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تباين الكروموسومات Y في الأكراد واليزيديين والتركمان في إقليم كردستان العراق

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المستخلص :

بسبب الاختلاط العرقي والهجرة ، فإن إقليم كردستان العراق لديه تنوع سكاني فريد من حيث العرق والدين منذ العصور القديمة. ونتيجة لذلك ، فإن سكان المنطقة عبارة عن فسيفساء عرقية حقيقية تضم الأكرادوالعرب والتركمان والآشوريين واليزيديين ومجموعات الأقليات الأخرى. نتيجة لذلك ، فإن الباحثين الأنثروبولوجيين والطب الشرعي مهتمون بعلم الوراثة لسكان كردستان الحديثة .

تم جمع ما مجموعه ٦٠ عينة من متطوعي إقليم كردستان العراق ينتمون إلى ثلاث مجموعات عرقية رئيسية ، وهي:الأكراد (عدد = ٢٠) ، واليزيدية (عدد = ٢٠) والتركمان (عدد = ٢٠) تم تحليلها بواسطة ١٠علامات

DYS481, DYS461, DYS460, DYS447, DYS439, DYS437, DYS426, DYS393):STR DYS390, DYS19)

وتم استخراج الحمض النووي من عينات دم جديدة باستخدام مجموعة استخراج الحمض النووي .

الكلمات المفتاحية : سلاسل الكروموسوم Y ، حجم الأليل ، تردد الأليل ، التنوع الجيني للسكان.

Y-chromosomal STR Variation in Kurds, Yazidis and Turkmans Populations in Iraq's Kurdistan Region

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Abstract:

Background: Since ancient times, the Kurdistan Region of Iraq has had a diversified population in terms of race and religion due to racial mixing and immigration. The population of the area is therefore a true ethnic mosaic made up of Kurds, Arabs, Turkmens, Assyrians, Yazidis, and other marginalized groups. As a result, the genetics of the inhabitants in contemporary Kurdistan are of interest to anthropological and forensic specialists.

Methods: A total of 60 samples were collected from Kurdistan Region of Iraq volunteers belonging to three major ethnic groups, namely: Kurds (n=20), Yazidis (n=20) and Turkmen (n=20) were analyzed by 10 Y-STRs markers (DYS19, DYS390, DYS393, DYS426, DYS437, DYS439, DYS447, DYS460, DYS461, DYS481). DNA was extracted from fresh blood samples using DNA extraction Kit.

Results: A number of genetic parameters such as mean number of alleles, allele frequency, gene diversity, polymorphic information content, genetic distance and phylogenetic were measured in this study. The loci DYS 437 and DYS 439 had the highest diversity (GD = 0.870, 0.880 and 0.900 respectively), while loci DYS 390 and DYS437 had the lowest (GD = 0.700,0.715 and 0.735 respectively). The Dendrogram separated the populations into two main cluster, Kurds and Yazidis in one cluster and Turkmen in second cluster, then Kurd and Yazidis were separated into different sub-cluster with some admix some dendogram leaves between the Kurd and Yazidis group.

Conclusions: The aim was to study a number of genetic parameters of Kurd, Yazidis and Turkmen populations in Kurdistan region of Iraq. The results showed a great convergence between the Kurdish and Yazidi groups, while the Turkmen group was far from them by the discriminating power of high-resolution Y-STR typing.

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Keywords: Y-chromosome STRs, PIC, Allele size, Allele frequency, Genetic diversity of population.

Materials and Method:

1. Collection of Samples:

The total of 60 blood samples from unrelated males of three groups were collected from Kurdistan Region of Iraqi, the Kurds, Yazidis and Turkmen, Samples were collected from December 2021 to February 2022. Informed consent and with approval of the University of Zakho was obtained for all cases. Genealogical information's about the geographic origin, native language, date of birth, blood group and tribe for each person was recorded.

2. Extraction of DNA:

Genomic DNA was extracted from blood samples using the Dongsheng blood Mini Kit (Dongsheng Biotech Company, China). In this study, ten STR primers were used as shown in table 1.

