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RESEARCH ARTICLE

Y-chromosomal Short Tandem Repeat Variation in Kurd, Assyrian, and Armenian populations in Iraq Kurdistan

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ABSTRACT

Background and objectives: North central Middle Eastern countries Iran, Iraq, Turkey, and Syria all have persistent Kurdish regions. Over thousands of years, several ethnicities have immigrated, settled, or resided in the region, including Turks, Persians, Arabs, Kurds, Armenians, Assyrians, Chechens, and Azeris. The aim of the present study is to use a set of Y-STR to characterize the genetic structure of the Kurd, Assyrian, and Armenian population and build a phylogenetic tree among them.

Methods: Eleven Y-chromosome STRs were evaluated in a total of 90 unrelated males from the Kurds, Armenians, and Assyrians populations in the Kurdistan region of Iraq (DYS19, DYS390, DYS393, DYS426, DYS437, DYS439, DYS447, DYS460, DYS461, DYS481, and DYS576). Using a DNA extraction kit, total DNA was isolated from leukocytes. PCR products were run on 8% polyacrylamide gel with a 50bp ladder as DNA marker to size the bands, and silver staining was used to identify the DNA bands. Power Marker V3.25 software was used to determine variety of genetic parameters, including total allele number, allele frequency, gene diversity, polymorphic information content (PIC), and phylogenetic tree was constructed by MEGA-X software.

Results: The total number of alleles identified in the three populations was 380. The sizes of the alleles ranged from 87bp to 275bp.The most diverse loci were DYS447 and DYS576 (GD: 0.949), whereas DYS426 showed the least diversity (GD:0.896). The Phylogenetic tree divided the populations into two main clusters; The Kurdish and Armenian clades in one cluster and the Assyrian in another cluster. Few of dendrogram leaves from the three examined groups were admixed with each other.

Conclusions: This study confirms the high-resolution Y-STR typing's ability to discriminate. It concludes that the genetic distance between Kurd and Armenians is less than the genetic distance between the Kurd and the Assyrians, meaning that the Armenians population are genetically closer to the Kurds population.

Keywords: Genetic diversity, population genetics of Iraqi Kurdistan, Y-chromosome STRs

INTRODUCTION

The Kurds are one of the indigenous peoples of the Mesopotamian plains. The adjacent Kurdish areas of Iran, Iraq, Turkey, and Syria are located in the Mid East's northern central region. Various ethnic groups, including Turks, Persians, Arabs, Kurds, Armenians, Assyrians, Chechens, Azaryan and others have immigrated to, established in, or lived there naturally over the centuries. A significant amount of archaeological evidence suggests that this region is the site of the Neolithic transition (Dogan *et al*., 2017). Assyrians are an ethnic group indigenous to Assyria, a region located in the Middle East (Oppenheim,1964). The tribal areas that form the Assyrian homeland are parts of present-day northern Iraq, south eastern Turkey, north western Iran, and north eastern Syria (Nisan, 2002). They are speakers of the Neo-Aramaic branch

isolated population who historically inhabited a region in the Near East bounded by the Mediterranean and Black seas and the Caucasus. They have a complex history including a major geographic displacement during World War I (Haber *et al*., 2016). Armenian is genetically related to Indo-European languages such as Hittite, Sanskrit, Avestan, Greek, Latin, Gothic, and Slavicas. They have an independent branch of the Indo-European language family. The Armenian and the Kurds historically are living together in one empire or divided between the neighboring empires (Martirosyan, 2014). Numerous DNA-based data are utilized to examine the phylogeography, origins, and demographic history. Numerous Y chromosome polymorphism studies have been conducted for forensic purposes, prenatal testing, and human migrations (Jobling and Tyler-Smith 2000;

