6		AM6		Ch6		Status
D8S1179	11	13	13	14	11	13	match
D21S11	30	32.2	30	30	30	30	match
D7S820	11	12	8	12	8	12	match
CSF1PO	12	12	12	12	12	12	match
D3S1358	17	17	15	15	15	17	match
TH01	6	7	6	9	6	9	match
D13S317	12	12	10	10	10	12	match
D16S539	9	9	11	11	11	11	mismatch
D2S1338	17	17	17	21	17	17	match
D19S433	13.2	14	12	14	12	13.2	match
VWA	16	16	16	16	16	16	match
TPOX	8	11	8	9	8	9	match
D18S51	12	19	12	17	12	19	match
D5S818	9	14	10	12	9	10	match
FGA	22	22	20	24	20	22	match
amloginine	X	Y	X	X	X	Y	

In the seventh mutation case at the D21S11 locus, which can be explained simply: Ch7 (29) =AF7 (30) - 1 repeat (allele size reduction). Where the mutation happened in the paternal allele, this mutation can be represented as the deletion of 1 repeat (one-step mutation process) (Table 13 appendix 7).

Table (13) The mutation case at the D21S11 locus observed in family 7 of the paternity test. (Ch = child, AF = alleged father, AM = alleged mother).

Locus	AF7		AM7		Ch7		Status
D8S1179	12	15	13	15	15	15	match
D21S11	30	30	32.2	32.2	29	32.2	mismatch
D7S820	10	11	8	10	8	10	match
CSF1PO	11	12	10	12	11	12	match
D3S1358	15	16	18	18	16	18	match
TH01	9	10	9.3	9.3	9	9.3	match
D13S317	11	12	11	13	12	13	match
D16S539	11	14	12	12	12	14	match
D2S1338	16	17	20	24	16	24	match
D19S433	14	15.2	13	13.2	13	15.2	match
VWA	16	19	14	18	14	19	match
TPOX	8	11	9	11	8	9	match
D18S51	13	22	16	18	13	18	match
D5S818	12	12	8	11	8	12	match
FGA	22	23	21	24	22	24	match
amloginine	X	Y	X	X	X	Y	

Other mutations were found in duos families, one of which was a fatherless test (table 14, appendix 8) and the other a motherless test (table 15, appendix 9). In the fatherless mutation case at the D8S1179 locus, the maternal allele mutation is represented. This mutation can be represented as a deletion of 2 repeats (two-step mutation process): Ch8 (12) = AM8 (14) – 2 repeats (allele size reduction).

Table (14) The mutation case at the D8S1179 locus observed in family 8 of the paternity test. (Ch = child, AF = alleged father, AM = alleged mother).

Locus	AM8		Ch8		Status
D8S1179	14	14	10	12	mismatch
D21S11	29	31.2	31.2	33.2	match
D7S820	9	10	8	9	match
CSF1PO	9	10	9	12	match
D3S1358	16	16	15	16	match
TH01	9	9	7	9	match
D13S317	8	11	11	11	match
D16S539	10	11	11	13	match
D2S1338	17	20	17	17	match
D19S433	13	14	13	14	match
VWA	14	19	14	18	match
TPOX	8	11	8	8	match
D18S51	14	16	12	16	match
D5S818	10	12	12	12	match
FGA	23	26	23	24	match
amloginine	X	X	X	Y	

In the motherless mutation case at the FGA locus, representing paternal allele mutation. This mutation can be represented as the insertion of 1 repeat (one-step mutation process): Ch9 (24) = AF9 (23) + 1 repeat (allele size extension).

Table (15) The mutation case at the FGA locus was observed in family 9 of the paternity test. (Ch = child, AF = alleged father, AM = alleged mother).

Locus	AF9		Ch9		Status
D8S1179	12	14	12	14	match
D21S11	29	31	29	29	match
D7S820	11	12	10	11	match
CSF1PO	10	13	10	10	match
D3S1358	16	17	15	16	match
TH01	7	9	7	9.3	match
D13S317	12	14	14	14	match
D16S539	12	13	13	13	match
D2S1338	17	25	17	25	match
D19S433	14	14	12	14	match
VWA	15	17	17	18	match
TPOX	11	11	9	11	match
D18S51	12	15	15	19	match
D5S818	11	12	12	12	match
FGA	23	23	20	24	mismatch
amloginine	X	Y	X	Y	

4.2. Steps and gender origin of STR mutations

We found four presumably null alleles, all of which were found in trio cases, and most of the mutation cases were in trio families. Six cases out of the nine detected allelic changes were one-step mutations, two cases were two-step mutations and one case was three-step (Table 16).

We observed that mutations occurred in paternal alleles more frequently than mutations that occurred in maternal alleles where the ratio between them was 6:3 (Table 18). We observed that mutations caused by the deletion of a repetitive unit occurred more frequently than mutations caused by the insertion of a unit, where the ratio between them was 5:4 (Table 16).

D7S820, D3S1358, TH01, D13S317, D19S433, D2S1338, and VWA were all found to be mutation-free. (Table 19)

Table (16) Overall mutation events observed in all families and its source during paternity tests

Cases	Locus	Type of mutation	Maternal / Paternal
Family 1	D21S11	one-step (allele size reduction)	M
Family 2	D8S1179	one-step (allele size extension)	P
Family 3	TPOX	one-step (allele size extension)	M
Family 4	CSF1PO	one-step (allele size reduction)	P
Family 5	D18S51	three-step (allele size reduction) or drop allele	P
Family 6	D16S539	two-step (allele size extension)	P
Family 7	D21S11	one-step (allele size reduction)	P
Family 8	D8S1179	two-step (allele size reduction)	M
Family 9	FGA	one-step (allele size extension)	P

4.3. Mutation rates

We found that the mutation rate for each STR locus was as shown in Table 17.

Table (17) Number of meiosis, number of mutations detected and mutation rates studied in 15 STR loci in the Libyan population.

Locus	No. of Meiosis	No. of Mutation	Mutation rate	95% CIs
D8S1179	219	2	0.00913242	0.0011-0.0326
CSF1PO	219	1	0.00456621	0.0001-0.0252
D18S51	219	1	0.00456621	0.0001-0.0252
D16S539	219	1	0.00456621	0.0001-0.0252
D21S11	219	2	0.00913242	0.0011-0.0326
FGA	219	1	0.00913242	0.0011-0.0326
TPOX	219	1	0.00456621	0.0001-0.0252
Total	3285	9	0.00273972	0.0013-0.0052

4.4. Combined Paternity index (CPI)

We estimated the combined paternity index (CPI) as shown in Table 18. CPI was calculated for cases twice: once when there was no mutation rate estimated for the Libyan population by elimination of the mutated loci, and the second time when using the mutation rate of the American population, which inserted in KIn CALc 4.0 software to illustrate the change in the value of paternity index of cases.

Table (18) CPI without mutated loci and CPI with mutation loci

Families	CPI without mutated loci	CPI with mutated loci
Family 1	3.10 E+13	7.40 E+11
Family 2	1.60 E+15	9.30 E+13
Family 3	6.20 E+11	7.90 E+07
Family 4	4.80 E+11	2.70 E+09
Family 5	1.50 E+10	6.40 E+06
Family 6	8.00 E+08	1.60 E+06
Family 7	9.10 E+12	1.90 E+11
Family 8	2.50 E+04	1.90 E+01
Family 9	4.00 E+04	2.70 E+02

4.5. Correlation analysis between mutation rate and heterozygosity

Expected heterozygosity (Hexp) for each locus and polymorphism information content (PIC value) is shown in Table 19, where genotype data was obtained from 172 families.

