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Y- chromosomal STR Variation in Arab, Soran and Behdinan Kurds population in Kurdistan region of Iraq

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Abstract

Background: Over than eight tenant groups make up Kurdistan region of Iraq local populations. Overall majority of the inhabitants are Muslim Kurds, followed by Yezidi Kurds. Alternative groups including Armenians, Assyrian, Chaldea Syriacs as well as a little minority of Arab and Turkmen individuals.

Methods: A total of 60 unrelated males from three population groups in Kurdistan region of Iraq Arabs, Soran and Behdinan kurd were successfully analyzed for ten Y-chromosome STRs (DYS19, DYS390, DYS393, DYS437, DYS439, DYS447, DYS460, DYS461, DYS481, DYS576). Whole DNA has been extracted from the blood samples using DNA extraction kit. PCR products were run on 8% polyacrylamide gel with a 50bp DNA ladder marker to size the bands for each sample. silver staining was used to identify the DNA bands. Power Marker V3.25 software was used successfully to determine a variety of genetic parameters which include total allele number, allele frequency, gene diversity and polymorphic information content (PIC). Phylogenetic tree was constructed by MEGA-X software.

Results: The total number of alleles identified in the three populations was 155. The size of the alleles ranged from 112bp to 245bp. The DYS19 had the highest diversity (GD: 0.941), whereas DYS393 locus had the lowest value among all (GD: 0.813). The Dendrogram split the populations into two main clusters: Arabs in one cluster, whereas Soran and Behdinan in another cluster.

Conclusions: This study validates that the discrimination potential of high-resolution Y-STR typing and supports the main datasets on the samples from Kurdistan region of Iraq. The comparison of two group of Kurds and Arab datasets offers an intriguing total pattern of Kurd groups. Meaning that Sorans are genetically closer to the Behdinan population.

Keywords: Y- chromosome STRs, Genetic diversity, population genetics of Kurdistan region of Iraq.

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Introduction

Kurds are an indigenous people of the Mesopotamian plains and also highlands of what is now south-eastern Turkey, north-eastern Syria, north-western Iran and south-western Armenia. Moreover, they are an Indo-European speaking group. Upwards of eight tenant groups make up Kurdistan region of Iraq local populations. Overall majority of the inhabitants are Muslim Kurds, followed by Yezidi Kurds. Alternative groups including Armenians, Assyrian, Chaldea Syriacs as well as a minority of Arab and Turkmen individuals (Dogan *et al*., 2017). 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, parental 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). Earlier genomic analyses using classical markers found Kurds to be genetically comparable to other Middle Eastern populations. Investigated mtDNA sequences variability for Kurmanji-speaking Kurds in Georgia (Caucasus) discovered near European connections among Kurds mtDNA groups (Lazim *et al*., 2020). The researchers looked at the mtDNA sequencing variation across 52 Kurds from Eastern Turkey and discovered that some of the mtDNA haplotypes detected through Kurds samples apparently originated in Europe, and have been related with back-migrations from Europe to the Near East (Peng *et al*., 2008). In another study by Nasidze and his co-workers on three Kurdish groups which are Zazaki and Kurmanji speakers from Turkey and Kurmanji speakers from Georgia. They found that Kurdish groups are most similar genetically to other West Asian groups, and most distant from Central Asian groups, for both mtDNA and the Y-chromosome and show a closer relationship with European groups than with Caucasian groups based on mtDNA, indicating some differences in their maternal and paternal histories.

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The first aim of this study was to apply a number of microsatellites marker sets for the genetics characterization of Arabs, Soran and Behdinan Kurds populations in Erbil, Mousl and Duhok provenances. The second aim was to study a number of genetic parameters within the populations.

Material and Methods

1. Sampling

A total of 60 blood samples from unrelated males of three population groups (Arab, Soran and Behdinan) from Erbil, Mosul and Duhok province in Kurdistan region of Iraq were successfully collected. Donors genealogical information were also gathered. In addition to that the informed consent with approval of the university of Zakho was obtained for all cases. The age of the, donors were between 18 to 70 years old.