Quintana-Murci *et al*., 2001). With a small amount of mutation and gene conversion, the Y-STR markers are passed from generation to generation without recombination (Rozen *et al*., 2003; Trombetta *et al.*, 2010). These markers aid to understand the geographical population dynamics and its demographic history (Roewer *et al*., 2005) and also provide data on the male lineage relationship (Lowery *et al.*, 2013). Due to its ability to distinguish between distinct genetic variants and the highly informative Y chromosome STR haplotypes they formed, Y-STR genotyping has become an important tool in forensic investigations. Non- recombining Y chromosomal markers are more sensitive to founder effects and genetic drift, hence Y-STRs are particularly effective in identifying genetic variations between populations (Heraclides *et al.*, 2017; Li *et al*., 2016). To illustrate, the Indo-European Kangju and Wusun people were displaced by the male-biased westward expansion of the Xiongnu nomads from the eastern steppe, which resulted an extensive admixture of east Eurasian lineages (Damgaard *et al*. 2018). Following the formation and blending of many nomadic Turkic states, gene flows between various groups of the former Hunnic empire occurred (Damgaard *et al*., 2018; Gnecchi Ruscone *et al.*, 2021). The aim of the present study is to use a set of microsatellite markers to characterize the genetic structure of the Kurdish, Assyrian, and Armenian populations in Duhok province. Secondly, is to examine various genetic parameters and build a dendrogram among these three populations in order to determine which primers will be most useful for further population genetic studies.

SUBJECTS AND METHODS

1. Sampling

During the period of 1 December 2021 to 1 March 2022, a total of 90 blood samples were obtained from unrelated males from the Kurds, Armenians and Assyrians populations that reside in Duhok province/Kurdistan Region of Iraq. Informed consent

and with approval of the University of Zakho was obtained for all cases. The genealogical data were also obtained from the donors. The age of participants ranged from 15 to 69 years old.

2. DNA Extraction

Genomic DNA was extracted from blood samples using the blood Mini Kit provided by a Chinese company called Dongsheng Biotech. In this research, eleven STR primers were employed as shown in table1.

3. PCR amplifications

The following were the PCR cycle parameters: one cycle of initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94 °C for 1 min. Annealing at 56.5 °C for DYS19, DYS390, DYS460 primers; 60 °C for DYS437, DYS439, DYS481; 61°C for DYS426, DYS447, DYS461 and 59.5 for DYS393, DYS576 for 40 seconds. Extension at 72°C for one minute, and then one cycle of two final extensions at 72 °C for seven minutes.

4. Typing

The initial step in running the amplified products was on a 1% agarose gel electrophoresis to identify successful amplifications. Then 8% polyacrylamide gel electrophoresis (PAGE) was used to electrophorese the PCR products. PCR products were run with a 50bp ladder DNA as marker to size the bands. Silver staining was used to identify the DNA bands (Bassam and Gresshoff, 2007).

5. Data analysis

Power Marker software version 3.25 was used to compute the allele frequencies and other parameters for each locus. The genetic relationship parameters

calculated according to Reynold (1983) statistics. The similarity matrix was used to construct the dendrogram using the unweighted pair group method arithmetic averages (UPGMA) procedure (Sokal and Michener, 1958). Phylogenetic tree construction was carried out using MEGA-X.

Table 1: Characteristics of Y-STR markers used in the study.

STR markers	*Primer Sequences*	*Repeat motif*	*Annealing Tm. °C*	*Expected Size(bp)*	*Reference*
DYS19	F; 5-'CTACTGAGTTTCTGTTATAGT-3' R; 5'-ATGGCCATGTAGTGAGGACA-3'	(TAGA)n	56.5	176 - 212	Naji. 2020
DYS390	F; 5'-TATATTTTACACATTTTTGGGCC-3' R; 5'-TGACAGTAAAATGAACACATTGC-3'	(GATA)n (GACA)n	56.5	201-242	Bai *et al.* ,2016
DYS393	F; 5'-GTGGTCTTCTACTTGTGTCAATAC-3' R; 5'-AACTCAAGTCCAAAAAATGAGG-3'	(GATA)n	59.5	109-133	Butler *et al.*, 2002