In this study, the Spearman correlation coefficient “R” between Hexp and the STR mutation rate was 0.107 ($p = 0.352$). The findings indicated that there was no significant relationship between mutation rates and corresponding He values at 14 loci. (Figure 1)

Table (19) Heterozygosity, PIC values, number of alleles and mutation number studied in 15 STR loci in the studied population

Locus	No. of allele	No. of mutation	mutation rate	He	PIC values
D8S1179	11	2	0.00913	0.823	0.799
D21S11	13	2	0.00913	0.809	0.784
D7S820	8	0	0	0.779	0.746
CSF1PO	8	1	0.00456	0.730	0.680
D3S1358	9	0	0	0.768	0.728
TH01	8	0	0	0.788	0.754
D13S317	8	0	0	0.773	0.739
D16S539	7	1	0.00456	0.775	0.739
D2S1338	13	0	0	0.857	0.841
D19S433	14	0	0	0.837	0.817
VWA	9	0	0	0.811	0.782
TPOX	7	1	0.00456	0.668	0.615
D18S51	13	1	0.00456	0.879	0.865
D5S818	10	0	0	0.753	0.716
FGA	15	1	0.00456	0.862	0.845

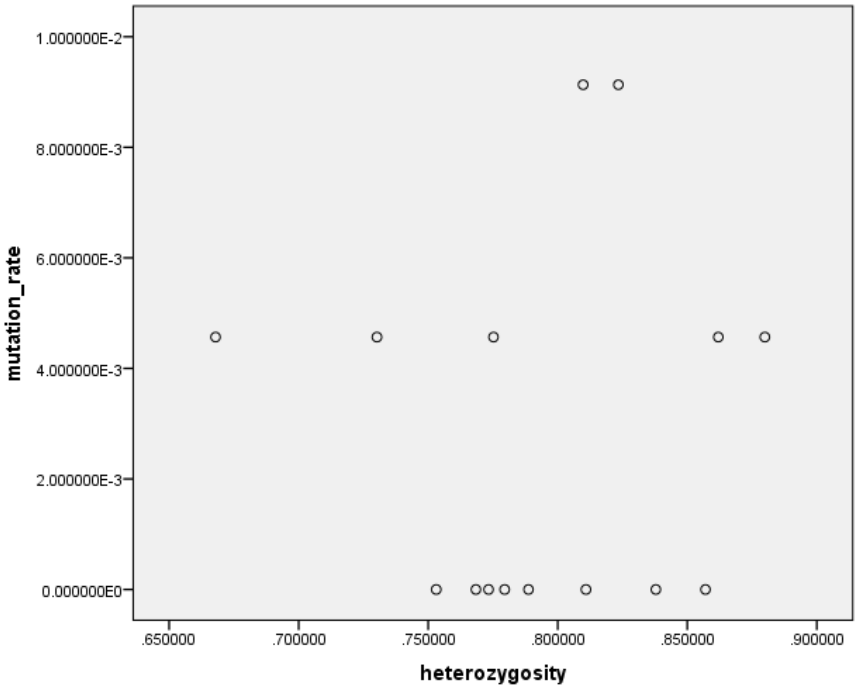

Figure (1) Correlation analysis between the mutation rate of 15 STR and the corresponding heterozygosity by spearman test (P=0.352, R=0.107).

5. DISCUSSION

In forensic laboratories, autosomal STR analysis is the primary method for determining parentage. A child's STR gene type is determined by his father and mother. Mutational events may be responsible for one or two STR loci mismatches and may lead to false conclusions.

The current work investigates the mutation events and rates in 15 autosomal STR markers used in paternity testing in 172 Libyan families. This study used Applied Biosystems HID kits: AmpFlSTR® IdentifilerTM and AmpFlSTR® IdentifilerPlusTM.

To reduce the impact of genotyping errors, all mutations were confirmed by re-genotyping both parents and children.

5.1. Overview of mutation events detected, steps and gender origin of 15 STR loci

In the 172 parenthood testing cases that have been investigated in the present study, only nine events of mutation in autosomal STR were detected. This study's sample size, which equals 317 persons, is composed of families from different Libyan cities, for example: Tripoli, Al Zawia, Benghazi, Misrata, Zliten, some of the Nafosa mountain cities, AL Jamail, and some of villages in the south. The study's samples were not specifically collected for the study of mutation, but rather were referred to the Libyan DNA center for paternity investigation. Therefore, we faced some limitations, such as sample type (duos sets more than trios sets), the size of the sample may appear to be small, and the geographical distribution of the study sample may not cover all the Libyan population in terms of different ethnicities. However, it is sufficient for a preliminary study of STR mutations in Libya because no such study has ever been conducted in the country. In a study performed by Huang *et al.* (2021), mutation rates of 28 autosomal STRs were determined from 8708 paternity testing cases in the Chinese Han population, As China's population is nearly 1,45 billion (Worldometer, 2022), 91% of the population was Han Chinese (Song, 2022). In a similar study that was conducted in Korea, the sample size was 545 trio sets, and 36 mutations were observed in 14 STR loci (Kim *et al.*, 2021). The current population of the Republic of Korea is more than 51 million (Worldometer, 2022). To ascertain the prevalence of locus-specific mutations in the Bosnian-Herzegovinian population, 583 parenthood testing cases were studied. Of the total cases, 14 mutation events were discovered at 11 loci (Zametica *et al.*, 2018), whereas the Bosnian-Herzegovinian population is more than 3 million (Worldometer, 2022)

As can be seen from the above cited analysis of different population data, the sample size of all the studies is small in comparison to their large populations, but it is sufficient to provide a good indication of the presence and rate of mutations in autosomal STR markers.

In this study, 7 out of 9 mutational events occurred in trio sets. Some potential mutational events may be overlooked in duos, resulting in an underestimated mutation rate in the population.

The majority of mutational events (6 events) were single-step mutations, accounting for 66.6% of total mutations. According to the strand-slippage replication mechanism and stepwise mutation model (SMM), single-step mutation was strongly preferred over multiple-step mutation (Amos, 2016). Based on previous findings, more than 90% of mutational events were one-step mutations (Sun *et al.*, 2014). Also, the study by Vieira *et al.* (2017) confirmed the previous finding, which found that 96.2% of the mutations observed in the Brazilian group were single-step mutations, which is consistent with the general notion that most mutations consist of single-step repeat gain or loss because of strand slippage during replication (Diegoli *et al.*, 2014).

There were two events of mutations that were double-step, one event was triple-step mutation, and there was no detection of four-step mutation among the multiple-step mutational events.

In terms of parental origin, paternal origin mutations (66.6%) were more common than maternal origin mutations (33.3%). Due to the rapid cell divisions that occur in the male gamete precursor cells during spermatogenesis, the greater number of spermatogenetic divisions of precursor cells in comparison to oogenesis results in more frequent paternal mutations. Mutation rates in paternal lineage cells are approximately five times higher (Drożdziok *et al.*, 2018). Vieira *et al.* (2017) agreed with our observation of a greater paternal contribution to the burden of mutations in the offspring.

Furthermore, in this study, it was found that overall mutations with losses were slightly higher than mutations with gains. This is consistent with the findings of Dauber *et al.* (2003) and the data reported by Xu *et al.* (2019), who both discovered that mutations with one to two complete repeat unit losses cause more mutations than mutations with one to two complete repeat unit gains. But this was inconsistent with the finding of Gusmo et al. who found that mutations with gains in the STR core were more common than

mutations with losses (Gusmao *et al.*, 2005). The ratio of loss/gain increased as the length of the repeat unit increased, indicating that as the number of repeat units increased, the repeat unit became easier to lose (Zhao *et al.*, 2015).