3. Amplifications of PCR:

The PCR program parameters as follows cycle: one cycle of initial denaturation at 94˚C for 5min, then (38) cycles of denaturation at 94˚C for 1 min, annealing temperature at 56˚C for DYS19, DYS390 and DYS460 primers; 60˚C for DYS393, DYS437, DYS439 and DYS481primers; 61˚C for DYS426, DYS447 and DYS461 primers for 40 sec. The extension was at 72˚C for 1min then followed by one cycle of two final extensions at 72˚C for 7min.

4. Typing and data analysis:

The PCR product, first was run on 2% agarose gel to check the amplified bands, then the PCR product was run on 8% polyacrylamide gel (PAGE). 50 bp ladder DNA marker was used with PCR products for size measurement of the bands. Silver-nitrates solution was used to stain the DNA bands (Qingzhi *et al*, 2014). The power marker V3.25 program was used to calculate the all-molecular genetics parameters and the phylogenic association according to Nei's statistics. The dendrogram was created using the similarity matrix and the unweighted pair group method of arithmetic

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averages (UPGMA) algorithm. (Sokal and Michener, 1958). Phylogenetic tree was construction using MEGA-X software.

Table 1: Information about Primers groups that were used in this study.

Primer name		Primer sequence	Repeat motif	Annealing Temp. °C	Expected Size (bp)	Ref.
DYS19	F R	5′-CTACTGAGTTTCTGTTATAGT-3′ 5′-ATGGCCATGTAGTGAGGACA-3′	(TAGA) TAGG	**56C**	170-200	NIST,2017
DYS390	F R	5′-TATATTTTACACATTTTTGGGCC-3′ 5′-TGACAGTAAAATGAACACATTGC-3′	(TCTA) (TCTG)	**56C**	189-233	Kayser *et al*,1997
DYS393	F R	5′-GTGGTCTTCTACTTGTGTCAATAC-3′ 5′-AACTCAAGTCCAAAAAATGAGG-3′	AGAT	**60C**	137-151	Kayser *et al*,2001
DYS426	F R	5′-CTCAAAGTATGAAAGCATGACCA-3′ 5′-GGTGACAAGACGAGACTTTGTG-3′	GTT	**61C**	92-98	Jobling et al,1996
DYS437	F R	5′-GACTATGGGCGTGAGTGCAT-3′ 5′-AGACCCTGTCATTCACAGATGA-3′	TCTA	**60C**	186-202	Ayub et al ,2000
DYS439	F R	5′-TCGAGTTGTTATGGTTTTAGGTCT-3′ 5′-GTGGCTTGGAATTCTTTTACCC-3′	GATA	**60C**	204-224	Yussup *et al*,2017
DYS447	F R	5′-GGTCACAGCATGGCTTGGTT-3′ 5′-GGGCTTGCTTTGCGTTATCTCT-3′	(TAATA)n (TAAAA)	**61C**	206-241	Redd et al,2002
DYS460	F R	5′-CAAATTTGCCAAACTCTTTC-3′ 5′-TCTATCCTCTGCCTATCATTTATTA-3′	ATAG	**56C**	162-182	White *et al*,1999
DYS461	F R	5′-AGGCAGAGGATAGATGATATGGAT-3′ 5′-TTCAGGTAAATCTGTCCAGTAGTGA-3′	(TAGA) CAGA	**61C**	174-190	NIST,2017

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DYS481	F R	5′-TTCTGTGAGAGTGTTGCGAGA-3′ 5′-ACCCAAGAAGAGCCACACAG-3′	CTT	**60C**	141-165	Becky *et al*,2012

Results:

The total number of alleles identified in the three populations was 218 alleles. The alleles sizes of the ranged from 80 bp to 272 bp (Table 2).

Table 2: Range of allele size of three population Kurds, Yezidis and Turkmans.