DYS426	F; 5'-CTCAAAGTATGAAAGCATGACCA-3' R; 5'-GGTGACAAGACGAGACTTTGTG-3'	(GTT)n	61	92-98	Butler et al., 2002
DYS437	F; 5'-GACTATGGGCGTGAGTGCAT-3' R; 5'-AGACCCTGTCATTCACAGATGA-3'	(TCTA)n	60	195-229	Bai et al., 2016
DYS439	F; 5'-TCGAGTTGTTATGGTTTTAGGTCT-3' R; 5'-GTGGCTTGGAATTCTTTTACCC-3'	(AGAT)n	60	198-225	Butler. 2006
DYS447	F; 5'-GGTCACAGCATGGCTTGGTT-3' R; 5'-GGGCTTGCTTTGCGTTATCTCT-3'	(TAATA)n (TAAAA)1	61	206-241	Redd et al., 2002
DYS460	F; 5'-CAAATTTGCCAAACTCTTTC-3' R; 5'-TCTATCCTCTGCCTATCATTTATTA-3'	(ATAG)n	56.5	162-182	Bosch et al., 2002
DYS461	F; 5'-AGGCAGAGGATAGATGATATGGAT-3' R; 5'-TTCAGGTAAATCTGTCCAGTAGTGA-3'	(TAGA) CAGA	61	174 - 190	(NIST) USA in 2017
DYS481	F; 5'-CTGTTTGAGAGTGTTGCGAGA-3' R; ACCCAAGAAGAGCCACACAG-3'	(CTT)n	61	119-164	Moon et al., 2022
DYS576	F; 5'-TTGGGCTGAGGAGTTCAATC-3' R; 5'-GGCAGTCTCATTTCCTGGAG-3'	(AAAG)n	59.5	183-207	Geppert et al., 2009

RESULTS

Using power marker V3.25 software, molecular diversity and population genetic structure such as mean allele number, allele frequency, gene diversity, polymorphic information content, and genetic distance were analysed with three different populations Kurds (n=30), Armenians (n=30) and Assyrians (n=30). The total number of alleles identified in the three populations was 380 alleles. The sizes of the alleles ranged from 87 to 275bp as shown in Table 2.

Table 2: Range of allele size of three population Kurd, Armenians and Assyrians

Primer		***Range of allele size, bp***	***Primer***		***Range of allele size, bp***
DYS19	Kurd	185—219	DYS447	Kurd	208—254
	Armenian	175—197		Armenian	221—275
	Assyrian	176—195		Assyrian	219—268
DYS390	Kurd	195—240	DYS460	Kurd	165—183
	Armenian	181—200		Armenian	162—190
	Assyrian	202—233		Assyrian	160—178
DYS393	Kurd	106—127	DYS461	Kurd	163—183
	Armenian	118—133		Armenian	165—180
	Assyrian	118—136		Assyrian	173—190
DYS426	Kurd	88—100	DYS481	Kurd	200—246
	Armenian	88—98		Armenian	208—250
	Assyrian	87—98		Assyrian	225—273
DYS437	Kurd	170—186	DYS576	Kurd	175—208
	Armenian	168—200		Armenian	167—204
	Assyrian	153—176		Assyrian	173—192
DYS439	Kurd	200—225	Range of all allele size bp		87 — 275
	Armenian	166—220			
	Assyrian	191—225			

The number of alleles per locus in the Kurd population ranged from 7 alleles at locus DYS437 to 16 alleles at locus DYS390, with an average of 10.91 alleles per locus. Allele frequency varied from 0.133 in DYS19 and DYS576 to 0.333 in DYS437 with mean of 0.197. The gene diversity ranged from 0.798 in DYS437 to 0.922 in DYS576 with a mean of 0.873, indicating a high level of diversity as shown in Table 3.

Table 3: Allele frequency, Allele number, availability, Gene Diversity, PIC in the Kurd population.