2. DNA Extraction

DNA was extracted from the whole blood samples using a DNA extraction kit. The DNA samples were extracted according to the instructions provided by the supplier company (Dongsheng Biotech Company, China). Ten STR primers were used during this study, namely: DYS19, DYS576, DYS393, DYS460, DYS390, DYS437, DYS439, DYS481, DYS461 and DYS447.

3. PCR amplifications

The cycles of PCR parameters were as following: one cycle of initial denaturation at 94˚C for 5 minute then 36 cycles of denaturation at 94˚C for 1 minute after that annealing at 56˚C for DYS19, DYS460 and DYS390; 59.5 ˚C for DYS393 and DYS576; 60˚C for DYS437, DYS439 and DYS481; 61˚C DYS447 and DYS461 for 40 seconds then extension at 72˚C for 1 minute and one cycle of two final extensions at 72˚C for 7 minute. The properties of the Y-STR markers used in this study are shown in table 1.

4. Typing

The amplified PCR products were run on 1.5% agarose gel to detect successful amplifications. Next step PCR products were electrophoresed on 8% polyacrylamide gel (PAGE). To detect the size of the DNA bands a 50bp DNA ladder was run with products of PCR. The DNA bands were visualized by silver staining (Bassam and Gresshoff, 2007).

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5. Data analysis

The molecular genetics parameters and phylogenic relationship were calculated using the power marker V3.25 software according to Reynold (1983). 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 was construction using MEGA-X software.

Table 1: Characterizations of Y-STR markers that are used in this study

Article I. STR markers	**Article II. Primer Sequences**	**Article III. epeat motif**	**Article IV. nnealing Tm. °C**	**Article V. xpected Article VI. ize(bp)**	**Article VII. ef.**
Article VIII. YS19	Article IX. F; 5-'CTACTGAGTTTCTGTTATAGT-3' Article X. R; 5'-ATGGCCATGTAGTGAGGACA-3'	Article XI. TAGA)n	Article XII. 6.5	Article XIII. 76 - 212 Article XIV.	Article XV. aji. 2020
Article XVI. YS390	Article XVII. F; 5'-TATATTTTACACATTTTTGGGCC-3' Article XVIII. R; 5'-TGACAGTAAAATGAACACATTGC-3'	Article XIX. GATA)n Article XX. GACA)n	Article XXI. 6.5	Article XXII. 01-242	Article XXIII. ai *et al.* ,2016
Article XXIV. YS393	Article XXV. F; 5'-GTGGTCTTCTACTTGTGTCAATAC-3' Article XXVI. R; 5'-AACTCAAGTCCAAAAAATGAGG-3'	Article XXVII. GATA)n	Article XXVIII. 9.5	Article XXIX. 09-133	Article XXX. utler *et al.,* 2002
Article XXXI. YS437	Article XXXII. F; 5'-GACTATGGGCGTGAGTGCAT-3' Article XXXIII. R; 5'-AGACCCTGTCATTCACAGATGA-3'	Article XXXIV. TCTA)n	Article XXXV. 0	Article XXXVI. 95-229	Article XXXV ai *et al.*, 2016
Article XXXVI YS439	Article XXXIX. F; 5'-TCGAGTTGTTATGGTTTTAGGTCT-3' Article XL. R;	Article XLI. AGAT)n	Article XLII. 0	Article XLIII. 98-225	Article XLIV. utler. 2006

