Nia NH, et al., Cell Mol Biol 2020, 66:2

Cellular and Molecular Biology

Research Article Open Access

Genetic screening of Y-STR (DYS389I/II and DYS385) in a Random Population of Iranian Kurdish Men

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Received: May 25, 2020; **Accepted:** July 31, 2020; **Published:** August 03, 2020

Citation: Nia NH, Keshavarzi F, Sadeghizedeh M (2020) Genetic screening of Y-STR (DYS389I/II and DYS385) in a random population of Iranian Kurdish men. Cell Mol Biol. 66:160.

Abstract

Introduction: Human Y-chromosome short tandem repeats (Y-STR) are powerful markers in forensic genetics. The objective of this study was to determine the haplotypes and allele frequency of DYS385 and DYS389I/II microsatellites in a random population of Iranian Kurdish men.

Materials and methods: Genomic DNA was extracted from 192 Kurdish volunteers using a cinnaclon kit (DN8115c.cn). Genotyping of Y-chromosomal STRs (DYS385 and DYS389I/II) was done by HRM method. Arlequin Software v.3.5 was used to calculate allele number, haplotype frequency, gene diversity (GD) and haplotype diversity (HD).

Results: The allelic frequency and number of alleles of DYS385, DYS389I and DYS389II STRs were 0.73, 0.66, 0.37 and 7, 7, 4, respectively. A total of 23 haplotypes were recorded, 8 of which were unique. 9 haplotypes have the greatest frequency in each of the four provinces. In addition, 5 and 3 unique haplotypes were observed in Kurdistan and Kermanshah provinces, respectively. Average locus diversity was 0.887 ranging from 0.01 for DYS385 to 0.659 for DYS389I.

Conclusion: The results of N.e (effective number of haplotypes) showed that amongst the four provinces, the Kurdish population of Kurdistan Province had the most effective haplotype. Comparative results demonstrated great similarity to the Kurdish population of Iraq. These loci can be used in forensic medicine since they have high variation and polymorphism.

Keywords: DYS385; DYS389I/II; Iranian Kurdish men; HRM

Introduction

Short tandem repeats (STRs) are short sequences of deoxyribonucleic acid (DNA), usually with a length of 2-6 bp repeated frequently in a head-tail manner. In humans, they are estimated to be approximately comprising of 2% of the genome. Y-STRs are taken specifically from the male Y-chromosome. Y-STRs are located on the entire length of the tall arm of the Y-chromosome. It consists of three distinctive regions on the Y-chromosome: two pseudo autosomal regions and a heterochromatic region known as a non-recombining region (NYR) [1]. Y-STRs are used in separating different groups of the human population and in genetic studies. These STRs are often used in forensics, missing person's investigations, historical investigations, some paternity testing sceneries and genetic genealogy [2]. Even though they are often utilized for suggesting which haplogroups an individual match, STR analysis typically provides a person's haplotype [3]. According to the variation of their repeats, STRs show a wide range of polymorphisms. Thus, they can be seen differently in multifarious populations.

As Y-STRs do not have any homologues on the X chromosome, their specific haplotypes have an important role in demographic and genealogic studies [4-5]. Many studies on STR have been undertaken. The use of Y-chromosomal polymorphisms for male identification in forensics began in 1966 with the analysis of whole Y-chromosome length polymorphisms to detect inclusion constellation in paternity cases [6]. Then, in 1992, Lutz Roewer reviewed the first Y-STR (Y-27H3) which is currently known as the DYS19 [7]. Online Y-chromosomal short tandem repeat haplotype reference database (YHRD) was published for Asian, European and U.S. populations in 2002.YHRD represents the largest collection of male-specific genetic profiles [8-10]. Other studies have analyzed Y-STR (including 385, 389I, 389II) for evaluating haplotype and genetic diversity [11,12]. Allelic frequencies were obtained for this locus and the highest gene diversity was observed at DYS385 [11,13,14]. In 2007, HRM was developed to identify many genetic variations such as point mutations and multiple genotyping [15]. Moreover, HRM was used for Y-STR genotyping for forensic genetic screening [16,17]. In Iran, there have been few studies in this regard.

In the current study, we present the haplotype and allelic frequency distribution in populations of the provinces of Kurdistan, Kermanshah, Elam, and West Azerbaijan based on a set of three Y-STR polymorphism loci (385, 389I, 389II); describe the value of forensic genetics and compare with similar markers in other Kurdish populations around the globe. In this study, the HRM method was

used to detect alleles which were carried out in a shorter time with greater precision compared to other common methods such as capillary electrophoresis. The basis of HRM method is finding the differences in a nucleotide sequence or in another word, genotyping. The objective of this study was to determine the haplotypes and allelic frequency of DYS385 and DYS389I/II loci of Y-chromosome amongst Kurdish male Iranians.