Marker	*Allele. Frequency*	*Sample Size*	*Allele No*	*Availability*	*Gene Diversity*	*PIC*
DYS19	0.133	30	14	1	0.916	0.909
DYS390	0.167	30	16	1	0.916	0.910
DYS393	0.267	30	9	1	0.829	0.808
DYS426	0.200	30	8	1	0.858	0.841
DYS437	0.333	30	7	1	0.798	0.772
DYS439	0.167	30	10	1	0.873	0.860
DYS447	0.167	30	12	1	0.889	0.879
DYS460	0.200	30	9	1	0.856	0.839
DYS461	0.233	30	11	1	0.871	0.859
DYS481	0.167	30	9	1	0.878	0.865
DYS576	0.133	30	15	1	0.922	0.917
Mean	**0.197**	**30**	**10.91**	**1**	**0.873**	**0.86**

In Armenian population, the number of alleles per locus ranged from 8 at DYS426 to 18 alleles at DYS447 locus with average of 12.18 alleles per locus. The range of allele frequency ranged from 0.133 in DYS447 and DYS576 to 0.3 in DYS390 and DYS461 with mean of 0.212. The gene diversity ranged from 0.829 in DYS461 to 0.929 in DYS447 with mean of 0.876 which is higher than Kurds population as shown in Table 4.

Table 4: Allele frequency, Allele number., availability, Gene Diversity, PIC in the Armenian population

Marker	*Allele Frequency*	*Sample Size*	*Allele No*	*Availability*	*Gene Diversity*	*PIC*
DYS19	0.233	30	14	1	0.869	0.857
DYS390	0.300	30	12	1	0.853	0.841
DYS393	0.200	30	12	1	0.882	0.871
DYS426	0.233	30	8	1	0.851	0.834
DYS437	0.233	30	10	1	0.853	0.837
DYS439	0.167	30	14	1	0.898	0.889
DYS447	0.133	30	18	1	0.929	0.924
DYS460	0.167	30	11	1	0.889	0.878
8DYS461	0.300	30	9	1	0.829	0.809
DYS481	0.233	30	11	1	0.873	0.861
DYS576	0.133	30	15	1	0.909	0.902
Mean	**0.212**	**30**	**12.18**	**1**	**0.876**	**0.864**

In Assyrians population, number of alleles per locus ranged from 8 at DYS426 to 15 alleles at DYS19 and DYS390 locus with average of 11.545 alleles per locus. The allele frequency ranged from 0.133 in DYS19 and DYS393 to 0.34 in DYS439 with mean of 0.215. The gene diversity ranged from 0.824 in DYS461 to 0.907 in both DYS447 and DYS447 with mean of 0.867 which is lower than Kurds and Armenians population as shown in Table 5.

Table 5: Allele frequency, Allele No., availability, Gene Diversity, PIC in the Assyrian population

Marker	*Allele Frequency*	*Sample Size*	*Allele No*	*Availability*	*Gene Diversity*	*PIC*
DYS19	0.133	30	15	1	0.907	0.899
DYS390	0.267	30	15	1	0.880	0.871
DYS393	0.133	30	12	1	0.893	0.884
DYS426	0.233	30	8	1	0.829	0.807
DYS437	0.200	30	11	1	0.880	0.868
DYS439	0.300	30	11	1	0.831	0.813
DYS447	0.167	30	14	1	0.907	0.899
DYS460	0.267	30	9	1	0.833	0.813
DYS461	0.267	30	9	1	0.824	0.803
DYS481	0.167	30	11	1	0.884	0.873
DYS576	0.233	30	12	1	0.871	0.859
Mean	**0.215**	**30**	**11.55**	**1**	**0.867**	**0.854**

In order to obtain accurate data analysis, the value of availability (number of observed alleles per number of individuals sampled) was calculated and found to be high, with an average of 1.00 in all the three populations as shown in Tables 3,4, and 5. The polymorphic information content (PIC) values for overall genetic variability also calculated for all primers and in all three populations as shown in Tables 3,4, and 5. The values ranged from 0.887 for the least informative marker, DYS426, to 0.947 for the most informative marker, DYS447.Phylogenetic analysis results are shown in Figure 1. The dendrogram which separated the populations into two main cluster, Assyrian cluster and Kurd-Armenian cluster. The latter, in turn, is divided into two sub-cluster: The Kurd and the Armenians except few individuals were admixed with another cluster or sub-cluster from all the three populations (figure No.1).