	5'-GTGGCTTGGAATTCTTTTACCC-3'				
Article XLV. YS447	Article XLVI. F; 5'-GGTCACAGCATGGCTTGGTT-3' Article XLVII. R; 5'-GGGCTTGCTTTGCGTTATCTCT-3'	Article XLVIII. TAATA)n Article XLIX. TAAAA)1	Article L. 1	Article LI. 06-241	Article LII. edd *et al.,* 2002
Article LIII. YS460	Article LIV. F; 5'-CAAATTTGCCAAACTCTTTC-3' Article LV. R; 5'-TCTATCCTCTGCCTATCATTTATTA-3'	Article LVI. ATAG)n	Article LVII. 6.5	Article LVIII. 62-182	Article LIX. osch *et al.,* 2002
Article LX. YS461	Article LXI. F; 5'-AGGCAGAGGATAGATGATATGGAT-3' Article LXII. R; 5'-TTCAGGTAAATCTGTCCAGTAGTGA-3'	Article LXIII. TAGA) Article LXIV. AGA	Article LXV. 1	Article LXVI. 74 - 190	Article LXVII. NIST) USA in 2017
Article LXVIII YS481	Article LXIX. F; 5'-CTGTTTGAGAGTGTTGCGAGA-3' Article LXX. R; ACCCAAGAAGAGCCACACAG-3'	Article LXXI. CTT)n	Article LXXII. 1	Article LXXIII. 19-164	Article LXXIV oon *et al.,* 2022
Article LXXV. YS576	Article LXXVI. F; 5'-TTGGGCTGAGGAGTTCAATC-3' Article LXXVII. R; 5'-GGCAGTCTCATTTCCTGGAG-3'	Article LXXVIII AAAG)n	Article LXXIX. 9.5	Article LXXX. 83-207	Article LXXXI eppert *et al*., 2009

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Results

The experimental results showed that total number of observed alleles in all populations was 155 (Table 6). The size of alleles ranged from 112bp to 250bp (Table 2).

Primer	populations	allele size, bp	Primer	populations	allele size,bp
DYS19	Behdinan	178—205	DYS447	Behdinan	224—230
	Arab	126—191		Arab	208—245
	Soran	218—240		Soran	212—242
DYS390	Behdinan	185—200	DYS460	Behdinan	162—174
	Arab	190—200		Arab	159—185
	Soran	190—220		Soran	158—177
DYS393	Behdinan	112—132	DYS461	Behdinan	161—178
	Arab	115—136		Arab	165—185
	Soran	108—124		Soran	166—174
DYS437	Behdinan	174—185	DYS481	Behdinan	228—246
	Arab	166—178		Arab	230—250
	Soran	160—195		Soran	224—245
DYS439	Behdinan	195—226	DYS576	Behdinan	170—198
	Arab	200—228		Arab	174—198
	Soran	190—215		Soran	175—202
			Average allele size bp		112 — 250

Table 2: Range of allele size of all three populations Arab, Soran and Behdinan Kurds

The number of alleles per locus in Behdinan population ranged from 4 alleles at locus DYS460 to 12 alleles at locus DYS461 within an average of 7.8 alleles per locus. Alleles frequency ranged from 0.15 at DYS461 and DYS576 loci to 0.50 in locus DYS447 within an average of 0.31 alleles. Whereas the gene diversity varied from 0.66 at DYS447 locus to 0.90 at DYS461 locus with a mean of 0.79 indicating a high level of diversity. Polymorphism Information Content (PIC) values ranged from 0.61 in DYS393 and DYS447 loci to 0.89 in DYS461 locus. The PIC average was 0.76 (Table 3).