Primer		**Range of allele size, bp**	**Primer**		**Range of allele size, bp**
DYS19	Kurd	180-200	DYS439	Kurd	220-252
	Yezidis	180-202		Yezidis	228-272
	Turkmen	162-178		Turkmen	174-200
DYS390	Kurd	205-240	DYS447	Kurd	171-201
	Yezidis	225-240		Yezidis	180-195
	Turkmen	186-202		Turkmen	215-260
DYS393	Kurd	108-136	DYS460	Kurd	168-180
	Yezidis	112-133		Yezidis	159-186
	Turkmen	140-148		Turkmen	121-139
DYS426	Kurd	92-116	DYS461	Kurd	174-189
	Yezidis	80-98		Yezidis	180-195
	Turkmen	132-152		Turkmen	156-168
DYS437	Kurd	180-189	DYS481	Kurd	156-171
	Yezidis	162-186		Yezidis	145-157
	Turkmen	201-210		Turkmen	121-145
Range of all allele size bp					80-272

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In the Kurds population (Table 3), allele frequency varied from 0.150 in DYS447 to 0.350 in DYS460 with mean of 0.245. The number of alleles per locus ranged from 4 alleles at DYS437 locus to 12 alleles at DYS439 locus with an average of 7.5 alleles per locus. The gene diversity ranged from 0.735 in DYS437 to 0.900 in DYS439 with a mean of 0.824, indicating a high level of diversity. Polymorphism Information Content of a Molecular Marker (PIC) measures the ability of a marker to detect polymorphisms, and it has enormous importance in selecting markers for genetic studies. The PIC values ranged from 0.753in DYS481 to 0.89 1in DYS439 with average of 0.799 which is more than 0.5 in all DNA markers used in this study.

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
DYS 19	0.250	20	6	1	0.815	0.789
DYS390	0.250	20	8	1	0.840	0.821
DYS393	0.300	20	8	1	0.825	0.804
DYS426	0.200	20	8	1	0.855	0.838
DYS437	0.300	20	4	1	0.735	0.685
DYS439	0.150	20	12	1	0.900	0.891
DYS447	0.150	20	11	1	0.890	0.880
DYS460	0.350	20	5	1	0.750	0.709
DYS461	0.200	20	7	1	0.840	0.819
DYS481	0.300	20	6	1	0.785	0.753
Mean	**0.245**	**20**	**7.5**	**1**	**0.824**	**0.799**

In Yazidis population the allele frequency ranged from 0.150 in DYS447 to 0.400 in DYS390 with mean of 0.260. The number of alleles per locus ranged from 4 alleles at DYS390 locus to 10 alleles at DYS437 and DYS439 loci with an average of 7.5 alleles per locus. The gene diversity ranged from

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0.700 in DYS390 to 0.880 in DYS437 with mean of 0.816. The PIC values ranged from 0.645 in DYS390 to
0.868 1in DYS437 with average of 0.790 (Table 4).

Table 4: Allele frequency, availability, gene diversity, and PIC in the Yazidis population.

Marker	Allele Frequency	Sample Size	Allele No.	Availability	Gene Diversity	PIC
DYS19	0.20	20	8	1	0.850	0.832
DYS390	0.40	20	4	1	0.700	0.645
DYS393	0.30	20	8	1	0.830	0.811
DYS426	0.30	20	5	1	0.760	0.719
DYS437	0.15	20	10	1	0.880	0.868
DYS439	0.20	20	10	1	0.870	0.856
DYS447	0.15	20	9	1	0.875	0.862
DYS460	0.20	20	9	1	0.855	0.838
DYS461	0.35	20	7	1	0.790	0.763
DYS481	0.35	20	5	1	0.745	0.704
Mean	**0.26**	**20**	**7.5**	**1**	**0.816**	**0.790**

While in Turkmen s population the range of allele frequency ranged from 0.20 in DYS439 locus to 0.40 at DYS390 locus with mean of 0.30. The number of alleles per locus ranged from 5 alleles at DYS19, DYS390 and DYS393 loci to 9 alleles at DYS439 and DYS447 loci with an average of 6.8 alleles per locus. The gene diversity ranged from 0.715 in DYS390 to 0.870 at DYS439 with mean of 0.795.
The PIC values ranged from 0.668 in DYS390 to 0.856 in DYS437 with average of 0.766 (Table 5).

