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Original article

Genetic Analysis of Autosomal Short Tandom Repeats Loci in The Libyan Western Mountain Population

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ABSTRACT

There is a little investigation of the genetic diversity of the Libyan population. This study aimed to explore Short tandem repeat (STR) loci in the Libyan western mountain population to analyse its genetic landscape. Allele frequency for 15 autosomal STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA) included in the Identifiler®Plus kit were investigate in 120 random unrelated individuals from the Westerner Mountain area of Libya. No deviations from Hardy-Weinberg equilibrium were seen, with the exception of D8S1179, D21S11, D3S1358, TH01 and FGA loci. A total of 129 alleles were observed with the corresponding allelic frequencies ranging between 0.00417 and 0.47917 of the studied sites were observed, and that alleles 8 and 12 are the highest frequency for all studied areas for the TPOX and D5S818 sites respectively had a value of (0.47917). D18S51 was also observed to be the most diverse site (Expected heterozygosity: 0.88312 and probability of matching: 0.0354). The results indicate that the 15 STR loci have a significant genetic diversity among studied individuals and are suitable for personal identification in the criminal field, and the importance of this work lies in the creation of a database of frequency distribution of allelic frequencies to be used for criminal identification, identification of missing persons and paternity tests.

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INTRODUCTION

Nucleic acids are polymers composed of repeating units called nucleotides. Nucleic acids are of two types: deoxyribonucleic acid, which is symbolized by the abbreviation DNA, and ribonucleic acid, which is symbolized by RNA. The basic unit of nucleic acid (nucleotide) consists of a nitrogenous base and a pentose sugar. In addition to a phosphate group [1]. The genetic material in most living organisms appears in the form of a double helix of DNA, which is characterized by the following specifications: stability so that the genetic information performs its function in the living organism, and the ability to repeat and multiply and allows the genetic information to be transferred to new cells [2]. This acid is found in all nuclei of the cells of the human body except for red blood cells, which do not have a nucleus. DNA is estimated to be more than 99.9% similar, and DNA analysis has become one of the most widespread methods for determining human identity in forensic science, paternity testing, and missing persons in human disaster investigations. What contributed to this rapid development is the discovery of the restriction enzyme for the technique of restricting multiple fragment lengths, abbreviated as (RFLP) [3], and this method targets variable sites in repetitive

patterns in the DNA chain by employing these enzymes to cut them into pieces of different lengths, and then they are spread across a gel using electrophoresis [4]. At this stage, the DNA remains invisible to the naked eye, as Jeffrey probes were discovered, a special part of DNA equipped with a radioactive tip that binds to the DNA in the membrane gel, and at sites with similar repetitive patterns, the target site in the DNA chain remains a specific site at all times [5], This method had defects and problems during its development, which were represented in the behavior of DNA samples on the electrophoresis device, such as the appearance of some impurities, and the displacement of the lines of genetic sites (band shifting) as a result of the mechanism of displaying the sites, which was carried out manually at the beginning of the use of fluorescent materials, but most of these problems were overcome by employing the Capiliary electrophoresis technique, and the genetic sites were displayed using (Automated fluorescent detection) devices, and the diagnosis of many genetic sites that appear in the form of allelic ladders for them (Allelicladder) and the calculation of their power of discrimination (Power of discrimination) as well as their allele frequency rate [6]. Hence, the Short tandem repeat (STR) technique has gained the attention of researchers in DNA analysis and the activation of this technique in most laboratories specialized in genetic fingerprint analysis and forensic laboratories [7,8].

The importance of such as a study lies in the fact that it represents one of the few studies through which the allelic frequency of genetic sites of some residents of the Western Mountain region in the Libyan community has been evaluated, and that the results that will be obtained from this study have great value for forensic medicine and forensic science, which many countries in the world have been keen to identify and refer to such results in criminal cases and judicial authorities. Study of allele frequencies for fifteen somatic sites of short repetitive sequences in random samples of some residents of the Western Mountain regions in Libya with the aim of obtaining statistical values for allele frequencies using STR technology and benefiting from them in judicial authorities and criminal cases.

METHODS

Ethical statement

Written and verbal consent was obtained from all participants ensuring privacy and confidentiality under the Declaration of Helsinki. Consents were obtained from all studied individuals for taking samples and conducting DNA analysis after explaining to them the purpose of this study. Some of the experiments of this study were conducted at the National Center for Disease Control and others at the DNA Center in Libya.