The possible variations of mutational events in STRs discussed in this study may be due to sample limitations, as 125 out of the 172 cases were duos; additional research on a larger scale of samples will be required to solve this problem.

5.2. Mutation rate

The overall germline mutation rate for the Libyan population across the 15 STR loci was 2.74×10^{-3}, which was consistent with the study by Kim *et al.* (2021) in Korea, where the overall mutation rate was 1.4×10^{-3}, but was inconsistent with previous studies in Brazil by Vieira *et al.* (2017), where the overall germline mutation rate was 2.1×10^{-4} and in the study of Xu *et al.* (2019), where the overall mutation rate of the 21 loci was 0.9×10^{-3}. This might be explained by the sample limitations in the number and types of family sets (mostly composed of duos). These mutation rates are an approximation that could be used in future local studies on STR mutation rates.

In this study, the D8S1179 and D21S11 loci exhibited the highest mutation rate, followed by CSF1PO, D18S51, D16S539, FGA and TPOX. These values were not within the range of what was expected for the STR markers in the American Association of Blood Banks, 2008 (AABB) (AABB.org, 2020). A summary of the mutation rate for each STR locus is provided in Appendix 10 (NIST, 2022). We hypothesize that the excess over the threshold for these loci was due to a sample size limitation and most of samples set were duos.

We compared the mutation rates obtained for the 13 STR loci for Libyan individuals with previous studies for some different populations in the world, as shown in Table 20. Mutations at D8S1179 and D21S11 loci were found more frequently in our study, but mutations at D18S51 and vWA loci were found more frequently in Han Chinese (Huang *et al.*, 2021). The study findings are consistent with Hohoff *et al.* (2009), a study in Nigeria, where mutations at D21S11 loci were found at the highest rate. However, in Poland, it was in the vWA locus (Drożdziok *et al.*, 2018), and in Brazil, it was in the FGA locus (Mardini *et al.*, 2013).

The analyzed datasets also revealed some similarities, such as the lowest mutation rates for TH01 in the six compared populations and for TPOX in the five compared populations, with the exception of the Libyan population.

The observed differences between Libyan and other countries' data at the mutated loci may also be due to the different allelic structures of these genetic markers in different populations. As shown in the STRBase database, some of these markers may have alleles of the same length but different repetitive sequences. Ballantyne *et al.* discovered that the mutability of a STR allele is dependent on its structure and that alleles with a high number of identical repetitive units are more likely to be mutated (Ballantyne *et al.*, 2010).

Table (20) Comparison of mutation rates between our study and data from five different populations.

Locus	Mutation rate x 10^{-3}					
	This study	Han Chinese (Huang *et al.*, 2021)	Beijing Chinese (Bi *et al.*, 2017)	Poland (Drożdziok *et al.*, 2018)	Nigeria (Hohoff *et al.*, 2009)	Brazil (Mardini *et al.*, 2013)
CSF1PO	4.566	1.455	1.260	0.000	0.000	1.506
D13S317	0.000	1.455	0.770	2.400	0.000	0.958
D16S539	4.566	0.342	0.450	0.600	4.292	1.095
D18S51	4.566	2.139	1.570	0.600	0.000	1.688
D21S11	9.132	1.027	1.390	1.200	5.882	1.597
D3S1358	0.000	1.198	0.870	0.600	2.066	0.593
D5S818	0.000	0.856	0.590	1.200	0.000	1.460
D7S820	0.000	0.428	0.800	0.000	2.092	1.049
D8S1179	9.132	1.198	0.770	4.200	2.941	1.551
FGA	4.566	2.054	2.160	2.400	2.075	2.281
TH01	0.000	0.273	0.100	0.000	0.000	0.046
TPOX	4.566	0.000	0.070	0.000	0.000	0.137
vWA	0.000	2.310	1.190	5.400	4.149	2.236
Total	2.74	1.143	0.923	1.434	1.611	1.246

5.3. Combined Paternity index (CPI)

Our findings show that mutations in the STR loci used for paternity testing can occur, so determining the PI value of all STR loci is required to ensure the accuracy of paternity testing.

As reported by Huang *et al.* (2021), the presence of STR mutations frequently affects the determination of parenthood in forensic paternity test cases. As a result, the mutation rate should be considered when calculating the cumulative paternity index (CPI).

According to a study by Xiao *et al.* (2018), support for maternity or paternity is only given if the cumulative paternity index (CPI) is greater than 10,000 and it would deny maternity or paternity if the cumulative paternity index (CPI) was less than 0.0001. If the cumulative paternity index (CPI) ranged from 0.0001 to 10,000, additional autosomal STR loci should be used until the CPI based on all tested loci was content to make the decisions.

In our work, the CPI values of seven trio cases with mutations showing a CPI > 10,000 and two duos mutants showing a CPI < 10,000. These findings suggest that more STR loci testing would be required. However, high CPI values can sometimes lead to incorrect conclusions, like in complex close-relative cases.

The study by Li *et al*. suggests that other methods, such as mtDNA sequencing, X/Y chromosomal STR typing, and other markers (e.g., RFLP, SNP), can be used to ensure paternity testing accuracy if necessary (Li *et al.*, 2011).

Finally, given the possibility of autosomal STR loci mutations, we see that increasing the number of required STR loci and supplementing the triplet samples is necessary. In this way, identification errors could be greatly reduced.

5.4. Correlation analysis between mutation rate and heterozygosity

Multiple factors can influence the mutation rate of STRs, including heterozygosity, allele size, tandem repeat structure, sex, and parental age. Our findings showed that there was no significant relationship between mutation rates and corresponding heterozygosity values at 15 loci. This was consistent with the findings of the study of Xu *et al.* (2019) where it was reported that there was no significant association between mutation rates

and the heterozygosity values at 21 loci (P=0.114). These results were also attributed to the possibility of limited case types (trios and duos) and limited sample size. Even in our study, limitations in case types (trios and duos) and sample size might be responsible for this result, and further investigations will be needed to clarify this issue.

In previous reports by (Lu *et al.*, 2012; Jin *et al.*, 2016; Xiao *et al.*, 2018), where a linear correlation between mutation rate and heterozygosity was found for 19, 24 and 33 STR loci, a positive linear relationship between heterozygosity and mutation rate was found by Huang *et al.* (2021), meaning the mutation frequency increases with STR loci heterozygosity.

5.5. Null alleles

Mutations in the primer-binding region cause null alleles, which can lead to allele dropout (García-Aceves *et al.*, 2018). To rule out incorrect identification of silent alleles as mutations, all genetic inconsistencies between parents and children were screened for false homozygosity.

Individuals who were heterozygous for both a null allele and a visible allele were scored as homozygous for the visible allele (Dakin and Avise, 2004).

We found four presumably null alleles, all of which were found in trio cases, and most of the mutation cases were in trio families. This could be explained by the sample's composition, which was mostly made up of duos, because some potential mutation events may be overlooked in duos, resulting in underestimated mutation rates in the population.

6. CONCLUSION

6.1. Conclusion

This is the first study in Libya to describe mutation events of 15 autosomal STR loci by using the Ampf/STR PCR reagents Identifiler, Identifiler plus based on 172 DNA paternity tests performed by a Libyan laboratory, which can be taken as a reference in the future for more studies of STR mutations in Libya.

