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***Corresponding author**

Hazha Jamal Hidayat
Hazha.hidayat@su.edu.krd

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Genetic Diversity in Hypervariable Segment I of Mitochondrial DNA Among the Kurdish Population Erbil City- Iraqi Kurdistan Region

Srwa yousif Anwer[1], Shakhawan Anwar Ali[2], Hazha Jamal Hidayat [1*]

[1] Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq
2 Department of Pathological Analysis, Paytakht Technical Institute, Erbil-Kurdistan Region, IRAQ.

ABSTRACT

Analysis of human sequence polymorphisms of mitochondrial DNA (mtDNA) hypervariable segment 1 (HV1) has become a significant technique in forensic casework and is used for genetic diversity and human evolution evaluation, consanguinity identification, and mitochondrial illness diagnosis. The present study aimed to provide insights into the demographic history and origins of the Kurdish people in the Iraqi Kurdistan region by analyzing mitochondrial DNA (mtDNA) sequence variations, particularly focusing on hypervariable segment 1 (HVS I). The HVS I of mtDNA was sequenced in 120 unrelated individuals from the Kurdish population by using Sangar sequencing. The study's findings reveal significant genetic diversity among the Kurdish population's HVS I of human mitochondrial DNA (mtDNA). The sequence of HVS I within the non-coding region had 413 mutations across 402 sites, resulting in 111 different haplotypes, as compared to the revised Cambridge Reference Sequence (rCRS) to gauge genetic diversity and community variety. Comparing this population sample to rCRS, the mutations found were (17.055%) transition and (0.921%) transversion. The phylogenetic tree for the Kurdistan region revealed a considerable degree of community variety. In order to determine the Kurdish ancestry and demographic history in the territory of Iraqi Kurdistan, the results also showed that the mtDNA haplotypes were classified into mtDNA haplogroups. The most frequent haplogroups were U, H, J, and HV, with frequencies of 22.5%, 19.2%, 14.5%, and 12.9%, respectively; nevertheless, T and N had a moderate prevalence of 8% and 6.4%, respectively. The haplotype results exhibited that Europe and India had the most genetic diversity in common with the Kurdish people of Iraqi Kurdistan. The findings will contribute to a better knowledge of Kurdish mitochondrial genome diversity, as well as to demographic history and forensic science.

1. Introduction

Mitochondrial DNA (mtDNA) analysis has emerged as a crucial technique in population genetics, anthropology, and forensic research (Sultana and Sultan, 2018). Due to its distinctive features, including maternal inheritance, high mutation rate, and lack of recombination, mtDNA functions as a potent molecular marker for examining genetic diversity, human migratory patterns, and ancestry (Kivisild, 2015). One of the most informative regions of mtDNA is the hypervariable region 1 (HVR1), which is located in the non-coding displacement loop (D-loop). This region rapidly acquires mutations across generations, rendering it especially valuable for tracing maternal lineages and assessing genetic links among populations (Johnson, 2013).

The HVR region is a part of the mtDNA D-loop, and mitochondrial function depends on polymorphisms in the non-coding D-loop region (Kwaśniewski et al., 2023). The elevated mutation rate characteristic of these hypervariable regions serves a significant purpose in the fields of population genetics and human identification assessments. The current analysis focuses on specific hypervariable segments of mtDNA, namely HVI (nucleotide positions 16024-16365), HVII (nucleotide positions 73-340), and HVIII (nucleotide positions 438-576), which were evaluated to enhance the understanding of genetic diversity within the population (Verma et al., 2018). mtDNA haplogroups facilitate the classification of HVR1 and HVR2 haplotypes into associated categories. The high-throughput sequencing of mtDNA from distinct human lineages has been made possible by the advent of new molecular biology methods. These developments have proven useful in tracing the matrilineal descent of modern humans from Africa, who were afterward distributed to Asia and Europe (Kapoor et al., 2017).

The Kurdish people represent one of the largest ethnic groups in the Middle East without a sovereign state. Despite their significant presence in countries such as Iraq, Iran, Turkey, and Syria, the genetic background of the Kurdish population remains underexplored. Given the complex migratory history and cultural diversity of the region, investigating the mitochondrial genome of the Kurdish people can provide new insights into their maternal lineage, genetic affiliations, and evolutionary background.