Data collection

This work was conducted on a sample of 120 randomly selected individuals from different areas of the Western Mountains who were not related and apparently healthy. Blood samples were collected on FTA cards (Fast Technology for Analysis of nucleic acids) [9,10].

Estimation of DNA quantity

Although, this step can be avoided if samples are collected using FTA Card, this step was done to know the concentration of DNA inside the samples in order to compensate for any loss in the volumes of reagents for the PCR and Genetic Analyzer stages, where the Nano Drop Lite spectrophotometer was used as previous described [5,10,11]. In brief, 10 µl of the extracted DNA sample (0.5-1.5 ng/µl) was added to each tube containing the reaction mixture **to** make a final volume of 25 µl.

PCR multiplex amplification

DNA amplification was performed using the Gene AMP PCR System 9700 Thermal cycler (Applied Biosystems) with the Identifiler®Plus Kit. The amplification reactions included AmpFlSTR™ Identifiler™ Plus were done under conditions recommended by the manufacturer (AppliedBiosystems; Thermo Fisher) [1].

STR typing

15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA, and AMEL) were typed using the 3500 Genetic Analyzer (Applied Biosystems) with the Identifiler®Plus Kit. DNA profiles were analysed using Gene Mapper®ID software (Version V1.4x.) (Figure 1) [3,12].

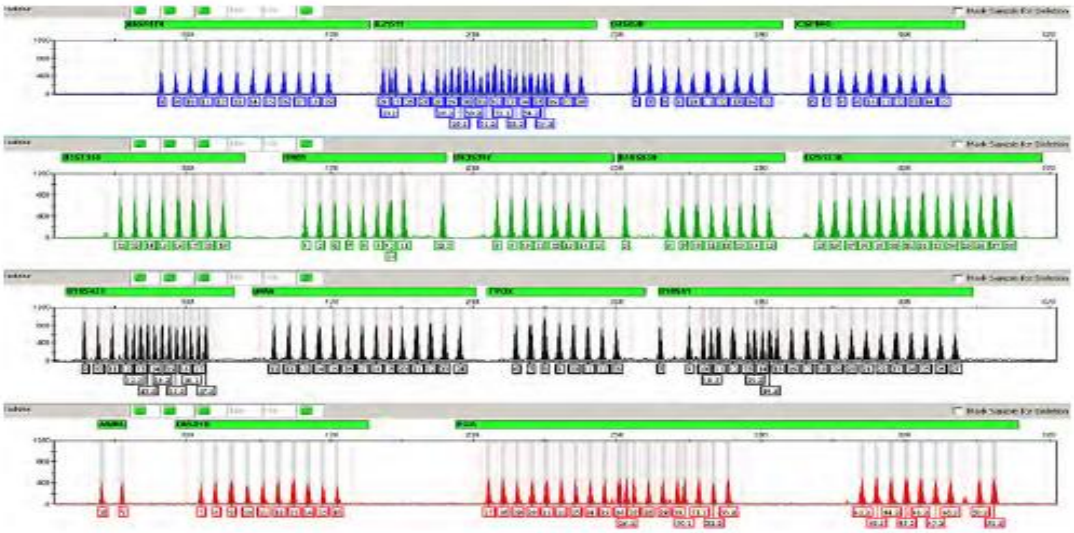

Figure 1. Ladder of alleles for fifteen somatic loci plus the sex locus for a set of reagents. Ampf/STR PCR Identifiler® Plus Kit.

Statistical analysis

Statistical analysis of the study results was performed for a sample of 120 individuals from the western mountain regions of Libya using Arlequin 3.5 software to calculate allelic frequencies, homozygotes, observed and expected heterozygotes, discriminatory power, exclusion power, deviation from Hardy-Weinberg equilibrium, probability of matching, paternity index, and polymorphism content.

RESULTS

This study relied on the STR technique for 15 somatic sites, and these sites are D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA. After collecting a random sample of 120 individuals from some areas of the Western Mountain and after obtaining their written consent, their DNA was extracted and doubled using the commercially available operating materials Ampf/STR PCR® Identifiler, Identifiler plus. The results showed the following:

Genotyping of study samples

The results were shown for the genotypes of fifteen sites for a sample of 120 individuals, where the results appear in the form of alleles. Table 1 shows the frequency of alleles for the studied sample.

Allele Frequencies of autosomal genetic loci Calculations

By calculating the number of times, the allele is observed over the total number of alleles, the frequency of alleles in the study samples is calculated. This is known as allele frequencies. Table (3.2) shows an example of a number of alleles for the TH01 site, provided with their allelic frequencies.

Table 1. Frequencies of 15 STR loci in 120 unrelated samples.