The observed mutation rates across the 15 STR loci ranged from 0.0000 to 0.00913, and the average mutation rate across all the 15 loci was 2.74×10^{-3}. The majority of mutational events (6 events) were single-step mutations, accounting for 66.6% of total mutations, and paternal origin mutations (66.6%) were more common than maternal origin mutations (33.3%).

The findings of this study are critical for determining the correct likelihood ratio in the Libyan population if mutation rates are to be used. Therefore, it has been determined that more research with a larger population is required for the interpretation of STR loci where no mutations have been identified.

The findings also provide highly informative polymorphic data for forensic genetics and population genetics research on Libyan populations.

To summarize, this report on detected mutations across the 15 STR loci is important not only in terms of statistics, but also in terms of its great value to Libyan practicing experts in the forensic field.

6.2. Recommendations

This findings suggest that knowing the rates of STR mutation in different populations could be useful for calculating the probability of relationship in disputed paternity testing and that such calculations should be based on population-specific mutation rates, at least for some STR markers commonly used in forensic genetics. The findings support the use of population and geographic data in future genetic applications.

The present research recommends in future that expanding the study and increasing the size of samples and select it as trios sets to confirm our results and get more reliable estimation of mutation rate specially for different Libyan population races.

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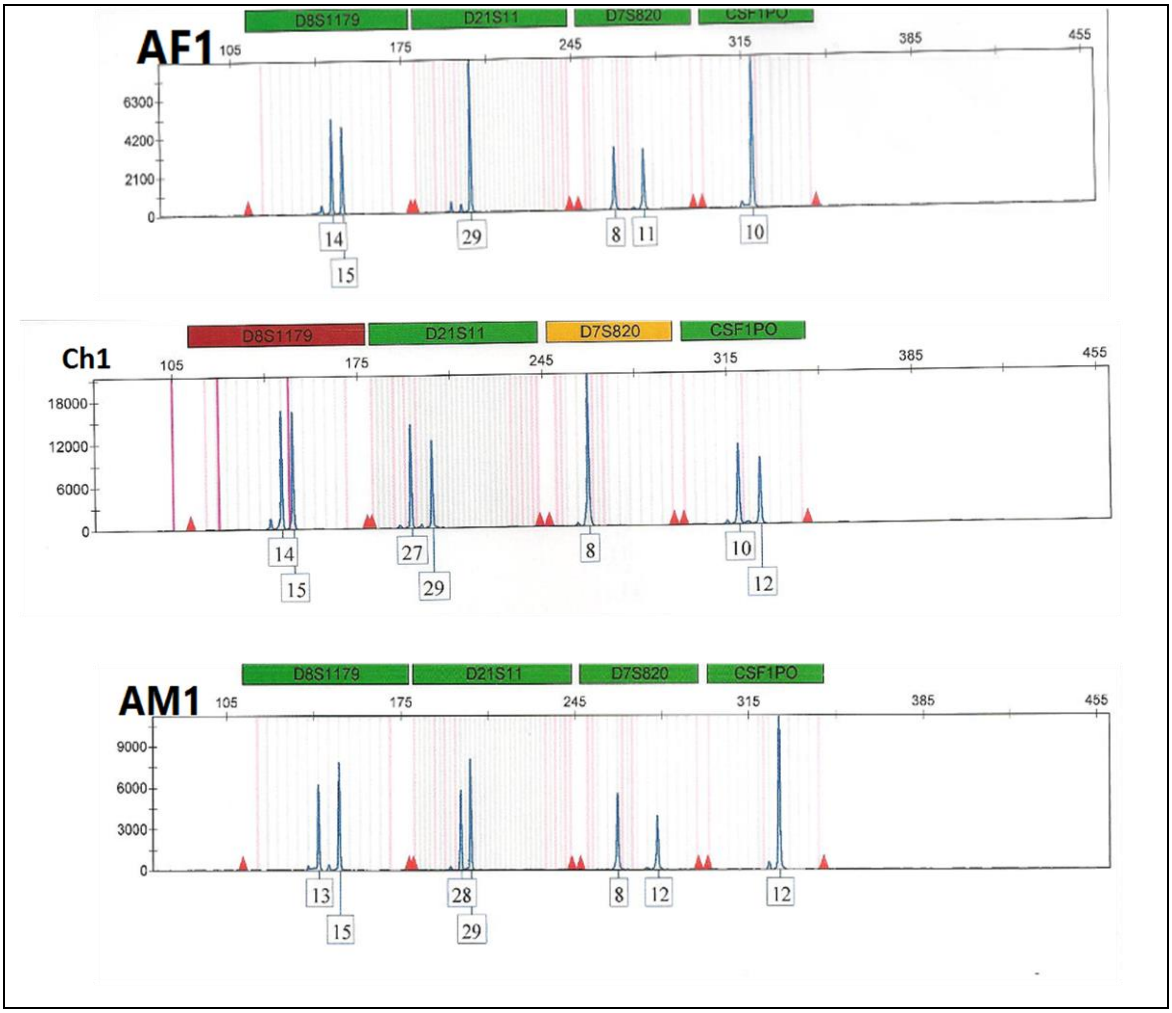
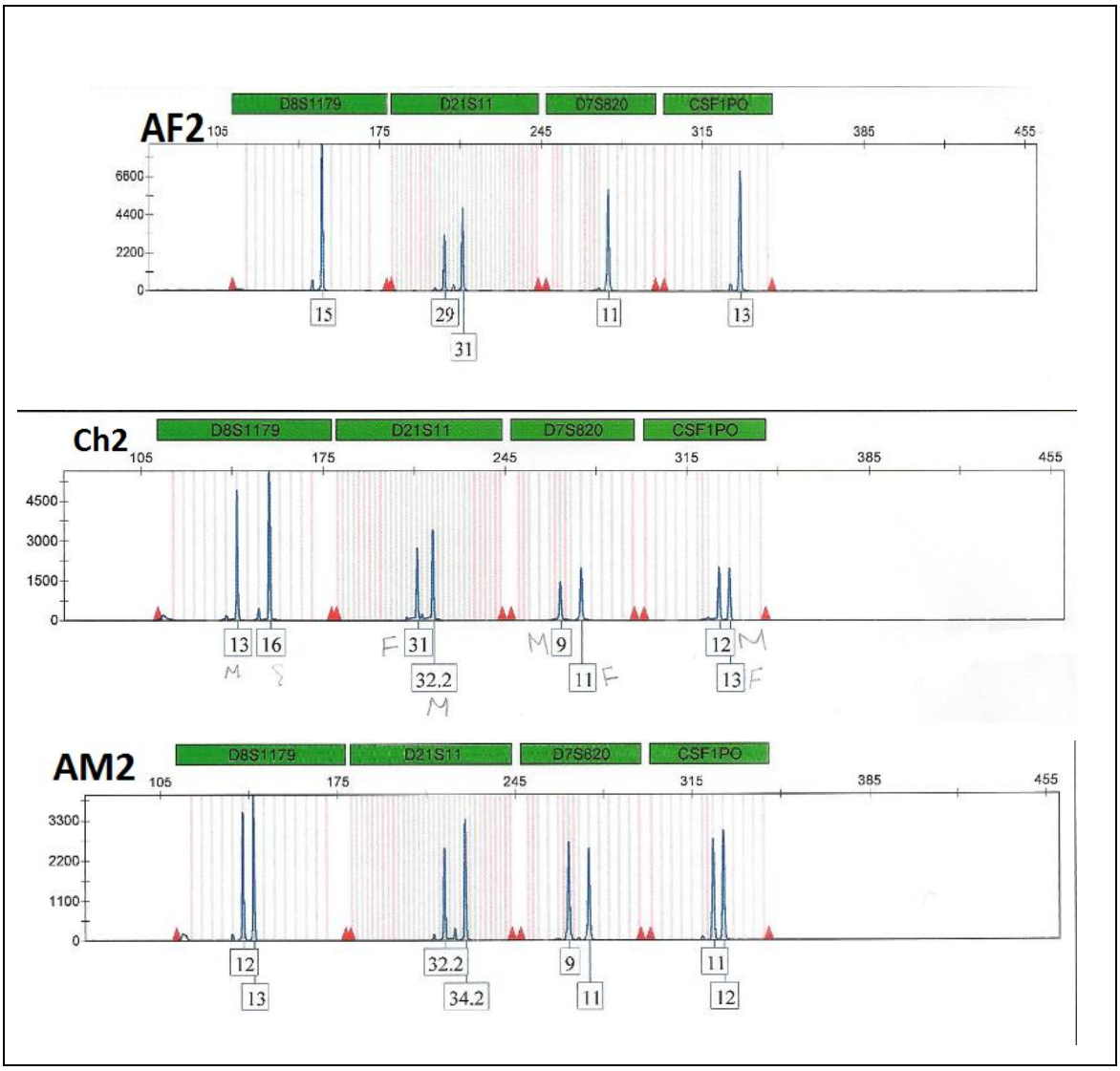
APPENDIX (1)