Material and methods

Sampling

After obtaining informed consent, blood samples were obtained from 192 unrelated Iranian Kurdish males from Kurdistan, Kermanshah, Ilam, and West Azerbaijan provinces in Iran.

DNA extraction

Genomic DNA was extracted from the whole peripheral blood samples using the standard extraction protocols kit (DN8115c.cn). All extracted DNA was quantified by Nano drop method.

HRM analysis

HRM PCR Kit was purchased from Sinagene and STR primers (DYS385; fdw-5'-GACAGAGCTAGACACCATGCC and Rev-GATCTATCTATTCCAATTACATAGTCCTC-3', DYS389I/II; 5'-AGCTACTTCTGTATCCAACTCTCACCTG-3' and Rev; 5'-AAACTTGAGGAACACAATTATCCCTGA-3') was taken from the STRbase DNA database (http:// www.cstl.nist.gov/strbase). Each Monoplex- HRM PCR was prepared in a total reaction volume of 25.0 µL consisting of 3 ng genomic DNA, 1 µL mix primers (0.5 µL forward and 0.5 µL reverse), 5.0 µL 2X HRM PCR Master Mix (containing the fluorescent dye Eva Green) and 14 µl ddH2O. Polymerase chain reaction (PCR) were carried out by a Rotor-Gene Q thermal with a program consisting of an initial denaturation step of 93°C for 8 min followed by 35 cycles of 93°C for 45 s, annealing for 45 s, and 72°C for 60 s. In continuous process, melting curve data was taken or the HRM curve from machine and HRM algorithm was run performed using the Rotor-Gene software in comparison with the male genomic DNA standard provided by Dr. Zeinali's Lab in Iran.

Statistical analysis

Arlequin Software v.3.5 was used to calculate allele number, haplotype frequency, gene diversity (GD) and haplotype diversity (HD). Intra population Analysis (IPA), Fst Differ and NEI GD were computed using the GeneAlex Software, v.6.4. Mega software was used to plot the charts. HRM curve analysis was performed using the Gene mapper software (v.3.2, Applied Biosystems).

The allele frequencies were estimated by direct gene count. The gene diversity (GD) and haplotype diversity (HD) were measured with the help of the formula N (1 _Ppi2) / (N-1), and the GenAlex software analyzed the initial analysis of the samples and the frequency of allele in the population was investigated. The Arlequin software (v3.5.1.2) was used to check the haplotype frequency. Probabilities (p values, 10,000 permutations) were calculated to measure the genetic distance. The GenAlEx, Fst software was determined by the populations studied and compared the data obtained from the population compared with other published data from neighboring populations.

Results

The characteristics of markers are presented in Table 1 and also in Figures 1 and 2, the result of HRM of locus 389I / II and 385 are presented.

HRM technique is based on the melting temperature of PCR products. In fact, the melting temperature of each sample is due to the discovery of two strands of DNA and single strand due to differences in alleles and thus nucleotides, and the HRM technique is based on these temperature differences in Tm. Samples are sorted in different variants. In the graphs, the varieties are separated by different colors in the graph, and each color represents a variant. People in the same variant are shown in a single color, and they are separated from each other. Each color and consequently each variant correspond to one of the locus alleles. Therefore, the samples were categorized according to this chart and the allele frequency was obtained based on matching with samples whose genetic profile was taken from the molecular diagnostic laboratory.

The number of alleles in Table 2 was obtained by matching the alleles of the control samples from which a sample with a specified profile along with other specimens was placed on the PCR machine. According to the results, the allele frequency in locus DYS385 is 73%, in DYS389III locus is 66% and in DUS389I locus is 37%, which expresses high polymorphism in two loci DYS385 and DYS389II. In all four western provinces of the country, the highest replication of alleles in the DYS389I locus is related to allele 13, in locus DYS389II related to allele 30, and in locus DYS385 associated with allele 30.

Allelic frequency

The analysis of the samples was done with the help of GenAlex software and the frequency of the allele was studied in the population (Table 3).Table 3 shows the most allele frequency in each of the four provinces. Also, Chart 4 shows the prevalence of allele in the male population of the west of the country by province. As shown in Tables 3 and 4, the highest number of alleles in the DYS385 position with 7 alleles and the lowest number of alleles associated with the DYS389I position with 4 alleles. Also, at DYS385, the highest genetic diversity (GD) was 0.73, and the lowest genetic diversity was found for DYS389I and 0.38. Also, in DYS389I, the highest allele frequency is for allele 13, in DYS389II, with the highest allele frequency associated with allele 30, and at DYS385, the highest allele frequency is related to allele 12.

Table 5 shows haplotype frequency distributions of 3 Y-STR in Kurdish male populations of the Western provinces of Iran. The 23 haplotypes were observed in the population of which 9 haplotypes are specific (Figure 3). Haplotype #9 has the highest frequency in each of the four provinces. The results show that Kurdistan and Kermanshah provinces have the highest number of haplotypes. Specific haplotypes are also available in these two provinces.

