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Allele frequencies of 15 Autosomal STR loci in Some of Iraqi population

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Abstract

This study was aimed to establish a database of autosomal Short Tandem Repeat (aSTR) DNA allele frequencies for an Iraqi population living in Baghdad city as a reference, therefore, a total of 456 unrelated individuals were analyzed at 15 STR DNA markers (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D2S1338, D16S539) included in the Kit from Applied Biosystems. The obtained results revealed that the Combined Matching Probability (CMP) was estimated at 1 in 3.3287×10^{-18} , and the Combined Discrimination Power (CDP) was greater than 0.98600987, which is comparable to values obtained with the many other allele frequency databases used in forensic investigations. It can be concluded that for identification purposes, it can be considered the multi-locus STR panels as a useful forensic tool.

Keywords: Iraq, allele frequency, database, STR, DNA typing

تكرار الاليلات التابعة الى 15 موقعا من المؤشرات الجسمية (التكرار الترادفي القصير aSTR) عند بعض السكان العراقيين

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الخلاصة

هدفت هذه الدراسة إلى إنشاء قاعدة بيانات كمرجع لترددات أليل الحمض النووي للمؤشرات الجسمية (التكرار الترادفي القصير aSTR) لسكان عراقيين يعيشون في مدينة بغداد، ولاتمام ذلك تم جمع 456 عينة من متبرعين لا تربطهم أي صلة قرابة، أذ تم تحليل 15 موقع منتشر على الكروموسومات الجسمية وهذه المواقع هي: (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D2S1338, D16S539). جميعها متضمنة في عدة مختبرية معتمدة من شركة عالمية (applied Biosystem). أظهرت النتائج التي تم الحصول عليها أن درجة التقارب بين العينات (CMP) قدرت بـ 1 في 3.3287×10^{-18} ، وكانت قوة التماثل بين العينات (CDP) أكبر من 0.98600987، والتي يمكن مقارنتها بالقيم التي تم الحصول

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عليها مع العديد من قواعد بيانات تردد الاليلات الأخرى المستخدمة في تحقيقات الطب العدلي. يمكن الاستنتاج أنه لأغراض تحديد الهوية ، يمكن اعتبار التكرار الترادفي القصير متعددة المواضع كأداة مفيدة في تحليلات الطب العدلي.

Introduction

Iraq has a population of 40,222,493 according to the 2020 estimate of World Population Prospects. The majority of specimens were taken from Baghdad Provinces and used (AmpFISTR1 Identifile PCR Amplification Kit), which includes the 13 DNA markers that has strongly suggested by the Federal Bureau of Investigation (FBI) forensic labs and use as the locus of choosing for forensic purposes, known as combined DNA identification system (CODIS13). The recommendation of the CODIS13 system was applied in all forensic labs around the world, and the DNA identification can be compared to each other [1, 2].

In the field of forensic caseworks, there are many aspects that can be studied like digital forensics and detection of copy-move forgery [3,4], but still, DNA analysis has been used as a basic tool for a number of different DNA analysis evaluations, start from simple routine paternity testing to large-scale human remains identification to complex forensic casework analysis. [5]. The use of a set of short tandem repeat (STR) loci as markers resulted in significant advancement in forensic casework [6]. The same procedures used in a wide range of medical and genetic settings, such as diagnosis, population genetic studies, and gene mapping, are used in DNA typing for forensics, identification, and paternity testing. [7] As a result, the significance of understanding marker heterogeneity among various populations is consistently highlighted [8]. Genetic markers STR (short tandem repeat) are a commonly used technique in forensic genetics and biological anthropology. [9].

Short tandem repeats (STRs) markers have gained much popularity in forensic DNA analysis. The genomic characteristics such as short sequence lengths, high polymorphism, and amplifying minute quantities of template DNA make these STR useful genetic markers in forensic DNA typing [10, 11]. Allelic frequency data obtained from the unrelated individual in a population is essential. It is the key to obtain reliable results in an analysis of DNA profiles [12].

Specificity of Short tandem repeat (STR) genetic locus is highly polymorphic repeat sequences. Their importance rises from the fact that they are informative genetic markers providing high statistical capacity for discrimination and individualization in many forensic and judicial regulations [13] The polymorphic nature of STRs and their use for the purpose of human identification is widely acknowledged and documented in medical and ethno-genetic searches performed by numerous groups worldwide [14]. This study was performed to establish a genetic database of the Iraqi Arab population for forensic and paternity testing purposes.

