

Comparison Between Frequencies of Several STRs Loci in Jordan with Neighboring Countries

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Abstract

Objective: To investigate the frequencies of 9 autosomal Short Tandem Repeats (STR) loci in the Jordanian population and compare them with the frequencies in neighboring countries.

Methods: DNA was extracted from the blood of 270 unrelated Jordanian individuals. CSFIPO, TPOX, THO1, F13AO1, FESFPS, vWA, D16S539, D7S820, D13S317 Markers were detected by amplifying the extracted genomic DNA using GenePrint™ STR Multiplex system.

Results: For each Marker, 6-9 alleles were observed. Markers D7S820 and D13S317 had the highest Power of Discrimination (PD) values while TPOX was the least informative marker. Marker D16S539 is significantly different in Jordanian population compared to all other neighboring populations.

Conclusion: Marker D16S539 is very powerful for discriminating between Jordanian and other populations from neighboring countries. The combination between these 9 STR loci seems to be a powerful tool for individual identification in the region.

Keywords: Short tandem repeat (STR); Population data; Allele frequencies; Jordanian.

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Introduction

Short Tandem Repeats (STRs) are class of polymorphisms characterized by the occurrence of repetitive pattern of two or more nucleotides directly adjacent to each other. The length of the pattern can range from 2 to 10 base pair located in the non-coding region of the DNA.^{1,2}

STRs loci can be divided into two types, those that exist on autosomal chromosomes, called autosomal STRs, and those that exist on the sex chromosome and called either Y-STRs or X-STRs. By examining enough STRs loci and counting the numbers of repeats for specific STR sequence at a given locus, it is possible to create a unique genetic profile of an individual (called genetic fingerprinting).²

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Many studies are carried out to demonstrate the power of STRs in certain case scenarios and found out to be valuable for typing the individual's DNA components in mixed samples, determining paternal lineages and yielding a combined power of exclusion (PEX) in paternity testing and evolutionary relationships between different populations.³⁻⁶

In most of the populations, worldwide, several STR markers have been analyzed and their allele frequencies are assigned including several Arab populations and neighboring countries.⁷⁻¹¹ However, up to our knowledge, there is no reports regarding the frequency of these STRs in Jordanian Population. Therefore, we attempt in this study to identify the frequency and distribution of several autosomal STRs in Jordanian population.

The Hashemite Kingdom of Jordan, is an east Mediterranean country, with a population of 5.6 million.¹²

In this study, the frequencies of nine autosomal STRs were investigated and compared with the frequencies reported in other populations from the Middle East and neighboring regions.

Materials and Methods

Population: Venous blood was collected from 270 unrelated healthy Jordanian individuals.

DNA extraction: Genomic DNA was extracted from peripheral blood leukocytes using standard procedures described by Davis and Dibner (1996).¹³

PCR amplification: Co-amplification of CCT triplex (CSFIPO, TPOX and THO1), FFv triplex (F13A01, FESFPS and vWA) and Silver™STRIII triplex (D16S539, D7S820 and D13S317) loci were performed using GenePrint™ STR Multiplex system (Promega Corp., Madison, WI, USA) according to the manufacturers' recommendations. PCR reactions were carried out in a total volume of 25 µl using Brinkmann Gradient Eppendorf Mastercycler.

Primer sequences were selected according to previous studies.^{9, 11} The final reaction mix contained 0.25 µM of primers, 200 µM dNTPs, 1 U Taq Polymerase and 50 ng template DNA. Thermocycling conditions of FFv Triplex and Silver™STRIII Triplex were the following: initial denaturation at 95 °C for 2 min; 28 cycles: 95 °C for 1 min, 60 °C for 1 min, 70 °C for 1.5 min, and a final extension at 60 °C for 40 min. Furthermore, amplification conditions of CCT Triplex were the followings: initial denaturation at 96 °C for 2 min; 28 cycles: 95 °C for 1 min, 64 °C for 1 min, 70 °C for 1.5 min, and a final extension at 60 °C for 30 min.

Typing of STRs: Electrophoresis was carried out using 6% polyacrylamide gel electrophoresis, and visualization of bands was carried out using silver staining. To confirm allelic size ranges for the loci, pGEM® DNA molecular weight markers were used (Promega). In addition, amplified positive control (K562 DNA) and negative control were used in each gel.