DISCUSSION

In the present study, the Y-STRs were used to determine the allele frequency and genetic variation in 11 loci among Kurds, Armenians and Assyrians populations in Duhok province. The results revealed that within a total of 380 alleles their sizes range 87 to 275 bp as shown in Table 2. These results are similar to those previously reported for the Iraqi Arab families lives in middle Euphrates that their PCR product size of the DYS390 locus was 200 bp, DYS392 locus ranged 93 to125 bp, DYS393 locus was up to 100 bp and DYS19 ranged from176 to 212 bp (Naji,2020). Mean number of alleles per locus scored in this study (Kurd 10.91, Armenian 12.18 and Assyrian 11.55 alleles) showed to be higher than those published in fact sheet of National Institute of Standards and Technology (NIST) USA with an average of 9 alleles per locus. High number of alleles per each population suggests high amount of genetic diversity in the population. Fattah *et al* (2019) reported that the average number of alleles in Kurd population was 5.125, while in this study the number of alleles in Kurd population (10.91) was much higher, this may be due to the use of larger number and different primers. High number of alleles within each population indicates a great level of genetic diversity. The allele frequency in the three groups, Kurd, Armenian and Assyrian was not similar to each other. A study by Ohied and Al Badran (2022) in Basrah population with many similar primers used, showed high allele frequency in all studied loci. Another study by Imad and his colleagues (2013) in middle and south of Iraq population, five of the primers they used were similar to those been used in this study. Allele frequencies in all loci were higher than the results in this study except at DYS439 the value was 0.299. The data in tables 3, 4 and 5 indicate that the mean value of gene diversity in Arminian population is the highest (0.876) then followed by the mean gene diversity in the

Kurd population (0.873) then in Assyrian Khalid population

(0.867). Gene diversity in this study was much higher than those reported by Imad and his colleagues (2013) and Naji (2020), while it was similar to those results of Albarzinji (2022) who reported genetic diversity value between 0.848 to 0.392 in Sorani Kurds. In Northern Greece, similar genetic diversity value of 0.9992 also has been scored in 17 Y STR loci, five of these STRs were similar to those used in this study (Leda *et al*., 2008). The results also revealed that the genetic diversity in Armenian population was higher than those in Kurd and Assyrian populations as shown in Tables 3, 4, and 5. These variations in genetic diversity values in different populations may be attributed to the gene flow and migration during different time of the history. The Polymorphism Information Content (PIC) value is often used to measure the informativeness of a genetic marker. According to Botstein *et al*. (1984), values of PIC greater than 0.5 (PIC>0.5) are consider as highly informative primer. In this study the value ranged from 0.772 at DYS437 locus with 7 alleles in Kurd population to 0.924 at DYS447 locus with 18 alleles in Armenian population. All these primers used in this study therefore can be consider as informative due to their high values. These results are in agree with those of Fattah *et al*. (2019) whom they reported high PIC values. Naji (2020) as well found that DYS19 and DYS392 primers were the most polymorphic in compare to other primers. To evaluate the genetic differentiation and the distance between different populations, a phylogenetic tree was constructed. The phylogenetic tree as shown in figure 1 separated the populations into two major clusters; Assyrian group in one cluster and Kurd-Armenian groups in another cluster. The latter, in turn, is divided into two sub-clusters: The Kurd and the Armenians, except few individuals from one clad clustered to another clad in all the three populations. The genetic distance in three populations indicates that the genetic distance between Kurd population and the Armenians population is less than the genetic distance between the Kurd population and the Assyrians population, meaning that the Armenians are genetically closer to the Kurds. This suggesting long shared history. These groups are sharing the same homeland since thousands of years as well both populations are Indo-European nations while the Assyrian people are Semitic. Admixing of some individuals from one population to another population can be attribute to the long sharing history of living with other for thousands of years. Also, wars genocides, immigrations and gene flow have its role in admixing some of the individuals from clusters. Another explanation of this, that there will be always an unknown number of males with a similar Y-STR profile (de Knijff,2022). Tomory *et al*, (2007) in their study demonstrated that there has not been much genetic separation among Hungarian-speaking communities in the Carpathian Basin. The Hungarian gene pool has been impacted by migration and neighboring gene flow. Therefore, the gene flow may be one of the reasons of the admixture pattern results among these three populations.