Materials and method

Population: this study was included population of Baghdad city from Iraq, healthy individuals were chosen, randomly (and, therefore likely to be unrelated). Buccal swab or known as a buccal smear which is a method of obtaining DNA of cells from the inside of a healthy individual's cheeks (Sterile Omni Swab (Whatman International Ltd., Maidstone, UK) were used as the DNA source, collected from a total of 456 individuals (229 males and 227 females) All buccal swabs were air-dried and stored until DNA extraction process.

DNA extraction: DNA was extracted from collected samples using a PrepFiler Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA) according to the manufacture's instructions. DNA quantity and purity were determined by using a NanoDrop spectrophotometer (Thomson, Wilmington, DE).

PCR amplification: Applied Biosystems AmpFiSTR® Identifiler™ kit was used, to amplify Fifteen autosomal STR markers including D3S1358, vWA, FGA, D8S1179,

D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D2S1338, D16S539 [6]. Amplification was done By using the Applied Biosystems Veriti® PCR System, approximately 1ng of template DNA from each sample. And following the protocols described in the User's Manual of Applied Biosystems,

DNA Typing: the amplified products were analyzed by electrophoresis on the 16-capillary ABI Prism® 3100 Genetic Analyzer after being diluted to (1:15) in Hi- Di™ formamide and GS500-LIZ internal size standard (Applied Biosystems), while POP™-4 (Applied Biosystems) was utilized for higher resolution separations on a 36 cm array.

Data collection: Data Collection v. 2.0 software (Applied Biosystems) was used to collect data and samples were analyzed using GeneMapper v. 3.2 software (Applied Biosystems).

Statistical Analysis: The expectations of Hardy–Weinberg equilibrium and two-locus linkage equilibrium were evaluated using Genetic Data Analysis software

Allele frequencies, power of discrimination (PD), power of exclusion (PE), polymorphism information content (PIC), match probability (MP), and typical paternity index (TPI) were calculated for each locus of the Baghdad Iraqi Arab population. [15, 16].

Results and Discussion:

Allele frequencies were calculated and statistical evaluations were performed to determine the suitability of these results for forensic and paternity studies [17]. The highest allele frequency observed for the allele of the autosomal STR loci was at the TPOX locus (0.497) for the 8 alleles as shown in (Table 1).

Table 1-Allele frequencies of 15 STR loci in the Baghdad -Iraqi population.

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539
5						0.001		
6						0.255		
7			0.025	0.004		0.202		
8	0.012		0.157	0.003		0.125	0.156	0.044
8.3						0.001		
9	0.003		0.109	0.023		0.250	0.082	0.179
9.3						0.141		
10	0.076		0.245	0.277		0.024	0.067	0.076
11	0.084		0.263	0.294	0.001		0.306	0.291
12	0.112		0.182	0.338	0.001		0.276	0.260
12.2								
13	0.273		0.019	0.048	0.003		0.081	0.129
13.2								
14	0.191		0.001	0.010	0.053		0.030	0.022
14.2								
15	0.197			0.002	0.282		0.002	
15.2								
16	0.043				0.258			
16.2								
17								
17.2	0.005				0.251			

18	0.003				0.143			
18.2								
19					0.008			
19.2								
20					0.001			
21								
21.2								
22								
22.2								
23								
23.2								
24								
24.2								
25								
26		0.003						
27		0.012						
28		0.123						
29		0.201						
29.2		0.002						
30		0.258						
30.2		0.036						
31		0.049						
31.2		0.121						
32		0.009						
32.2		0.137						
33		0.002						
33.2		0.039						
34		0.001						
34.2		0.004						
35		0.002						

Table 1-continued

Allele	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
5							
6				0.003			
7				0.002	0.001		
8				0.497		0.007	
8.3							
9				0.140	0.003	0.054	
9.3							
10				0.093	0.008	0.099	