Forensic parameters of 3 Y-STR loci in the Kurdish male population of the western provinces are presented of Iran in Table 6. Kurdistan province has the highest amount of N_e and therefore has the most influential haplotypes on the population. Also, the highest amount of genetic diversity was observed in Kurdistan province.

Discussion

In this study, the allelic frequency and haplotypic abundance of 3 Y-STR sequences in 192 males from residents of the Kurdish provinces of

western Iran (Kurdistan, Kermanshah, West Azerbaijan and Ilam) were investigated. According to the results, the allele frequency in locus DYS385 is 73%, in DYS389III locus is 66% and in DUS389I locus is 37%, which expresses high polymorphism in two loci DYS385 and DYS389II. In all four Western provinces of the country, the highest repeat of allelic in DYS389I, DYS389II and DYS385 loci are related to alleles 13, 30, and 30, respectively. While according to previous studies [10] in Isfahan population, the highest replication of alleles in DYS389I associated with locus 13, in DYS389II, is 29, and in DYS385, the allele is 12, and therefore, these two populations are different in one of the three sites studied. In the population of Tehran, the highest replication of alleles in DYS389I was related to Locus 13, in DYS389II it was 30, and in DYS385, the highest allele frequency was related to allele 12 [9]. In the Kurdish population of Iraq, the highest repeat of ally in DYS389I is related to Locus 13, in DYS389II, it is 30, and in DYS385, the most frequent allele is allele-13 [11]. In the Turkish population, the highest frequency of alleles in Locus 385 of the allele 10 was in Locus DYS389I and related to the allele 13, and in the locus DYS389II, it was related to the allele 12.

Also, Table 5 shows the number of haplotypes, and in Figure 3, these haplotypes are compared in all four Kurdish provinces in western Iran. In the study population, 23 haplotypes were identified, with 9 specific haplotypes. Haplotype # 9 has the highest frequency in each of the four provinces. Table 5 shows that there are 5 dedicated haplotypes in Kurdistan province and 4 specific haplotypes in Kermanshah province.

According to Tables 6, the Fst values in each of the four Kurdish provinces of the west of the country are less than 0.25, indicating a low degree of differentiation among these populations and their similarity, but there is still a small difference between these populations. In fact, the results of Alley's abundance show that the Iranian Kurds have the most similarities. In addition, among the neighbors, the open-ended population most closely resembles the Iraqi Kurds, and this is more similar to that of Tehran and Isfahan (Table 6). This similarity between the alley repeats in all four provinces of the Kurdish province of the West indicates a high incidence among the Kurds, although the existence of specific haplotypes may indicate differences within the population of the girl under study. Comparing the results with studies of Turkey, China, Georgia and Kurdish groups in Europe, the Caucasus, the West and Central Asia, showed that there is a genetic similarity between the population studied and the Western Asian and Central Asian groups (Table 7).

Also, as shown in Table 7, DYS385 locus has the highest frequency of alleles in the Kurdish population of Iran and can be used in forensic medicine due to its high polymorphism, along with other markers. In a study by Vatan Doost and his colleagues in 2015 on 8 Locus STRs in the population of Isfahan, DYS385 was selected with the highest frequency as a polymorphism marker in forensic medicine [15]. In a study by Salimi and colleagues in 2010 on the prevalence of haplotypia and allele frequencies in Tehran's men's random population, DYS385 locus had the highest polymorphism [16]. In a study conducted by Karim et al. in 2015 on STR, DYS385 showed the highest polymorphism compared to other loci [17-23].

Conclusion

In the studied population, the Kurdish provinces of the West have high haplotypic variations and, despite differences with other ethnic groups, there are similarities in the population of these four provinces. Also, the Iranian Kurds are very similar to the Kurdish population of Iraq, which indicates the common cause of these populations and the desire of the Kurds to be inward.

Consent for publication

Not applicable.

Availability of data and material

Data and materials will be available on request from corresponding author.

Competing interests

There is no conflict of interest.

Funding

None

Ethics statement and an informed consent

The study was ethically approved by the Research Committee of Department of Genetics, Faculty of Advance Science & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran. Informed consent was obtained for intervening with volunteers that included some pivotal elements. After obtaining informed consent, blood samples were obtained from 192 unrelated Iranian Kurdish males from Kurdistan, Kermanshah, Ilam, and West Azerbaijan provinces in Iran.

Contribution of authors

Fatemeh keshavarzi designed the study and wrote the manuscript; Nooshin Hashami Nia, conceived the experiments, prepared the figures and collected the samples. Majid Sadeghizadeh has provided technical expertise for the study and had given advice on the project. All authors reviewed and approved the manuscript.

Acknowledgments

The authors would like to thank the volunteers who contributed samples for this study.

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