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18SS51	D5S818	FGA
6	-	-	-	-	-	0.19167	-	-	-	-	-	0.0125	-	-	-
6.3	-	-	-	-	-	0.00417	-	-	-	-	-	-	-	-	-
7	-	-	0.00833	0.00417	-	**0.2625**	0.00417	-	-	-	-	0.0125	-	-	-
7.3	-	-	-	-	-	0.00417	-	-	-	-	-	-	-	-	-
8	-	-	0.22083	0.02917	-	0.0875	0.09167	0.00417	-	-	-	**0.47917**	0.00417	0.05417	-
8.1	-	-	0.00417	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	0.07082	0.00833	-	**0.2625**	0.0625	0.12916	-	-	-	0.17917	-	0.04167	-
9.1	-	-	0.00417	-	-	-	-	-	-	-	-	-	-	-	-
9.3	-	-	-	-	-	0.15417	-	-	-	-	-	-	-	-	-
10	0.04583	-	**0.3**	0.26667	-	0.03332	0.075	0.0625	-	-	-	0.04583	0.02083	0.05833	-
11	0.07917	-	**0.25**	**0.30832**	-	-	**0.30417**	**0.32917**	-	0.0125	-	**0.25**	0.0125	**0.2375**	-
12	0.1	-	0.10417	**0.34167**	0.00417	-	**0.325**	**0.3**	-	0.10833	-	0.02083	0.10833	**0.47916**	-
12.1	-	-	0.00417	-	-	-	-	-	-	-	-	-	-	-	-
12.2	-	-	-	-	-	-	-	-	-	0.01667	-	-	-	-	-
13	0.175	-	0.02917	0.04167	0.00417	-	0.09582	0.1625	-	**0.25833**	-	-	0.1375	0.12083	-
13.2	-	-	-	-	-	-	-	-	-	0.04167	-	-	0.00417	-	-
14	**0.31667**	-	0.00417	-	0.07083	-	0.0375	0.0125	0.00417	**0.3**	0.07083	-	**0.16667**	0.00417	-
14.2	-	-	-	-	-	-	-	-	-	0.05	-	-	0.00417	-	-
15	**0.2625**	-	-	-	0.26667	-	0.00417	-	-	0.09167	0.12917	-	0.08333	0.00417	-
15.2	-	-	-	-	-	-	-	-	-	0.05833	-	-	0.00417	-	-
15.5	-	-	-	-	-	-	-	-	-	0.00417	-	-	-	-	-
16	0.02083	-	-	-	**0.27083**	-	-	-	0.075	0.02083	**0.31251**	-	**0.17916**	-	-
16.2									-	0.025	-	-	0.0125	-	-
17	-	-	-	-	**0.27083**	-	-	-	**0.24581**	-	**0.27083**	-	0.1125	-	0.00417
17.2	-	-	-	-	-	-	-	-	-	0.0125	-	-	-	-	-
18	-	-	-	-	0.1125	-	-	-	0.10417	-	0.1375	-	0.08333	-	0.00417
19	-	-	-	-	-	-	-	-	0.12917	-	0.07083	-	0.05	-	0.04583
20	-	-	-	-	-	-	-	-	**0.1625**	-	0.00833	-	0.0125	-	0.14167
21	-	-	-	-	-	-	-	-	0.06667	-	-	-	0.00417	-	0.14167
21.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00417
22	-	-	-	-	-	-	-	-	0.05417	-	-	-	-	-	**0.18333**
23	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	0.17081
24	-	-	-	-	-	-	-	-	0.07917	-	-	-	-	-	**0.19167**
24.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00417

25	-	-	-	-	-	-	-	-	0.025	-	-	-	-	-	0.05417
26	-	0.00417	-	-	-	-	-	-		-	-	-	-	-	0.04583
27	-	0.025	-	-	-	-	-	-	0.00417	-	-	-	-	-	-
28	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	**0.27083**	-	-	-	-	-	-	-	-	-	-	-	-	0.00417
30	-	**0.3125**	-	-	-	-	-	-	-	-	-	-	-	-	0.00417
30.2	-	0.02083	-	-	-	-	-	-	-	-	-	-	-	-	-
31	-	0.05419	-	-	-	-	-	-	-	-	-	-	-	-	-
31.2		0.075	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	0.00833	-	-	-	-	-	-	-	-	-	-	-	-	-
32.2	-	0.07083	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33.2		0.05833	-	-	-	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34.2	-	0.02083	-	-	-	-	-	-	-	-	-	-	-	-	-
35	-	0.02083	-	-	-	-	-	-	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	-	0.00833	-	-	-	-	-	-	-	-	-	-	-	-	-

DISCUSSION

The main objective of this study was to know the allele frequency distribution of short tandem repeat (STR) patterns for fifteen somatic loci and to shed light on the population genetic structure of some areas of the western mountain in the Libyan society, as the study was conducted on a random sample of 120 unrelated individuals and a blood sample was taken and placed on FTA cards after written consent of the volunteers for this study.