11		0.008	0.001	0.238	0.024	0.319	
12		0.081		0.026	0.132	0.296	
12.2		0.001					
13		0.239	0.002		0.175	0.212	
13.2		0.019					
14		0.241	0.082		0.167	0.012	
14.2		0.059					
15		0.139	0.099		0.129	0.002	
15.2		0.106					
16	0.049	0.039	0.241		0.116		
16.2		0.045					
17		0.013	0.295		0.105		
17.2	0.205	0.007					
18	0.109	0.001	0.186		0.093		0.014
18.2		0.001					0.003
19	0.150		0.081		0.029		0.064
19.2							0.001
20	0.121		0.012		0.013		0.098
21	0.055				0.002		0.161
21.2							0.002
22	0.046				0.002		0.150
22.2							0.002
23	0.128						0.164
23.2							0.002
24	0.061						0.180
24.2							0.001
25	0.063						0.101
26	0.012						0.033
27							0.007
28	0.001						0.005
29							0.011
29.2							
30							
30.2							
31							
31.2							
32							
32.2							
33							
33.2							
34							
34.2							
35							

This finding is comparable to what had been observed in a previous study of Iraqis from the southern and central regions of the country [14,15] and this allele 8 has been described as the predominant one in Arab populations [18, 19, 20]. As such, it cannot be considered a characteristic specific to the Baghdad Iraqi Arab population. Several additional alleles that had been previously found to be at high frequency in a southern and central Iraqi population [18, 21] were also found to be at high frequency in this sampling of 456 central (Baghdad) Iraqi Arabs (Table 2) and slight differences in specific allele frequencies may be rooted in the relatively smaller number of individuals previously genotyped and/or to a difference in the regions where sampling was performed.

Table 2-The highest and lowest alleles at each locus for 456 (912) Baghdad Arab Individuals of 456 (912 alleles) Iraqi Arab individuals.

STR Markers	highest Allele	Frequency	No. of observations	lowest Allele	Frequency	No. of observations
D8S1179	13	0.273	249	9, 18	0.003	3
D21S11	30	0.258	235	34	0.001	1
D7S820	11	0.263	240	14	0.001	1
CSF1PO	12	0.338	308	15	0.002	2
D3S1358	15	0.282	257	11,12,20	0.001	1
TH01	6	0.255	233	5,8,3	0.001	1
D13S317	11	0.306	279	15	0.002	2
D16S539	11	0.291	265	14	0.022	20
D2S1338	17	0.205	187	28	0.001	1
D19S433	14	0.241	220	12.2,17.2,18	0.001	1
vWA	17	0.295	269	11	0.001	1
TPOX	8	0.497	453	7	0.002	2
D18S51	13	0.175	160	7	0.001	1
D5S818	11	0.319	291	15	0.002	2
FGA	24	0.18	164	19.2	0.001	1

Nineteen alleles at a total of ten different STR DNA markers were detected in this sampling of Baghdad Iraqi Arabs. These additional alleles ranged from one new allele at D8S1179 to five new alleles at the FGA locus (Table 3). Observing previously undetected alleles is to be expected whenever there is an increased sample size of the database [22].

Table 3-Alleles frequency of STR Marker In Iraqi Population.

STR Markers	Allele	No. of Observations	Frequency
D8S1179	18	3	0.003
D21S11	26	3	0.003
	32	8	0.009
	33	2	0.002
D7S820	-	-	-
CSF1PO	-	-	-
D3S1358	11	1	0.001
	20	1	0.001
TH01	5	1	0.001
D13S317	-	-	-
D16S539	-	-	-
D2S1338	-	-	-
D19S433	17.2	6	0.0065
vWA	13	2	0.002

TPOX	7	2	0.002
D18S51	7	1	0.001
	21	2	0.002
	22	2	0.002
D5S818	15	2	0.002
FGA	21.2	2	0.002
	22.2	2	0.002
	23.2	2	0.002
	28	5	0.005
	29	10	0.01

No statistically significant departures from the expectations of Hardy Weinberg Equilibrium (HWE) were observed for any of the 15 loci for which data was available (Table 2). After applying a Bonferroni correction [23], no pairs of loci were found to have statistically significant departures from the expectations of linkage equilibrium (LE) [16, 24]. The lowest p -value for pairwise (LE) was found for the vWA/D3S1358 ($p=0.004$) pair of loci. The highest observed heterozygosity (H_o) was observed at the D18S51 locus ($H_o=85.96\%$) followed by the FGA locus ($H_o=85.75\%$) and lowest was observed at the TPOX locus ($H_o=66.67\%$) as shown in Table 4.