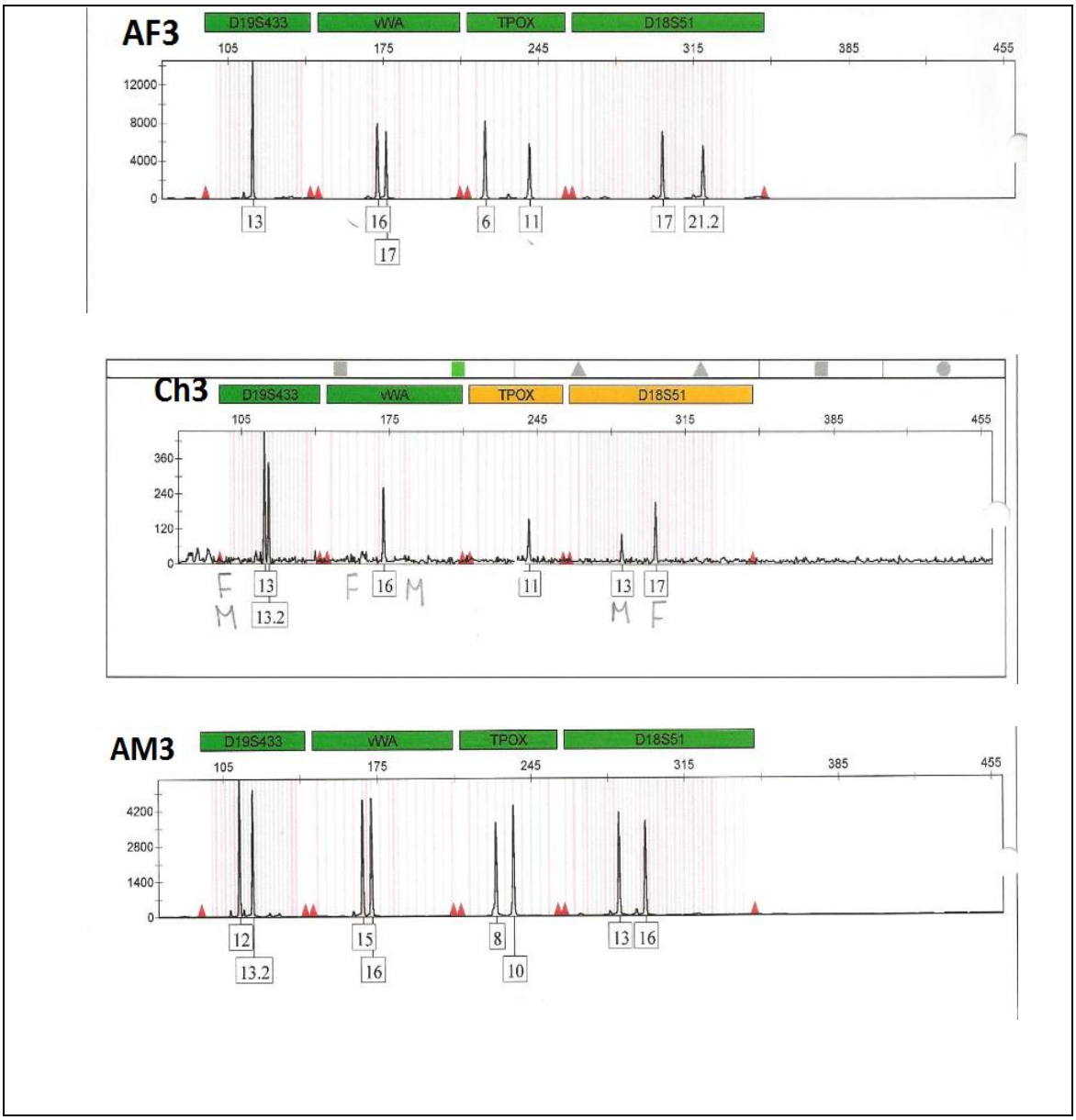
The electropherogram of first mutation case observed on D21S11 locus in the form of maternal allele mismatch

APPENDIX (2)



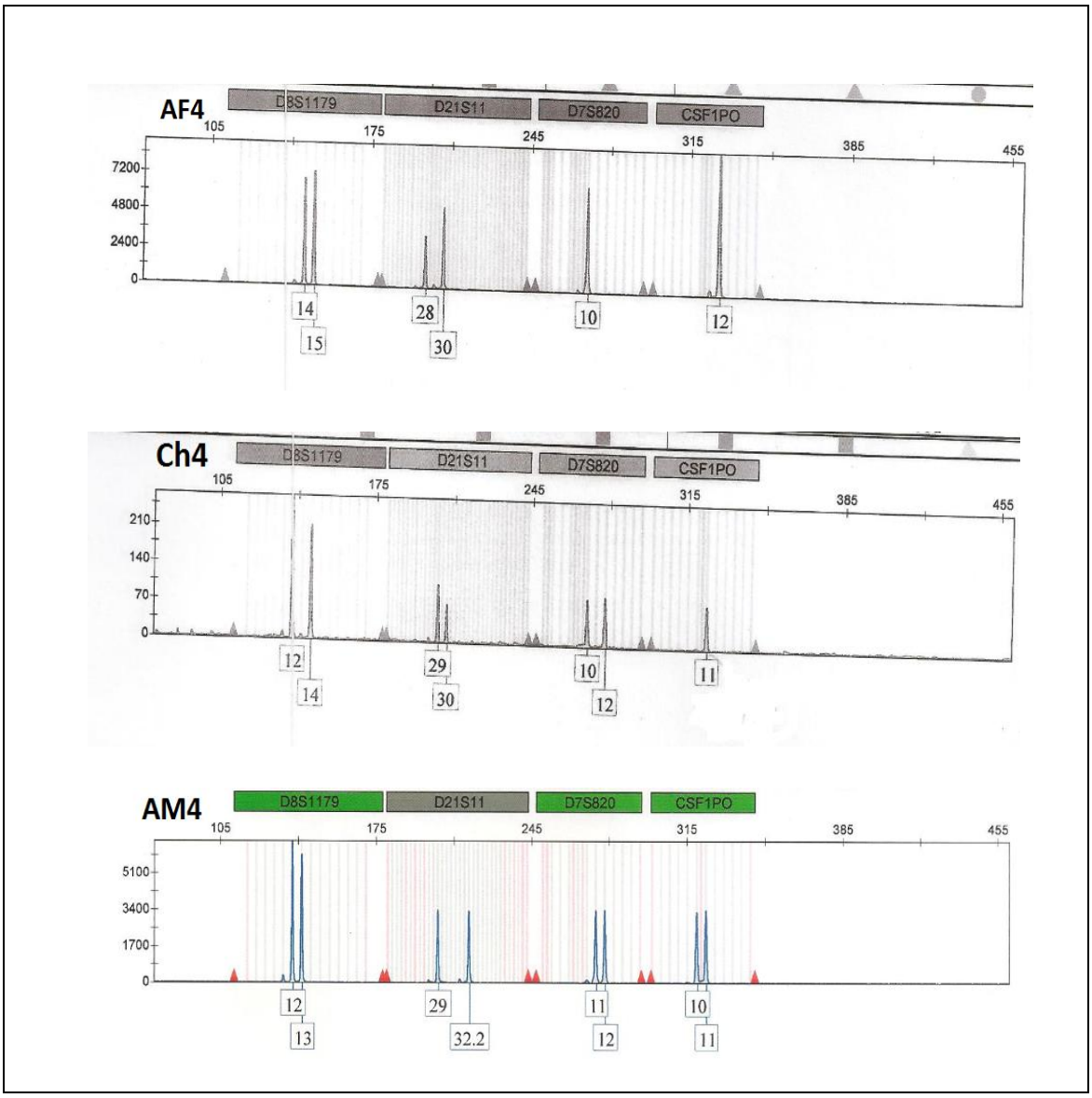
The electropherogram of second mutation case observed on D8S1179 locus in the form of paternal allele mismatch

