Research Article
Volume 13:6, 2022

Journal of Forensic Research

ISSN: 2157-7145 Open Access

Investigation of the Allelic Frequency of 7 Autosomal loci in the Kurdish Population of Iran

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Abstract

Introduction: The number of repeats of a single microsatellite may be different in different individuals, and this is the basis of their use in genetic fingerprinting. The aim of this study was to investigate of the allelic frequency of 7 autosomal loci in the Kurdish population of Iran.

Materials and Methods: Two fifty hundred men and women non-relatives of living Kurds in Kurdish provinces of Iran were randomly selected for the study of 7 autosomal markers (D16S539, D2S1338, D7S820, D21S11, D18S51, CFSIPO, and D13S317). After molecular analysis, allele's frequency distributions and other population genetic parameters were done.

Results: The D21S11 and CFSIPO markers had the highest (0.8324) and the lowest (0.7400) polymorphism in the studied population, respectively. All of the 7 autosomal loci studied in Iranian Kurdish population had above 0.7 and 0.6 polymorphism and heterozygosity, respectively.

Conclusion: The degree of differentiation power or PD for 7 autosomal markers was between 0.730 and 0.884, which indicates a high differentiation power for all 7 markers.

Keywords: Forensic medicine • Polymorphism • Autosomal markers • Short Chromosome Repeat Sequences (STR)

Introduction

Almost about 440 million STR markers exist in the genome of creatures that most of them have polymorphic alleles with different lengths. The difference in the length of alleles is due to the difference in the number of times that the main monomer sequence has been repeated. These polymorphisms include Single Nucleotide Polymorphism (SNP), Restriction Fragment Length Polymorphism (RELP), and minisatellite and microsatellite sequences [1]. There are many DNA polymorphism uses in different settings such as gene mapping and fingerprinting and identity tests [2]. Minisatellite and microsatellite markers due to multiple alleles are the best sequences used for these purposes. Polymorphism in these markers is like the repetition of sequences. The difference between these two markers is that in minisatellite markers, the length of repetition units is larger than microsatellite sequences with the repetitive sequence lengths of 1, 2, 3, 4, 5, and 6 nucleotides [3]. One of the most important uses of these markers is DNA fingerprinting and identification by DNA.

In both markers, the numbers of repetitive central sequences are different among people and this constitutes the basis for the frequent use of these genetic markers in fingerprinting by DNA. It means that different people through the difference in loci repeats of these markers can be separated from each other [4]. DNA fingerprinting was started in 1985 and at that time, Jeffry and colleagues studied minisatellite sequence in the genome. They used the RFLP technique and blotting and could use the numbers and repetitive patterns in the mentioned sequences for each person as individual indicators. Therefore, DNA fingerprinting was established in forensic labs. From 1991, the use of microsatellite markers became popular. Tracking these markers is based on the PCR technique. The use of these markers in identification tests has more advantages than minisatellite markers. Most of the samples that are used in identification tests, due to degradation in environmental conditions, contain a small amount of DNA [5].

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Received: 07-Nov-2020, Manuscript No. JFR-PreQc-20-001; **Editor assigned:** 23-Dec-2020, Pre QC No JFR- PreQc-20-001 (PQ); **Reviewed:** 05-Jan-2021, QC No JFR-PreQc-20-001; **Revised:** 12-Nov-2021, Manuscript No. JFR-PreQc-20-001 (A) ; **Published**: 19-Jan-2022, DOI: 10.37421/ 2157-7145.2020.13.001

Materials and Methods

Samples

This research is a case-control study. The study was ethically approved by Iranian Legal Medicine Research Center, Legal Medicine Organization, and Tehran, Iran. To conduct the study, 250 unrelated women and men were equally selected as samples. The participants lived in the Kurdish regions of Kurdistan province with Ardalani-Sorani-Kurmanji dialects and were selected completely randomly to study autosomal markers. In order to comply with the current ethical standards in research and to respect the rights of the participants, a letter of consent was designed which was signed by the candidates. Also, the objectives of the project were fully explained to them and all donors participated in the research with consent and full knowledge. Then, 5 ml of blood was taken using a 10 ml syringe which was later transferred into CBC tubes containing EDTA as an anticoagulant. All blood samples were kept at -20°C until DNA extraction.

DNA extraction

To extract DNA, the researcher used the DNA extraction kit of Kowsar Biotechnology Company, which employs the salting-out method.

Primers

Common primers for each locus of the site were selected from http://www.UniSTS and synthesized by MACRO GEN–seoul-korea before application (Table 1).

Table 1. The frequency of the primers.