Overall, this study analyzed the genetic diversity of the Kurdish population in Iraqi Kurdistan using mtDNA sequences from 120 unrelated individuals. Results showed a high level of genetic diversity, with 413 mutations and 111 distinct haplotypes. The most common haplogroups were U, H, J, and HV, with significant genetic similarity to Europe and India. This research contributes to understanding the Kurdish population's genetic diversity and maternal ancestry and has implications for forensic investigations and anthropological studies.

2. Materials and methods

2.1. Population sample

For the current investigation, blood samples were taken from 120 unrelated Kurdish individuals from Erbil city, Iraqi Kurdistan region. All donors have received informed consent and information about their ethnic background. The study included only native peoples.

2.2. HVSI amplification via PCR and genomic DNA extraction

The genomic DNA was extracted from the blood sample via the quick protocol of AddPrep Genomic DNA Extraction Kit (Cat. No. 10023, KOREA). The DNA extraction was conducted in the laboratory of IMMUNOGENE CENTER, Erbil –Iraqi Kurdistan region. Since there are multiple copies of mt-DNA in every cell, a simple method to extract and measure DNA was used to amplify HV1.

According to a study performed by Tsutsumi et al. (Tsutsumi et al., 2004), amplification from 15,997 nt to 16,401nt of HVSI was done by using (forward primer: 5’CACCATTAGCACCCAAAGCT-3 and reverse primer: 5’TGATTTCACGGAGGATGGTG-3). In this experiment, these primers were used by applying a Polymerase Chain Reaction (PCR) machine to amplify the HVS1 in the mtDNA control region. The primers were validated in silico against the human reference genome through the application of a Basic Local Alignment Search Tool (BLAST) utilizing the 2.2.27 release number.

The PCR conditions were established for the HVSI region, consisting of an initial denaturation step at 94 degrees Celsius for one minute, followed by an annealing step at 60 degrees Celsius for one minute, followed by a denaturation step at 94 °C for 1 min, and an extension step at 72 degrees Celsius for one minute. A final extension was conducted at 72 degrees Celsius for ten minutes. The thermocyclers were programmed for a total of 40 cycles. Subsequently, the PCR products were sequenced at the IMMUNOGENE CENTER utilizing the 3130 Genetic Analyzer (Applied Biosystems, Hitachi High Technologies, Tokyo, Japan).

2.3. Phylogenetic and statistical analysis

2.3.1. Multiple alignment

The electropherograms were read by the tool of BioEdit 7.1 (Hall, 1999). All sequences were aligned with the revised rCRS using the MUSCLE tool of MEGA 11 (Kumar et al., 2008). A phylogenetic tree was constructed to analyze the mtDNA haplogroups by PhyloTree 16 (Van Oven and Kayser, 2009). The error rate during haplogroup organization was lowered by implementing an algorithm in HaploGrep (Kloss-Brandstätter et al., 2011).

2.3.2. Haplogroup interpretation

MitoTool, a complimentary online bioinformatics application for examining mtDNA sequences (Ruiz-Pesini et al., 2007, Fan and Yao, 2011), was utilized to present the haplogroup results. MitoTool Clustal W was employed to align HVS1 sequences with rCRS and to identify the variations for each batch mode sequence. The precise coordination, whether optimal or close, governed the haplogroup via mt-DNA, considering its unique variation patterns. However, We independently verified the induced haplogroups using MitoTool's mthap software. Ultimately, we integrated the MitoTool and mthap categorization data, and our phylogenetic tree has been developed to address and reconcile the inconsistencies in the derived haplogroups (Johnson et al., 2015). Finally, phylogenetically described haplogroups or branches were employed to assign individuals a potential haplogroup when MitoTool characterization and mthap yielded inconclusive or ambiguous results (more than one probable haplogroup per individual).