Table 5: Allele frequency, availability, gene diversity, and PIC in the Turkmen population.

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Marker	Allele Frequency	Sample Size	Allele No.	Availability	Gene Diversity	PIC
DYS 19	0.35	20	5	1	0.740	0.695
DYS 390	0.40	20	5	1	0.715	0.668
DYS 393	0.35	20	5	1	0.745	0.704
DYS 426	0.30	20	6	1	0.785	0.752
DYS 437	0.25	20	7	1	0.820	0.795
DYS 439	0.20	20	9	1	0.870	0.856
DYS 447	0.25	20	9	1	0.855	0.839
DYS 460	0.30	20	7	1	0.800	0.773
DYS 461	0.25	20	7	1	0.815	0.790
DYS 481	0.35	20	8	1	0.805	0.783
Mean	**0.30**	**20**	**6.8**	**1**	**0.795**	**0.766**

For all population together the range of allele frequency ranged from 0.083 in DYS439 locus to 0.183 at DYS461 locus with mean of 0.132. The number of alleles per locus ranged from 13 alleles at DYS390 locus to 26 alleles at DYS439 locus with an average of 18.3 alleles per locus. The gene diversity ranged from 0901 in DYS390 to 0.946 at DYS439 with mean of 0.918. The PIC values ranged from 0.892 in DYS390 to 0.946 in DYS439 with average of 0.918 (Table 6).

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
DYS 19	0.117	60	17	1	0.925	0.920
DYS 390	0.167	60	13	1	0.901	0.892
DYS 393	0.133	60	19	1	0.925	0.920
DYS 426	0.133	60	16	1	0.919	0.913
DYS 437	0.117	60	18	1	0.927	0.923

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DYS 439	0.083	60	26	1	0.949	0.946
DYS 447	0.100	60	24	1	0.944	0.942
DYS 460	0.167	60	16	1	0.906	0.898
DYS 461	0.183	60	16	1	0.909	0.902
DYS 481	0.117	60	18	1	0.925	0.920
Mean	**0.132**	**60**	**18.3**	**1**	**0.923**	**0.918**

The value of availability (number of observed alleles per number of individuals sampled) was calculated, this value was found to be high in all the three populations Kurds, Yazidis and Turkmen with an average of 1. Results of phylogenetic analysis are shown in Figure 1. The dendrogram separated the populations into two main clusters, the Turkmen group in one cluster and Kurd and Yazidis groups in a second cluster. The latter, in turn, was divided into two sub-cluster: The Kurd and the Yazidis except few individuals from Kurd and the Yazidis populations were admixed with another in the sub-cluster.