APPENDIX (3)



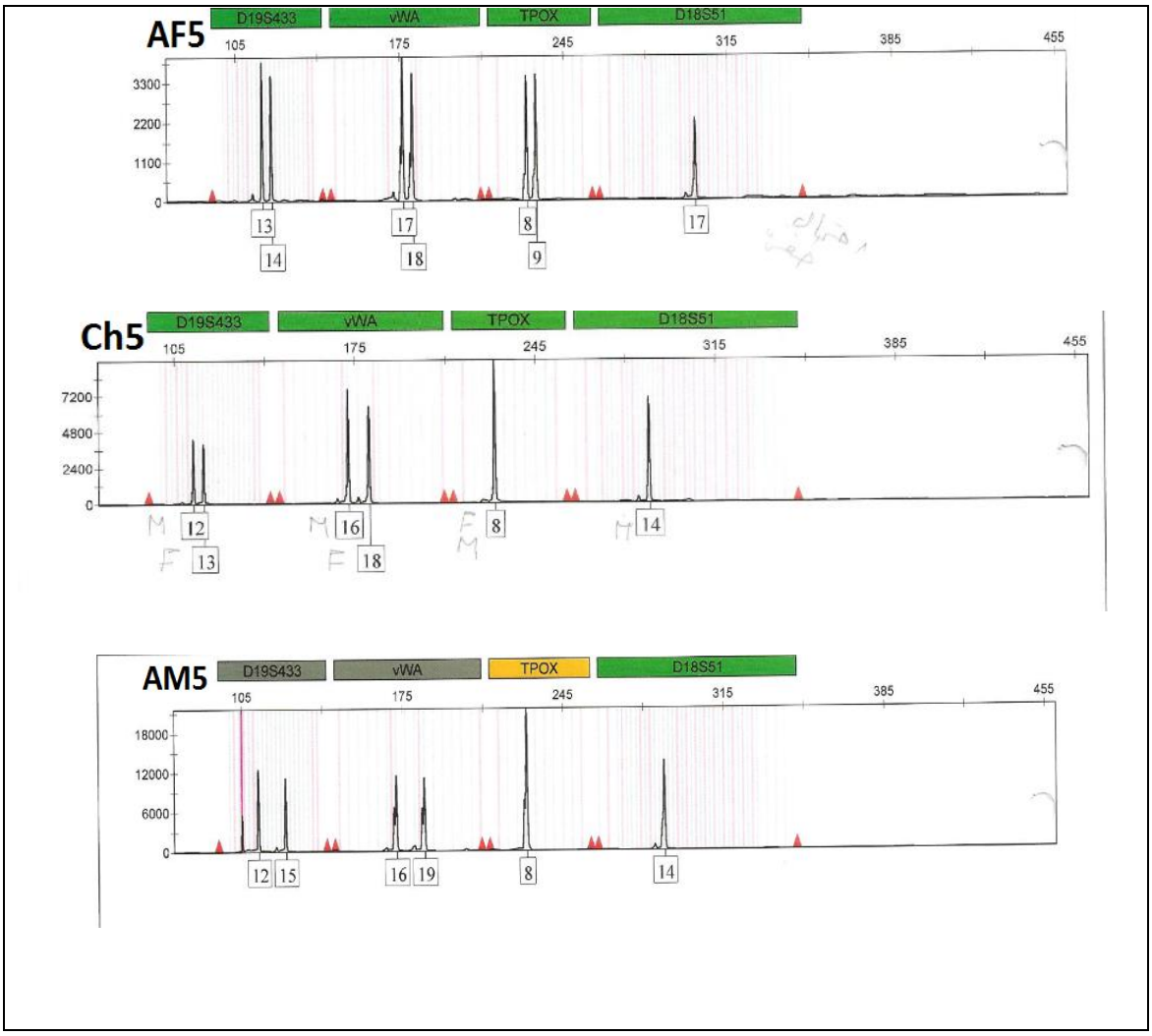
The electropherogram of third mutation case observed on TPOX locus in the form of maternal allele mismatch.

APPENDIX (4)