The name of the primer	The frequency of the primers
CSF1PO	Forward:5'-AACCTGAGTCTGCCAAGGACTAGC-3'
	Reverse: 5'-TTCCACACACCACTGGCCATCTTC-3'
D21S11	Forward:5'-ATATGTGAGTCAATTCCCCAAG-3'
	Reverse: 5'-GTT GTA TTA GTC AAT GTT CTC C-3'
D13S317	Forward:5'-ACAGAAGTCTGGGATGTGGA-3'
	Reverse: GCCCAAAAAGACAGACAGAA 5'--3'
D18S51	Forward:5'-CAAACCCGACTACCAGCAAC -3'
	Reverse: GAGCCATGTTCATGCCACTG 5'--3'
D7S820	Forward:5'-TGTCATAGTTTAGAACGAACTAACG--3'
	Reverse: 5'-CTGAGGTATCAAAAACTCAGAGG -3'
D16S539	Forward:5'-GATCCCAAGCTCTTCCTCTT-3'
	Reverse: 5'-ACGTTTGTGTGTGCATCTGT-3'
D2S1338	Forward:5'-CCAGTGGATTTGGAAACAGA-3'
	Reverse: 5'-ACCTAGCATGGTACCTGCAG-3'

PCR

For every 250 DNA samples extracted for 7 markers in Table 1, PCR was performed in a volume of 25 µl. Promega PCR kits were used for PCR. The PCR, which consisted of 22 µl (1 µl specific primer and 21 µl master mixes) and 3 µl DNA samples, was performed at the annealing temperature obtained for each marker in 35 cycles in a thermocycler. The quality of PCR products was evaluated using 3% agarose gel at 110 voltages for 2 and half hours in TBE buffer prepared by Sina Clone Company. The propagation of the desired locus was carried out well due to the appropriate annealing temperature, and sharp bands were created for each sample. For staining the gel, the researcher used DNA safe stain dye prepared by Sina Clone Company in a volume of one microliter [6].

Isolating of STR alleles and determining the band weights

Polyacrylamide gel was used to isolate alleles while silver nitrate was used to detect DNA molecules. The gel was soaked in 150 ml of 50% methanol+5% acetic acid for 20 minutes. It was then washed in a container with 150 ml of 50% methanol for 10 minutes. The gel was washed with water for 10 minutes and was incubated with 150 ml of 2% sodium thiosulfate for one minute and washed twice with water for one minute. Moreover, the gel was later immersed in 150 ml of 0.1% silver nitrate and 0.08% formalin for 20 minutes and washed twice with water for 1 minute. The gel was then incubated in 150 ml of 2% sodium carbonate and 0.04% formalin to achieve the desired staining intensity. Finally, it was rinsed in 150 ml of 5% acetic acid for 10 minutes and was washed again for another 5 minutes. When samples were run on the gel and the time required to separate the alleles elapsed, the Gel Analyzer 390 was used to determine the weight of the bands.

Results

The characteristics of D16S539, D21S1338, D7S820, D21S11 D18S51, CFSIPO and D13S317 autosomal loci. Also, other information inferred using statistical software according to the mentioned methods.

Sixteen different alleles were detected for Locus D21S11, which had heterozygosity as well as a high diversity (nei) (0. 0-0880-8496). The allele with the highest frequency was number 30 with a value of 0.2400.

About CSF1PO marker, 7 alleles were detected for this locus, ranging in size from 331 to 287 bp, with the allele 12 as the one with the highest frequency (0.3150). The rate of heterozygosity and its diversity was 0.7452 and 0.7400, respectively.

The number of alleles observed for D7S820 gene locus was 13 with a frequency of 0.005, which indicates the polymorphism of this gene locus. The highest allele frequency observed in locus

D7S280 was related to number 6 with a frequency of 0.256. In comparison the heterozygosity of this marker, i.e. 0.76, with other studies showed close relation with populations in Bosnia (0.706) and Palestine (0.690).

The number of alleles observed for the D18S51 gene locus was 19. In addition, the highest allelic frequency for locus D18S51 was related to allele 8 (0.285). Also, the number of alleles observed for D13S317 gene locus was 10 and the highest allelic frequency for locus D13S317 was related to allele number 6 with 0.205. The lowest allelic frequency for locus D18S51 was related to allele 4 whereas the least allele frequency for locus D13S317 was for allele 4.

Five different allele were detected for D2S1338 locus that had heterozygosity, diversity and polymorphism (0.7127-0/7522/0.8200). High diversity and polymorphism proved the usability of this locus in forensic studies and identification and many other applications in this population [7].

Also, 11 different alleles were detected for D16S539 locus that included heterozygosity, diversity and polymorphism (0.7805/0-8062/0-8500).