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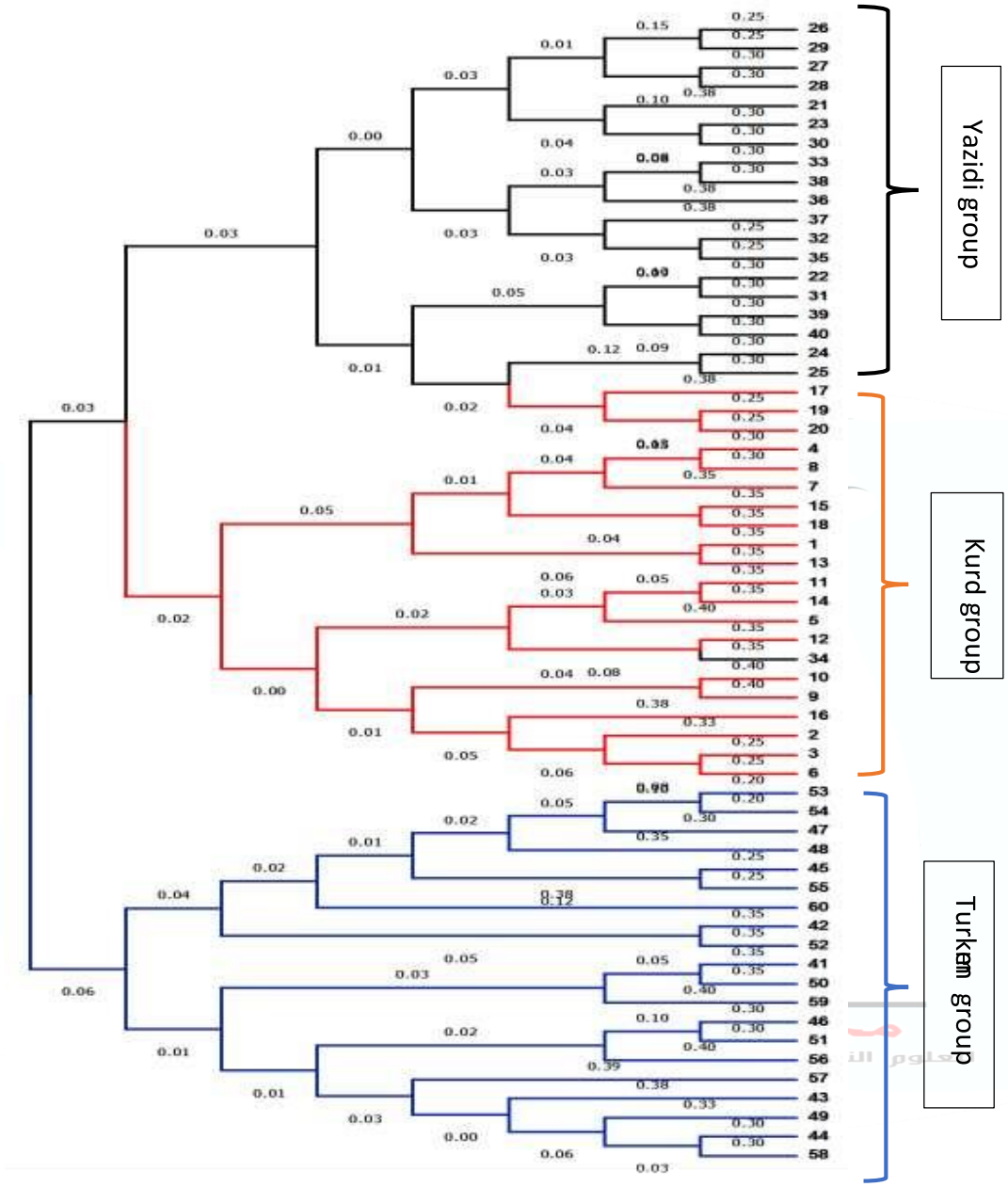

Figure 1: Dendrogram for the genetic relationship of Kurds, Yazidis and Turkmans populations.

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Discussion:

The Y-STR chromosomal variation pattern shown here offers strong genetic support for major variations across the three populations under study. This study's findings show that a total of 183 alleles have been discovered. The size of the alleles varied from 80 bp to 272 bp (Table 2). The PCR product sizes of the examined DYS loci ranged from 93 to 212 bp, which were previously reported for the Iraqi Arab families living in the middle Euphrates. (Naji,2020). Kurd and Yazidis had an average of 7.5 alleles per locus, and Turkmen had a mean of 6.8 alleles, which was lower than the average of 9 alleles per locus reported in a fact sheet by the National Institute of Standards and Technology (NIST) in the USA but higher than the average of 5.63 alleles per locus mentioned by Ohied and Al Badran in (2022). The allele frequency
represents the incidence of a gene variant in a population. The average of allele frequencies in Kurd and Yazidis groups were close to each other with value of 0.245 and 0.260 respectively while the average allele frequency in Turkmen group was much higher with value of 0.300 (Tables 3,4 and5). A study conducted in Basrah in 2022 by Ohied and Al Badran found an allele frequency value of 0.377, which is greater than the findings of this investigation. Allele frequencies were found to be higher than the findings in this study in a study conducted in 2013 by Imad and his colleagues in the middle and southern populations of Iraq. This finding could be explained by the fact that all three populations under study remained generally unmixed. A population's diversity of inherited traits is referred to as genetic diversity. A population must be able to adapt to its changing environment. The data in tables 3, 4 and 5 indicate that the mean value of gene diversity in the three population were 0.824, 0.816 and 0.795 in Kurd, Yazidis and Turkmen populations respectively. These results are much higher than those of Ohied and Al Badran (2022) and Imad *et al*. (2013). Chen *et al*. (2018) found findings that are similar to those of this study, with genetic diversity in three major Chinese ethnic groups in southwest China ranging from 0.2615 to 0.855. Kassab et al. (2020), who discovered that genetic diversity ranged from 0.222 to 0.807 in the Saudi population, obtained a similar result to our