Table 3: Allele frequency, Availability, Gene diversity, Allele number and PIC in Behdinan population

Marker	Allele. Frequency	Sample Size	Allele No	Availability	Gene Diversity	PIC
DYS19	0.20	20	11	1	0.88	0.87
DYS390	0.30	20	6	1	0.80	0.77
DYS393	0.45	20	6	1	0.67	0.61

DYS437	0.35	20	5	1	0.74	0.70
DYS439	0.30	20	10	1	0.84	0.82
DYS447	0.50	20	5	1	0.66	0.61
DYS460	0.35	20	4	1	0.72	0.66
DYS461	0.15	20	12	1	0.90	0.89
DYS481	0.30	20	9	1	0.82	0.79
DYS576	0.15	20	10	1	0.89	0.88
Mean	0.31	20	7.8	1	0.79	0.76

In the Arab population the number of alleles per locus differ from 3 alleles at locus DYS390 to 11 alleles at locus DYS447 with an average of 6.9 alleles per locus. The allele frequency ranged from 0.20 at DYS19 and DYS576 loci to 0.45 at DYS437 locus within an average of 0.325. This average indicated that the number of null allele (not amplified) was zero. The gene diversity in Arabs population ranged from 0.64 in DYS390 to 0.815 in DYS460 within average of 0.77 that are considered to be lower than Soran and Behdinan population. (PIC) values ranged from 0.56 at DYS390 locus to 0.86 at DYS19 and DYS447 loci (Table 4).

Table 4: Allele frequency, Availability, Gene diversity, Allele number and PIC in Arab population

Marker	M Allele Frequency	Sample Size	Allele No	Availability	Gene Diversity	PIC
DYS19	0.20	20	10	1	0.87	0.86
DYS390	0.40	20	3	1	0.64	0.56
DYS393	0.35	20	9	1	0.81	0.79
DYS437	0.45	20	4	1	0.65	0.58
DYS439	0.40	20	5	1	0.71	0.66
DYS447	0.25	20	11	1	0.87	0.86
DYS460	0.35	20	9	1	0.815	0.80
DYS461	0.30	20	5	1	0.775	0.74
DYS481	0.35	20	5	1	0.72	0.67
1DYS576	0.20	20	8	1	0.84	0.82
Mean	0.325	20	6.9	1	0.77	0.73

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In Soran population the number of alleles per locus varied from 6 at DYS393 and DYS439 loci to 11 for both loci DYS390 and DYS460 with a mean of 8.1 alleles per locus, While the allele frequency ranged from 0.20 in DYS437 locus to 0.40 at DYS447 locus with mean of 0.295. The gene diversity ranged from 0.755 in DYS439 to 0.865 in DYS437 and DYS460 loci with a mean of 0.817 which is considered to be higher than Arabs and Behdinan populations. (PIC) values ranged from 0.72 at DYS439 locus to 0.85 at DYS437 and DYS460 loci (Table 5).

Marker	Allele Frequency	Sample Size	Allele No	Availability	Gene Diversity	PIC
DYS19	0.25	20	7	1	0.805	0.78
DYS390	0.30	20	11	1	0.855	0.84
DYS393	0.30	20	6	1	0.785	0.75
DYS437	0.20	20	9	1	0.865	0.85
DYS439	0.40	20	6	1	0.755	0.72
DYS447	0.35	20	7	1	0.800	0.78
DYS460	0.25	20	11	1	0.865	0.85
DYS461	0.30	20	7	1	0.800	0.77
DYS481	0.35	20	9	1	0.805	0.78
DYS576	0.25	20	8	1	0.835	0.81
Mean	0.295	20	8.1	1	0.817	0.79

Table 5: Allele frequency, Availability, Gene diversity, Allele number and PIC in Sorani population

In order to achieve predictable data analysis, the value of availability which are number of observed alleles per number of individuals sampled was determined. This value has shown to be high in all the three populations: Arabs, Soran and Behdinan within an average of 1.000 (Tables 3,4 ,5 and 6).

For all population together the range of allele frequency ranged from 0.100 in DYS19 locus to 0317 at DYS439 locus with mean of 0.218. The number of alleles per locus ranged from 12 alleles at DYS393 and DYS437 loci to 22 alleles at DYS19 locus with an average of 15.5 alleles per locus. The gene diversity ranged from 0.813 in DYS393 to 0.941 at DYS19 with mean of 0.875. The PIC values ranged from 0.790 in DYS393 to 0.937 in DYS19 locus with average of 0.863 (Table 6).