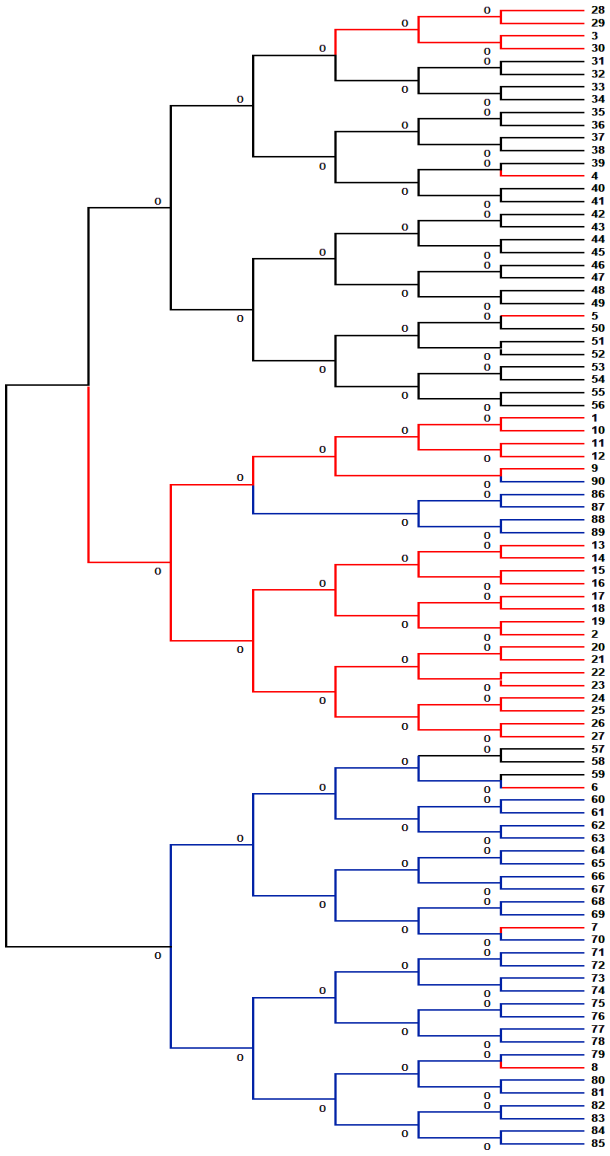

Figure 1: Phylogeny relationship of Kurd, Armenian, and Assyrian population using 11 Y STR- markers. The red color are Kurd individuals; the black color is Armenian individuals while the blue color are Assyrian individuals.

CONCLUSION

All investigated loci have high power of discriminating values, indicating that a DNA-based database can be created using these loci. DYS426 had the lowest gene diversity. DYS447 and DYS576 had a high degree of genetic diversity. The Kurdish community may use these 11 STR loci as a crucial tool for paternal typing and forensic identification based on statistical parameters. The phylogenetic tree separated the populations into two major clusters. Assyrian group in one cluster and Kurd-

Armenian group in another cluster. The latter, in turn, is divided into two sub-cluster: the Kurd and the Armenians, which indicate that the Armenians and the Kurd genetically are close to each other than the Assyrian group.

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