Table 4-Observed and expected homozygosity and heterozygosity for each of 15 autosomal STR loci for 456 Baghdad Iraqi Arab individuals:

Locus	Observed	Observed2	Expected	Expected3
	Homozygosity	Heterozygosity	Homozygosity	Heterozygosity
D8S1179	19.08%	80.92%	17.73%	82.27%
D21S11	17.54%	82.46%	16.06%	83.94%
D7S820	22.81%	77.19%	19.95%	80.05%
CSF1PO	28.29%	71.71%	28.04%	71.96%
D3S1358	26.10%	73.90%	23.20%	76.80%
TH01	20.61%	79.39%	20.47%	79.53%
D13S317	20.83%	79.17%	21.29%	78.71%
D16S539	23.90%	76.10%	20.88%	79.12%
D2S1338	17.11%	82.89%	12.28%	87.72%
D19S433	18.86%	81.14%	16.03%	83.97%
vWA	24.12%	75.88%	20.32%	79.68%
TPOX	33.33%	66.67%	33.24%	66.76%
D18S51	14.04%	85.96%	12.75%	87.25%
D5S818	23.25%	76.75%	24.71%	75.29%
FGA	14.25%	85.75%	13.32%	86.68%

An analysis of all possible pairwise comparisons of the 456 genotypes was performed, similar to the analysis performed in [25, 26]. The observed mean and standard deviation was not statistically significantly above what was observed in an analysis of a population of 5,000 simulated individuals with the same allele frequencies. Therefore, a theta correction factor of 0.01 for hidden population substructure would be appropriate [27].

All loci show high Power of Discrimination ($PD \geq 0.79$) and power of Exclusion (PE) is greater than 0.50 at all loci except TPOX (PE=0.33) CSF1PO (PE=0.44) and D3S1358 (PE=0.47). All STR loci with $PE > 0.50$ can be considered highly polymorphic [28].

Polymorphism Information Content (PIC) was found to be highest for the D2S1338 locus (PIC = 0.87) and lowest for the TPOX locus (PIC = 0.62) (Table 5).

Table 5- Forensic statistical parameters for each of 15 autosomal STR loci (the 13 CODIS core loci and D19S433 and D2S1338) in 456 Baghdad Iraqi Arab individuals:

Forensic Parameters						
Locus	Matching Probability	Power of Discrimination	Polymorphism Information Content	Power of Exclusion	Typical Paternity Index	Paternity Index Expected
D8S1179	0.0542	0.95	0.80	0.57	2.82	2.82
D21S11	0.0448	0.96	0.82	0.60	3.11	3.11
D7S820	0.2129	0.79	0.56	0.51	2.51	1.48
CSF1PO	0.1308	0.87	0.67	0.44	1.78	1.78
D3S1358	0.0926	0.91	0.73	0.47	2.16	2.16
TH01	0.0733	0.93	0.76	0.55	2.44	2.44
D13S317	0.0754	0.92	0.76	0.54	2.35	2.35
D16S539	0.0741	0.93	0.76	0.50	2.39	2.39
D2S1338	0.0272	0.97	0.87	0.61	4.07	4.07
D19S433	0.0442	0.96	0.82	0.58	3.12	3.12
vWA	0.0702	0.93	0.77	0.50	2.46	2.46
TPOX	0.1565	0.84	0.62	0.38	1.50	1.50
D18S51	0.0298	0.97	0.86	0.66	3.92	3.92
D5S818	0.1019	0.90	0.71	0.51	2.02	2.02
FGA	0.0323	0.97	0.85	0.66	3.75	3.75
Combined Matching Probability (CMP)			3.3287×10^{-18}			
Combined Discrimination Power (CDP)			1			
Combined Exclusion Probability (CEP)			0.98600987			

The Combined Matching Probability (CMP) estimated at 1 in 3.3287×10^{-18} and the Combined Discrimination Power (CDP) reaches about 1, which should be sufficient for matching between any individual even its most close relatives. The Combined Exclusion Probability (CEP) value for the fifteen loci considered together is greater than 0.98600987, These findings strongly support the use of this combination of genetic markers for paternity testing and human identification analysis [29, 30].

Conclusion

Statistical tests that were performed and the allele frequencies obtained from this study are established to confirm the suitability of markers of the 15 autosomal STR loci for forensic and paternity studies. In the Iraqi population, multi-locus STR panels may be a useful forensic tool which can be applied for identification purposes. Also, according to Hardy-Weinberg expectations all tested loci showed no significant deviation. The obtained results further suggest that proper DNA databases should be generated and employed for forensic genetics calculations to exonerate or convict a suspect from the crime scene.

Ethical Clearance

Informed consent was obtained from all individual participants involved in the study.

Conflict Of Interest

The authors have declared that no conflict of interest exists.

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