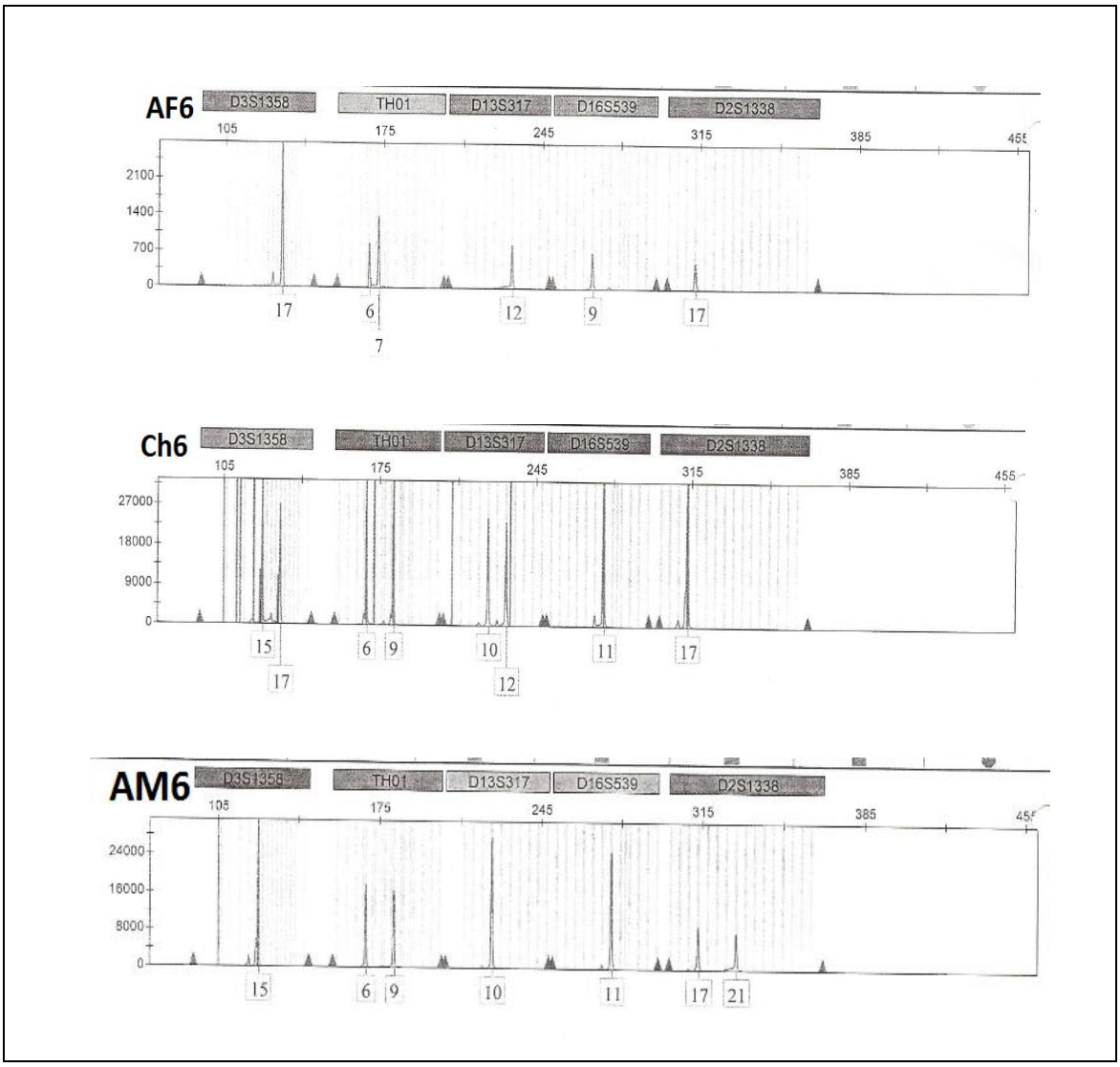
The electropherogram of fourth mutation case observed on CSF1PO locus in the form of paternal allele mismatch

APPENDIX (5)



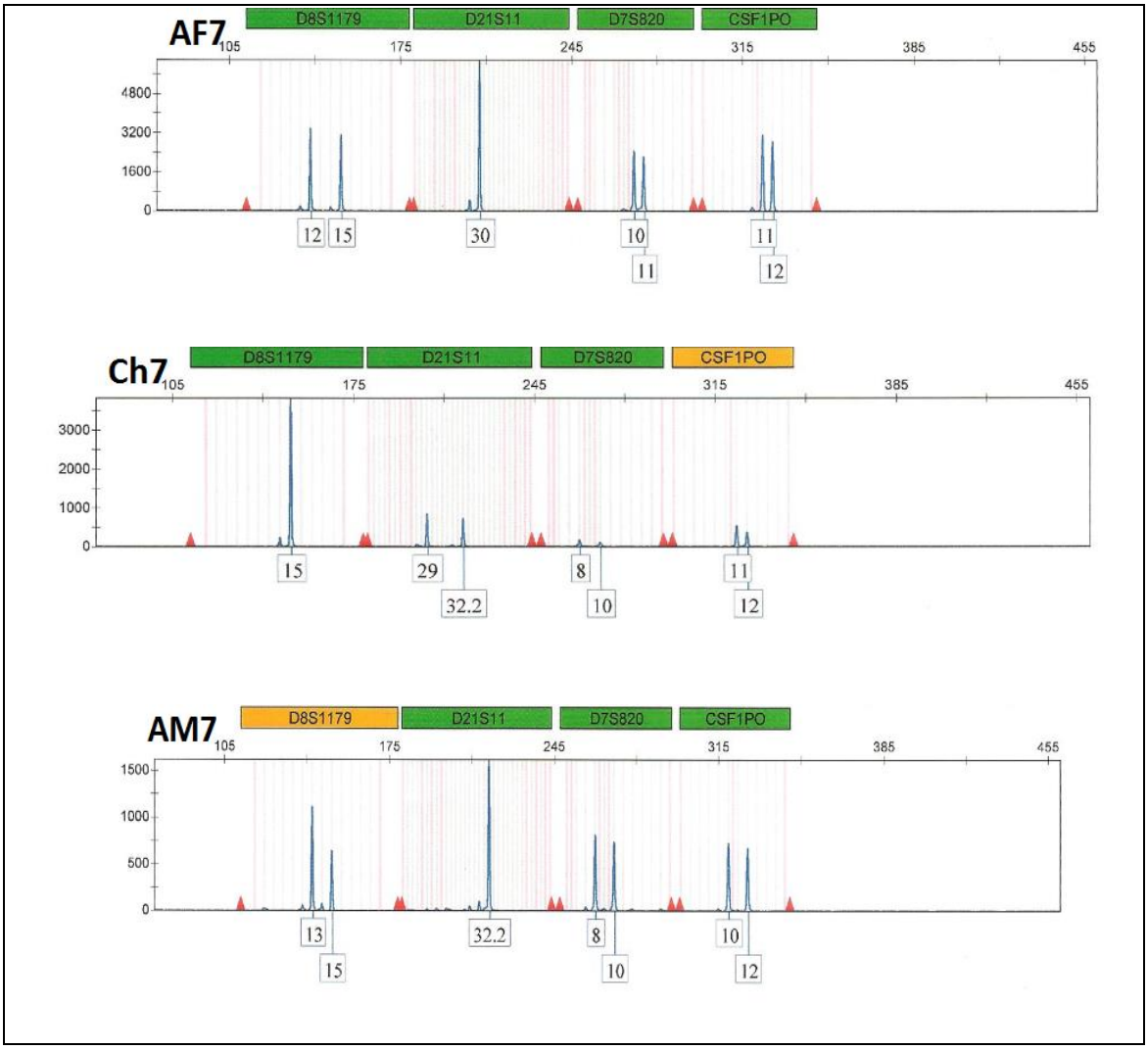
The electropherogram of fifth mutation case observed on D18S51 locus in the form of paternal allele mismatch

APPENDIX (6)