Analysis of data: Statistical analyses were conducted with the aid of the Statistical Package for Social Sciences SPSS® Version 15.0 (SPSS, Inc. USA). Compatibility of each marker was tested using Hardy-Weinberg Equilibrium (HWE) by a Chi-square test for goodness of fit. For each marker, 10000 simulated samples were drawn under the assumption of HWE. Statistical parameters such as Power of Discrimination (PD) and Chance of Exclusion (CE) for each locus were estimated as described by Huston (1998).¹⁴ The Polymorphic Information Content (PIC) was calculated according to Botstein et al.¹⁵ Minimum Allele Frequencies (MAF) for PCR-based loci, based on statistical and population genetics theory¹⁶ were determined.

Results

The allele frequencies of 9 autosomal loci in Jordanian population are shown in Table 1. The examined loci are: CSFIPO, TPOX, THO1, F13A01, FESFPS, vWA, D16S539, D7S820 and D13S317. Deviations from Hardy-Weinberg Equilibrium were observed in the CSFIPO, vWA and D7S820 Markers. Markers D7S820 and

D13S317 had the highest Power of Discrimination (PD) values while TPOX was the least informative marker.

Genetic comparisons between populations coming from the Middle East and neighboring countries were analyzed using previously published STRs data. Populations used were Syrian,¹⁷ Saudi,¹¹ Iraqi,⁹ Kuwaiti,⁸ Ashkenazi Jews,¹⁰ Maltase,¹⁸ and Iranian.¹⁹ Table (1) shows the inter-population comparison between Jordanian and other Middle East and neighboring populations.

Jordanian had significant differences with Syrian population in three out of seven loci (CSFIPO, D16S539 and D7S820), with Saudi in three out of six loci (THO1, F13AO1 and vWA), with Iraqi population in five out of seven loci (THO1, vWA, D16S539, D7S820 and D13S317) with Kuwaiti in four out of seven loci (TPOX, THO1, D16S539 and D7S820), with Ashkenazi Jews in one locus out of three loci (F13AO1), with Maltese in two out of three loci (THO1 and D16S539), and with Iranian in three out of seven loci (THO1, D16S539, and D7S820). Marker D16S539 is significantly different in Jordanian population compared to all other populations listed in Table (2).

Table (1): Allele frequencies and statistical parameters of nine tandem repeat loci in the Jordanian population (n = 540 chromosome).

Allele	CSFIPO	TPOX	THO1	F13AO1	FESFPS	vWA	D16S539	D7S820	D13S317
4				0.031					
5				0.220					
6		0.009	0.281	0.311					
7	0.019	0.002	0.100	0.270					
8	0.017	0.481	0.181	0.119	0.019		0.020	0.143	0.126
9	0.030	0.113	0.172	0.009	0.024		0.120	0.130	0.089
9.3			0.219						
10	0.322	0.117	0.026		0.343		0.339	0.207	0.056
11	0.287	0.235	0.020	0.017	0.317		0.094	0.339	0.298
12	0.270	0.043		0.006	0.220		0.206	0.159	0.252
13	0.039			0.017	0.056	0.030	0.128	0.022	0.146
14	0.017				0.022	0.109	0.023		0.033
15						0.157	0.043		
16						0.243			
17						0.280			
18						0.141			
19						0.037			
20						0.007			
N	270	270	270	270	270	270	270	270	270
MAF	0.01652	0.008853	0.01711	0.012763	0.0169302	0.013319	0.017116	0.017461	0.018930
OH	0.292	0.390	0.287	0.265	0.365	0.220	0.291	0.237	0.229
EH	0.265	0.325	0.201	0.348	0.271	0.196	0.203	0.221	0.202
PD	0.886	0.707	0.905	0.908	0.852	0.940	0.912	0.944	0.948
CE	0.666	0.599	0.769	0.753	0.613	0.746	0.741	0.740	0.722
PIC	0.69173	0.64274	0.77010	0.728695	0.6817049	0.7761162	0.775908	0.746712	0.77039
P	0.042	0.092	0.152	0.112	0.143	0.043	0.155	0.027	0.048

MAF: Minimum Allele Frequencies; OH: Observed Heterozygosity; EH: Expected Heterozygosity; PD: Power of Discrimination; CE: Chance of Exclusion; PIC: Polymorphic Information Content; P: Hardy–Weinberg Equilibrium.