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Table (6): Allele frequency, availability, gene diversity, and PIC in the all three populations collectively

Marker	Allele Frequency	Sample Size	Allele No	Availability	Gene Diversity	PIC
DYS19	0.100	60	22	1	0.941	0.937
DYS390	0.217	60	14	1	0.868	0.855
DYS393	0.300	60	12	1	0.813	0.790
DYS437	0.233	60	12	1	0.861	0.847
DYS439	0.317	60	15	1	0.844	0.830
DYS447	0.300	60	15	1	0.844	0.829
DYS460	0.167	60	15	1	0.889	0.879
DYS461	0.167	60	18	1	0.912	0.905
DYS481	0.233	60	15	1	0.856	0.841
DYS576	0.150	60	17	1	0.917	0.911
Mean	**0.218**	**60**	**15.5**	**1**	**0.875**	**0.863**

Phylogenetic results according to Reynold statistics (1983) are shown in Figure 1. The Dendrogram tree separated the populations into two main clusters: Arabs group in one cluster and Behdinan group in a second cluster. Each cluster has been divided into two sub-clusters. The Sorani group clads has been split between these two main clusters, half of the clads had clustered with Arab group and the other half of the clads with Behdinan group. Some individual leaves from all groups were admixed with other groups, especially the Sorani group with Behdinan group.

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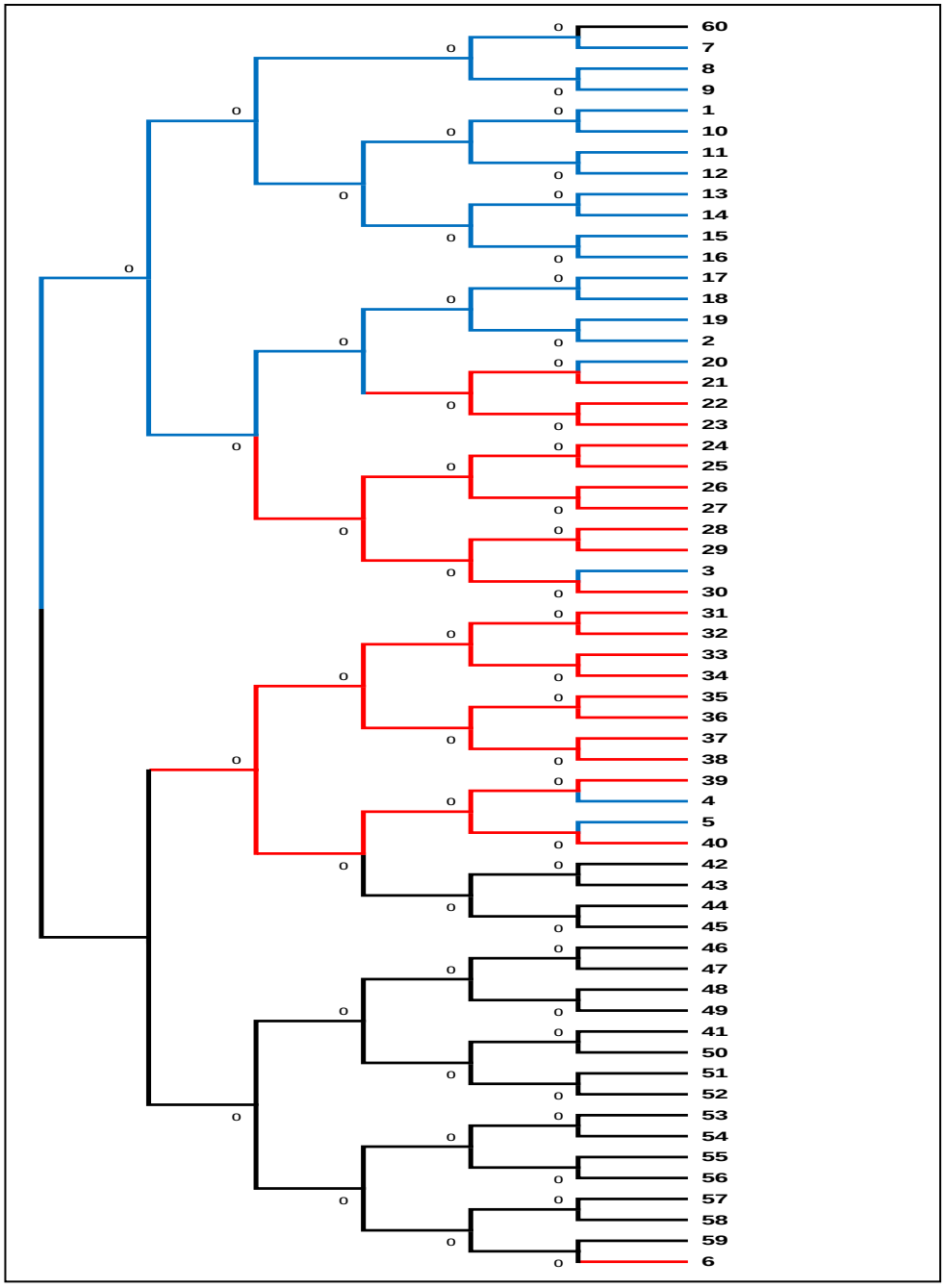