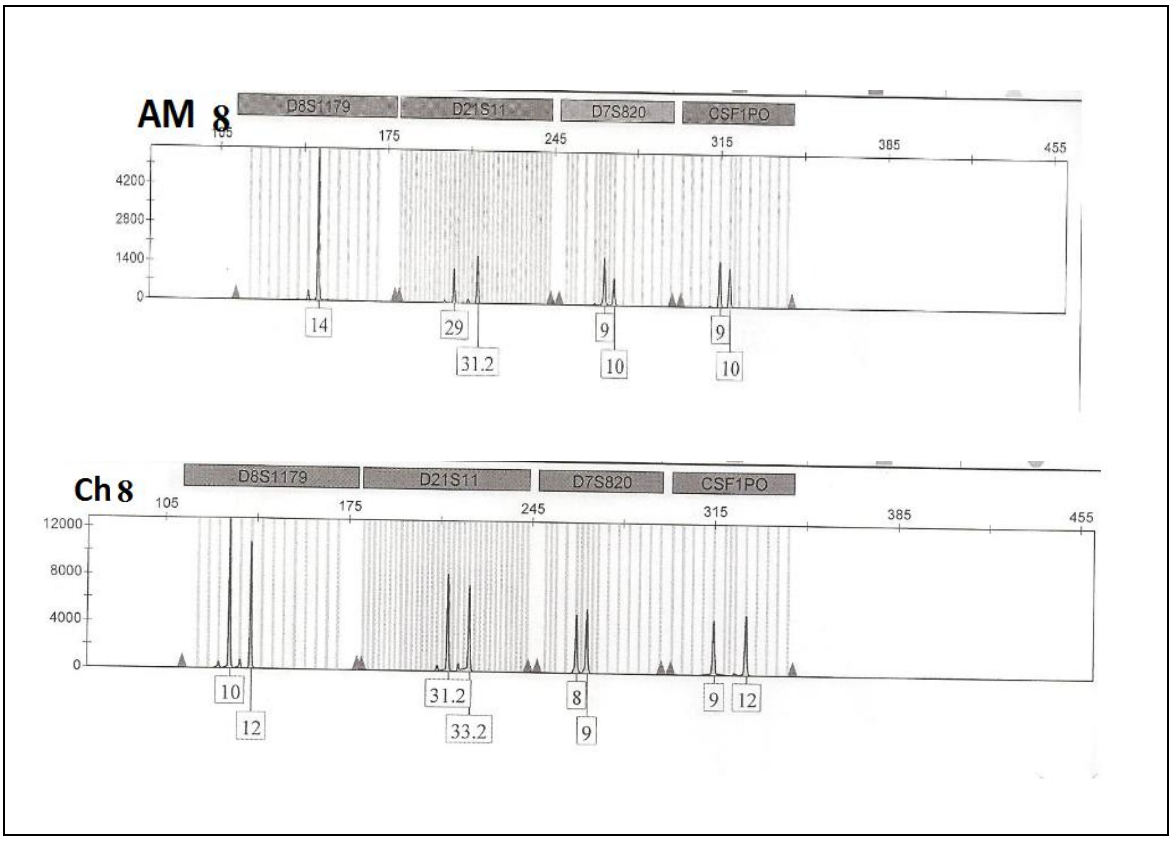
The electropherogram of sixth mutation case observed on D16S539 locus in the form of paternal allele mismatch

APPENDIX (7)



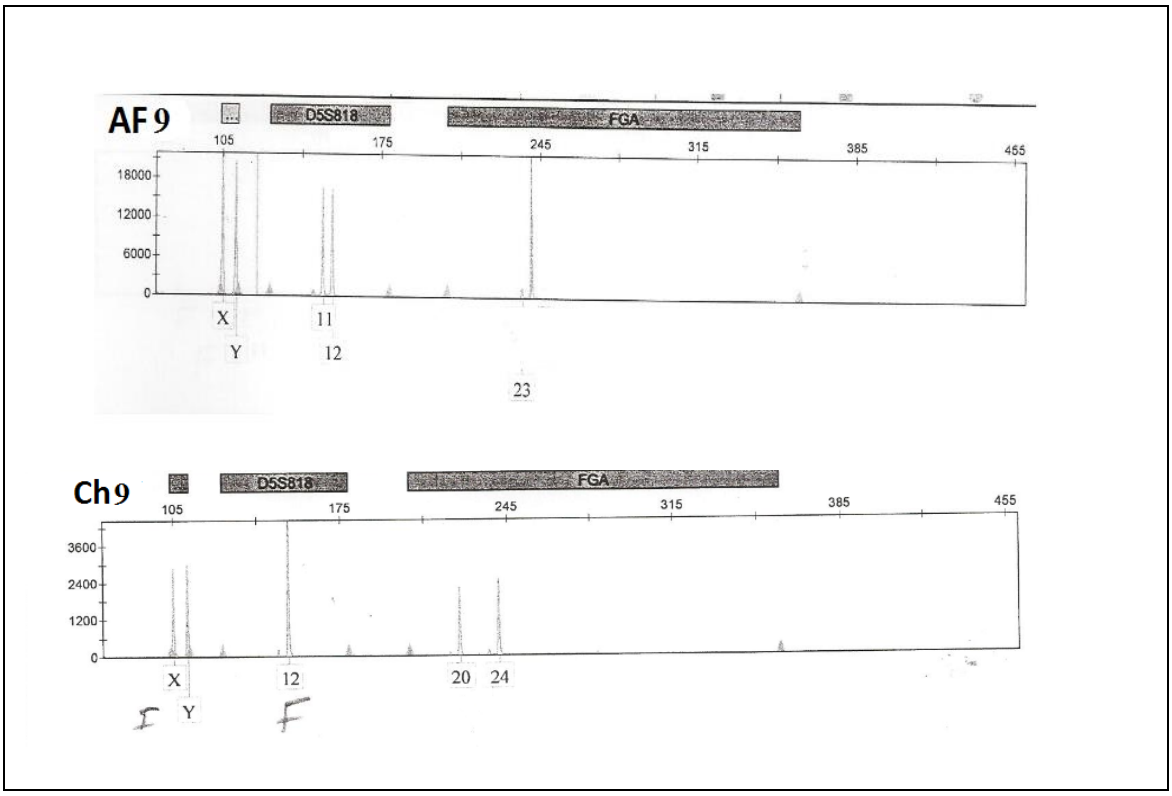
The electropherogram of seventh mutation case observed on D21S11 locus in the form of paternal allele mismatch

APPENDIX (8)



The electropherogram of eight mutation case observed on D8S1179 locus in the form of maternal allele mismatch

APPENDIX (9)



The electropherogram of ninth mutation case observed on FGA locus in the form of paternal allele mismatch

APPENDIX (10)

Apparent Mutations Observed at STR Loci in the Course of Paternity Testing (NIST, 2022)

STR System	Maternal Meioses (%)	Paternal Meioses (%)	Total Number of Mutations	Mutation Rate
CSF1PO	95/304,307 (0.03)	982/643,118 (0.15)	1,487/947,425	0.16%
FGA	205/408,230 (0.05)	2,210/692,776 (0.32)	3,125/1,101,006	0.28%
TH01	31/327,172 (0.009)	41/452,382 (0.009)	100/779,554	0.01%
TPOX	18/400,061 (0.004)	54/457,420 (0.012)	100/857,481	0.01%
VWA	184/564,398 (0.03)	1,482/873,547 (0.17)	2,480/1,437,945	0.17%
D3S1358	60/405,452 (0.015)	713/558,836 (0.13)	1,152/964,288	0.12%
D5S818	111/451,736 (0.025)	763/655,603 (0.12)	1,259/1,107,339	0.11%
D7S820	59/440,562 (0.013)	745/644,743 (0.12)	1,089/1,085,305	0.10%
D8S1179	96/409,869 (0.02)	779/489,968 (0.16)	1,239/899,837	0.14%
D13S317	192/482,136 (0.04)	881/621,146 (0.14)	1,558/1,103,282	0.14%
D16S539	129/467,774 (0.03)	540/494,465 (0.11)	1,041/962,239	0.11%
D18S51	186/296,244 (0.06)	1,094/494,098 (0.22)	1,746/790,342	0.22%

D21S11	464/435,388 (0.11)	772/526,708 (0.15)	1,816/962,096	0.19%
Penta D	12/18,701 (0.06)	21/22,501 (0.09)	57/41,202	0.14%
Penta E	29/44,311 (0.065)	75/55,719 (0.135)	163/100,030	0.16%
D2S1338	15/72,830 (0.021)	157/152,310 (0.10)	262/225,140	0.12%
D19S433	38/70,001 (0.05)	78/103,489 (0.075)	187/173,490	0.11%