study. According to Yepiskoposian et al. (2010), the genetic variability of the Yezidi community is much lower than that of the Kurds. This observation is in contrast to the study's findings because both the Kurdish and Yazidi populations have comparable levels of genetic diversity (Tables 3 and 4). Another study, by Dogan et al. (2017), which also includes the Turkmen population and showed comparable genetic diversity in the Kurd and Yazidi populations, validates these findings. The findings of this study indicate that Turkmen communities have less genetic diversity than other populations (Tables 3, 4 and 5). Polymorphism Information Content (PIC), which assesses a marker's capacity to identify polymorphisms, is crucial for choosing markers for genetic studies. (Lemos *et al.*,2020). Due to their strong genetic differentiation, easy selection of the highly informative Y chromosome STR markers, and usage in population genetics studies and forensic applications, Y-STRs are useful. The calculated Polymorphism Information Content (PIC) varied from 0.892in DSY390 which can be consider the least informative to 0.946 in DYS439 which can be consider the most informative primer (Table 6).

Because all values at all loci are greater than 0.5, all of the primers employed in this investigation can be regarded as highly polymorphic, according to Botstein et al. (1980). Whether the distance indicates time from a common ancestor or degree of differentiation, genetic distance is a measure of the genetic divergence between species or between populations within a species. (Nei, 1987). To evaluate the genetic differentiation and the distance between different populations, a phylogenetic tree was constructed. The phylogenetic tree (figure No.1) separated the populations into two major clusters. Turkmen population in one cluster and Kurd-Yazidis populations in another cluster. The latter, in turn, is divided into two sub-clusters: The Kurd and the Yazidis, except few individuals from Kurd and the Yazidis clusters were admixed with each other. The genetic distance in three populations indicates that the genetic distance between Kurd population and the Yazidis population is less than the genetic distance between the Kurd population and the Turkmen population, meaning that the Yazidis are genetically closer to the Kurds. This suggests a long-shared

history and the same homeland for thousands of years, as well as the fact that both Kurdish and Yazidi populations come from Indo-European countries. Around 700-900 years ago, Yezidis stopped marrying other Kurds outside of their community for religious reasons. Thus, resulting in the Yezidis becoming to some extend sort of a separate group. Another reason that the Yazidis are highly conserved social culture, religious and practice of intra marriages. Despite being successively ruled by many conquerors, such as the Armenians, Romans, Byzantines, Arabs, Ottoman Turks, and Persian (Kinnane, 1970), Kurdistan remained relatively unmixed by the influx of invaders due to their protected and hostile mountainous territory. The Turkmen population which they are new to the region in compare to the other population remained unmixed (Figure 1.). Explanation of this mainly because of their high social conserved culture and strict endogamy known to be practiced in this ethnic group.

Conclusions:

In conclusion, the Y-STR data presented in this study found that the 10 primers used are highly informatics and can be used for fathered genealogical studies and also these markers can provide very useful information for the analysis of forensic cases. The loci DYS439 had the highest gene diversity, while loci DYS390 had the lowest gene diversity. Phylogenetic tree analysis shows that the populations were divided into two main cluster the Kurds-Yazidis cluster and Turkmen cluster. The Phylogenetic tree revealed some convergence between the Kurdish and Yazidis population, while the Turkmen population was far from them.

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