Figure 1: phylogeny relationships of the Arabs, Soran and Behdinan populations. The blue color (1-20) indicates Behdinan group, the red color (21-40) is the Sorani group and black color (41-60) is Arab group

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Discussion

In the present study, the Y-STRs were used to determine the allele frequency and genetic variation in 10 loci among Arabs, Soran and Behdinan populations in Erbil, Mousl and Duhok provinces. The results revealed that within a total 155 alleles their sizes varied from 112bp to 245bp (Table 2). These results are similar to those previously reported for the Iraqi Arab families lives in middle Euphrates that their PCR products size was 200bp for the locus DYS390 while in DYS393 locus was up to 100bp and DYS19 locus varied from 126bp to240bp (Naji, 2020). The mean number of alleles per each locus was scored in this study (Arabs 6.9, Soran 8.1 and Behdinan 7.8 alleles) showed to be lower than those published in fact sheet of National Institute of Standards and Technology (NIST) with an average 9 alleles per each locus. High number of allele per each population suggests a high amounts of genetic diversity in that population. Fattah *et al* (2019) reported that the average number of alleles in Behdinan population was 5.125, whereas in this study the number of alleles in Behdinan population (7.8) was much higher, this may be due to use larger number of markers. The allele frequency in these three populations were not the same with each other. A study done by Balnd and his co-worker (2022) 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 area, five of the primers they used were similar to those that have been used in this study. Allele frequency through all loci was higher than those of this study. The data in tables 3, 4, 5 and 6 shows that the mean value of gene diversity in Soran population is 0.817, which considered to be the highest, while in Behdinan population was 0.79, then followed by the mean value of gene diversity in Arabs population which was 0.77. Genetic diversity in this study was much higher than those that are reported by Imad and his colleagues (2013) and Naji (2020) whereas it was identical to those results of Albarzingi (2020) who reported the value of gene diversity varied from 0.392 to 0.848 in Sorani population. In addition to that in Northern Greece, similar genetic diversity with value of 0.999 has been scored successfully for 17 Y-STR loci, six of these STR were similar to those that had been used in this study (Leda *et al*., 2008). The results also revealed that the gene diversity in Sorani population was higher than in Arab and Behdinan populations as well (Tables 3,4 and 5).