Discussion

In this study, 250 non-relative Kurds from Iran were investigated and analyzed for allelic diversity and heterozygosity of autosomal loci D21S11, D7S820, D18S51, D16S539, D21S1338, CFSIPO, and D13S317. All loci were in Hardy-Weinberg equilibrium and had high polymorphism and diversity. High diversity and polymorphism proved the usability of these loci in forensic studies and identification procedures besides many other applications on the investigated population. Sixteen different alleles were detected for Locus D21S11, which had a heterozygosity as well as a high diversity (nei) (0.0-0880-8496). The allele with the highest frequency was number 30 with a value of 0.2400, which can be explained as follows in comparison with the populations of other regions: In the Kurdish population of northern Iraq, the 29th allele has the highest frequency (0.224) and its heterozygosity is estimated to be 0.847. Nonetheless, allele 29 had an allelic frequency of 0.241 in the population of Azerbaijan.

As for the Philippines, the highest allelic frequency was related to allele 30 with an allelic frequency of 0.2351 and a heterozygosity of 0.8566. For the Palestinians living in Iraq, the allele frequency was 30.2 with a frequency of 0.330 and the highest allele and heterozygosity frequency was 0.8396. In the Iranian population, the allele with the highest frequency was allele 29 which had a frequency of 21.40%. In another comparison with the Iraqi Kurds, allele 30 with the frequency 0.2426 had the highest frequency with a heterozygosity of 0.881. In this study, allele 37 with a frequency of 0.2400 had the highest allelic frequency.

Also, 11 different alleles were detected for D16S539 locus that included heterozygosity, diversity and polymorphism (0.7805/0-8062/0-8500). High diversity and polymorphism proved the usability of this locus in forensic studies, identification process and many other cases. In this study, the allele with the highest frequency was number 11 with a frequency of 0.3000. In comparison, as regards the population of Azerbaijan, the allele with the highest frequency and 11 repetitions was the one with 0.259 allelic frequencies. Among Palestinians living in Iraq(14), allele 11 with a frequency of 0.292 had the maximum allelic frequency and a heterozygosity of 77.36%. In the population of Iranians, the most frequent allele was number 11 with a frequency of 31.10%. In another comparison with Iraqi Kurds, the allele with 11 replications and with a frequency of 0.3544 had the highest allelic frequency and a heterozygosity of 0.806.

Five different allele were detected for D2S1338 locus that had heterozygosity, diversity and polymorphism (0.7127-0/7522/0.8200). High diversity and polymorphism proved the usability of this locus in forensic studies and identification and many other applications in this population. The allele with the most frequency was 17 tetra nucleotides with a frequency of 0.3600, which is compared with the populations of other regions here. In the population of Azerbaijan, the allele with the highest frequency was the one with 15.2 replications and an allelic frequency of 0.199. Among Palestinians living in Iraq, allele 17 with a frequency of 0.349 had the foremost allelic and heterozygous frequency of 77.36%. In the Iranian population, the allele with the highest frequency was the 16th allele with a frequency of 26.30%. In another comparison with the Iraqi Kurds, the allele enjoying 17 replications with the maximum frequency of 0.3641 had the highest allelic frequency and a heterozygosity of 0.806.

CSF1PO is a simple tetra nucleotide repeat located in the e-fms proto-oncogene gene encoding the CSF-1 receptor on the long arm of chromosome 5 (5q33.1). So far, 23 alleles have been reported for different populations. In the population of current study, 7 alleles were detected for this locus, that comparison of this study with other populations showed in the Kurdish population of northern Iraq, the allele with the highest frequency was that of 11 alleles. For the population of the Philippines, Azerbaijan and Palestinians the allele with the biggest frequency was the numbers of 12, 10 and 11 alleles, respectively [8]. Allele 10 with a frequency of 30.80% had the highest frequency in the case of Iranian population. In another comparison with the Iraqi Kurds, the allele with the largest frequency was number 12.

Conclusion

The closeness and similarity of the results of comparisons among populations and also their differences can be attributed to the closeness and proximity and geographical connections, genetic origin, race and migration. Also, the degree of differentiation power or PD for 7 autosomal markers was between 0.730 and 0.884, which indicates a high differentiation power for all 7 markers.

Conflict of Interest

The authors declare that have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were by the ethical standards of the ethical Iranian Legal Medicine Research Center committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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How to cite this article: Homayounpour, Gholamreza, Farouq Karimpour and Fatemeh Keshavarzi. "Investigation of the Allelic Frequency of 7 Autosomal loci in the Kurdish Population of Iran." *J Forensic Res* 13 (2022): 001.