Table (2): Pairwise comparison of the allele frequency data from the current study to allele frequency data from neighboring populations.

Allele	Syrian ¹⁷	Saudi ¹¹	Iraqi ⁹	Kuwaiti ⁸	Ashkenazi Jews ¹⁰	Maltese ¹⁸	Iranian ¹⁹
CSFIPO	< 0.001	0.639	0.301	0.016	0.485	0.376	0.268
TPOX	0.128	0.094	0.500	< 0.001		0.964	0.723
THO1	0.080	0.007	0.008	< 0.001	0.486	< 0.001	0.001
F13A01		0.003			0.004		
FESFPS		0.828					
vWA	0.331	< 0.001	< 0.001	< 0.001		0.334	0.048
D16S539	< 0.001		< 0.001	< 0.001		0.000	< 0.001
D7S820	< 0.001		< 0.001	< 0.001		0.054	0.018
D13S317	0.404		< 0.001	0.111		0.698	0.376

Discussion

In Jordanian population, no information is available on the allele frequencies of STRs when compared to other populations in the Middle East^{5, 8-11, 18, 19}. The goal of this study was to make STRs profile for Jordanian population. The allele frequencies and population parameters of 9 autosomal loci in Jordanian population were determined. The examined loci (CSFIPO, TPOX, THO1, F13A01, FESFPS, vWA, D16S539, D7S820 and D13S317) were selected because they are commonly used in forensic and evolutionary studies.²⁰

In all of the examined alleles, frequencies were in Hardy–Weinberg Equilibrium except for the CSFIPO, vWA and D7S820 Markers. The lack of equilibrium in these alleles could be due to consanguineous marriage that is very common in the Jordanian population or could be due to the nature of the Jordanian population that has different ethnic groups and religions.

STRs have been shown to be valuable tools for identifying and typing the individual DNA component in mixed samples.²⁰ They have also been shown to be very powerful in paternity testing and in examining evolutionary relationships between different populations and ethnic groups.³⁻⁶ The result of our study demonstrates that marker D16S539 is significantly different in Jordanian population compared to all other populations. This allele can be used as a discriminating allele in forensic cases that involve individuals from Jordan and

individuals from neighboring countries.

In conclusion, a Jordanian population database has been established for the 9 STR studied loci. These loci have been shown to be a very useful tool for forensic testing and genealogical studies.

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مقارنة بين ترددات السلاسل القصيرة المترادفة (STRs) في الأردن والدول المجاورة

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الملخص

الهدف: دراسة ترددات القرائن لتسع من السلاسل القصيرة المترادفة (STRs) (THO1 و TPOX و CSFIPO و F13A01 و FESFPS و vWA و D16S539 و D7S820 و D13S317) المحمولة على الصبغيات (الكروموسومات) الجسمية على عينة مكونة من مائتين وسبعين فردا أردنيا.

الوسائل والطرق: تم مضاعفة القرائن باستخدام نظام طباعة المورث المتعدد بينما تم الكشف عن الأطوال المختلفة للسلاسل المضاعفة باستخدام الترحيل الكهربائي في الهلام الاكرالاميدي المتعدد.

النتائج والخلاصة: لوحظ من خلال هذه الدراسة أن المدلالات الوراثية D13S317 و D7S820 كان لهن أكبر قوة فصل من الناحية الإحصائية بنما كانت TPOX المدللة ذات الإخبار الأقل. كما شكلت هذه الدراسة السابقة الأولى التي تم من خلالها رسم المعالم لتسع من السلاسل القصيرة المترادفة للمجتمع الأردني ومقارنتها مع نظيراتها المنشورة عالميا للشعوب القاطنة في منطقة الشرق الأوسط.

الكلمات الدالة: السلاسل القصيرة المترادفة، ترددات القرائن الوراثية، بيانات مجتمع، الأردنيون.