The variation in the value of genetic diversity in different populations may be due to a significant gene flow and migration throughout different periods of historical events. The value of Polymorphism Information Content (PIC) is usually used to measure the informativeness of a gene marker. According to Botstein *et al*., (1984), the value of PIC greater than 0.5 (PIC>0.5) is considered as a highly informative primer. In the present study the value of PIC ranged from 0.563 in DYS390 which regarded to be the lowest informative primer and 0.857 in DYS19 which is considered to be the

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most informative primer in Arabs population. Whereas in Soran populations the PIC ranged from 0.723 in DYS439 which is the lowest and 0.852 in DYS460 its considered to be the most informative primer while in Behdinan populations the PIC varied from 0.61 in DYS393 and DYS447 which is the lowest informative primer and 0.89 in DYS461 that is the most informative primer. All these primers that are used during this study can be considered informative due to its high values. The studies' outcomes are in agreement with results that have been done by Fattah *et al*., (2019) they were reported high values of PIC. Furthermore, Naji (2020) found that DYS19 and DYS393 primers were the most polymorphic in comparing to other primers. In order to estimate the genetic distance and differentiation among different populations, a phylogenetic tree was constructed. The phylogenetic tree (Figure 1) separate the populations into two main clusters. Arabs group in one cluster and Behdinan in another clusters. The Sorani group clads as shown in figure 1. had clustered with both Arab and Behdinan groups.

The phylogenic tree shows that the genetic distance between Behdinan and Sorani populations is less than the genetic distance between the Behdinan and Arabs population. Both Behdinan and Sorani populations are Kurd and a branch of Indo-European nations and endogenous people to the region (Hennerbichler, 2012), while the Arab group are sematic people came to the region through expanding of Islam more than one thousand years ago. The Kurdish people are believed to be of heterogeneous origins. They are descendants of many ancient civilizations. These civilizations can be explained in waves as follows; Gutians, Hurrians, Mitanni, Phrygians and Medes. This can explain why the Sorani group and Behdinan group formed separate sub-clusters. Admixing of some individuals from one population to another population can be attribute to the long sharing history of living with each other. Also wars, genocides, immigrations and gene flow has its role in admixing some of the individuals from clusters to another. 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. Similar to these results the Kurd population is known to have inhabited inhospitable mountainous region for millennia and had remained relatively unmixed with the invaders (Grugni, 2012). In spite of that, the neighboring gene flow may be one of the reasons of the admixture pattern results among the Kurd populations. Another explanation of this, that there will be always an unknown number of males with a similar Y-STR profile (de Knijff,2022). Molecular genetic evidence concerning the origins of the Kurds is very limited, never the less we find some scattered papers. More molecular genetics studies are needed to unveil the origin of Kurd and other populations lives in Kurdistan. These future studies must include the whole genome sequences, haplogroups studies and

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establish good molecular genetics data to understand the origin of Kurd populations and its relations with neighboring populations.

Conclusion

This study validates that discrimination potential of high-resolution Y-STR typing and supports the main datasets on the samples from Kurdistan region of Iraq. The comparison of three groups (Arab, Sorani and Behdinan) DNA-based datasets can be creating by using these loci. The primers DYS19, DYS461 and DYS576 could be considering the greatest due to their strong PIC power. DYS393 had the lowest gene diversity and DYS19 has the highest degree of gene diversity. The Kurds (Soran and Behdinan) can use these ten STR loci as a crucial technique for prenatal typing and forensic identification according to statistical parameters. The phylogenetic tree separates the populations into two major clusters. Arabs group in one cluster and Sorani-Behdinan in another clade. The genetic distance in this three populations explain that the genetic distance between Behdinan and Sorani populations is less than the genetic distance among the Behdinan and Arabs population, which means that the Sorani group are genetically closer to the Behdinan populations. There was some admix leaves among some sub-clusters and clads.

Ethical clearance

This study is approved through the ethic committee in College of Science, Zakho University, Kurdistan region of Iraq. Written informed consent have been obtained among all the participants.

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Conflict of Interest: No conflict

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