A blood sample was taken and placed on FTA cards after obtaining written consent from the volunteers for this study, using special laboratory practical methods in the field of DNA analysis, and the results were analyzed with statistical programs specific to forensic science and population genetics, and based on these results. The results obtained showed that the 15 genetic loci of the study population showed no deviation from Hardy-Weinberg equilibrium in all regions except for Zintan city at locus D8S1179 and Finally, the FST genetic distance between the study areas was calculated and the farthest distance was between Qasr al-Hajj and Tigi with a distance of 0.05009 and the closest distance was between Shakshouk and Zintan with a distance of 0.00359, which indicates the existence of genetic differentiation between all areas.

The homozygosity in the fifteen STR loci for all the studied regions is low compared to the proportion of heterozygosity, which indicates a low level of inbreeding among the population of the studied regions, and that the community is in a state of random mating.This study confirme other studies showed that The results of a genetic divergence study of five western Mediterranean regions averaged over five STR loci all loci are in equilibrium with Hardy and Weinberg [13].

CONCLUSION

The main objectives of this work were to investigate the frequency distribution of alleles for short 15 STR loci and to shed light on the population genetic structure of some regions of the Western Mountain in Libyan society. The study recommends analyzing a large number of samples and different societies and ethnicities, supporting the results with some statistical information, documenting them and placing them in a database and banks of genetic fingerprints so that they can be easily used by criminal cases in detecting crimes and identifying the identity of unidentified corpses in wars and victims of natural disasters. we can conclude that the main findings of this work can be concluded that all studied loci were highly diverse based on the genotypes that emerged and the number of alleles observed was 129 and the number of genotypes was 4.84986×10^{23} and the highest frequency of allele 8 at locus TPOX with a frequency of 0.47917 followed by allele 12 at locus D5S818 with a frequency of 0.47916 with 47.9% each being the two most common alleles.

Conflicts of Interest

The authors declare no conflicts of interest.

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التحليل الوراثي لمواقع التكرارات الصبغية الجسدية القصيرة المترادفه في سكان الجبل الغربي الليبي

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المستخلص

هناك القليل من البحث في التنوع الجيني للسكان الليبيين. تهدف هذه الدراسة إلى استكشاف مواضع التكرار الترادفية القصيرة في سكان الجبال الغربية الليبية لتحليل المشهد الجيني الخاص بهم. تم التحقيق في تردد الأليل لـــ 15 موضعًا جسميًا متكررًا قصيرًا (D8S1179 و D21S11 و D7S820 و CSF1PO و D3S1358 و THO1 و D13S317 و D16S539 و D2S1338 و D19S433 و VWA و TPOX و D18S51 و D5S818 و FGA) المضمنة في مجموعة Identifiler®Plus في 120 فردًا عشوائيًا غير مرتبطين من منطقة الجبال الغربية في ليبيا. لم يتم ملاحظة أي انحرافات عن توازن هاردي وينبرج، باستثناء مواضع D8S1179 و D21S11 و D3S1358 و TH01 و FGA. وقد تم رصد 129 جينًا إجمالاً بترددات أليلية مقابلة تتراوح بين 0.00417 و0.47917 من المواقع المدروسة، وأن الأليلين 8 و12 هما أعلى تردد لجميع المناطق المدروسة لمواقع TPOX وD5S818 على التوالي بقيمة (0.47917). كما لوحظ أن D18S51 هو الموقع الأكثر تنوعًا (التغاير المتوقع: 0.88312 واحتمال المطابقة: 0.0354). تشير النتائج إلى أن مواقع STR الـ 15 لديها تنوع وراثي كبير بين الأفراد المدروسين وهي مناسبة للتعريف الشخصي في المجال الإجرامي، وتكمن أهمية هذا العمل في إنشاء قاعدة بيانات لتوزيع الترددات الأليلية لاستخدامها في التعريف الجنائي وتحديد الأشخاص المفقودين واختبارات الأبوة.

الكلمات المفتاحية. المواقع الوراثية، الترددات الأليلية التنوع الوراثي، قوة التمييز.