2.4. Statistical analyses

The evaluation of genetic diversity metrics and molecular variance analysis was performed using many criteria, specifically with Arlequin 3.5, as described by Excoffier and Lischer (Excoffier and Lischer, 2010). Moreover, various neutrality tests were implemented, including Tajima's D (Tajima, 1989), Fu's Fs (Fu, 1997), and R2 (Bektas et al., 2020). Furthermore, the mismatch distribution, as articulated by Harpending (Harpending, 1994), was assessed using two separate approaches that emphasize genuine demographic analysis. Finally, to determine and analyze the normal distribution data pertaining to abrupt population increases, the sum of squared deviations (SSDs) and Harpending's raggedness score were utilized.

3. Results and Discussions

3.1. Sequence Diversities and Neutrality Test Statistics of HVS1

The use of mitochondrial markers in forensic casework requires knowledge of the prevalence of a particular mtDNA haplotype in a given population. The nucleotide sequences of the mtDNA D-loop (nt 15997-16401) and HV1 were determined in this study in a total of 120 unrelated people from the Kurdish-native speakers in the Iraqi Kurdistan region. All samples had PCR products with a size of roughly 420 bps, excluding the primers (Anderson et al., 1981). As shown in (Table 1), which provides a statistical overview of the genetic diversity of the Kurdish population, with 111 distinct haplotypes detected among 120 individuals, overall diversity is fairly high. A grand number of 247 polymorphic sites were discovered in the Kurdish data collection. The low value of Tajima's D (-1.76378) suggests that the population is growing.

Moreover, Iraq (-25.27102) (Al-Zahery et al., 2011), Iran (-25.30270) (Nasidze et al., 2006), Turkey (-25.06957) (Mergen, 2004), Kuwait (-25.40242) (Theyab, 2010) are the four countries having populations with negative Fs values, which suggest population growth. The findings of the mtDNA diversity and neutrality measures for the Kurdish population are shown in (Table 2). The genetic diversity measure shows that the Kurdish population (0.9986) is similar to nearby populations in Iraq (0.9918) and Saudi Arabia (0.9905), Turkey (0.9851), Iran (0.9895), Syria (0.9881), Kuwait (0.9863), and Kurdistan/Iraq as well as kurmanje-T, Zazaki-T, (0.988), and (0.986). The Kurdish population's Tajima's D (-1.76378) and Fu's FS (-96.565) values are significant at P>0.05 and P>0.005, respectively. These results suggest that the Kurdish population may be seeing growth in a relatively short period of time.

Table 1. Shows mitochondrial DNA HV1 sequence variability among the Kurdish population.

Genetic features	**Statistical data**
No. of the samples	120
No. of haplotypes (h)	111
No. of variable sites (s)	247
Haplotype diversity (S.D)	0.9986 (0.0013)
Nucleotide diversity	0.06901 (0.00855)
Average no. of nucleotide difference (K)	24.912
Tajima's D	-1.76378
Fu's FS	-96.565
Total no. of sites	402
Invariable (monomorphic) sites	87
Variable (polymorphic) sites	274
Total no. of mutation	413
Singleton variable sites	49
Parsimony informative sites	225

3.2. Haplogroup Predication of HVS1

According to a study conducted in Iraq by Al-Rashedi and colleagues, Arabs constitute approximately 75–80% of the Iraqi population, while Kurds, Turkmen, and other minority groups make up the remaining 20–25% (Al-Rashedi et al., 2015). Due to the prevalence of unique genetic sequences among different populations, each ethnic group possesses a distinct genetic structure.

mtDNA haplogroups-such as U, H, and J-are key maternal lineage groups that represent branches of ancient human ancestry. These haplogroups originated at various times and locations across the globe and are characterized by unique genetic markers (Kristjansson et al., 2023). Haplogroup U, for instance, emerged around 55,000 years ago, likely in the Middle East or Central Asia, and is now widespread across Europe, Western Asia, and North Africa. Its subclades, including U2, U4, and U5, are especially significant, with U5 representing one of the earliest mtDNA lineages found among indigenous European populations (Sahakyan et al., 2017).

Haplogroup H is believed to have originated between 20,000 and 25,000 years ago in Southwest Asia or Europe. Today, it is the most common mtDNA haplogroup in Europe, comprising 40–50% of the population, and is also present in North Africa and the Middle East (Sahakyan et al., 2017). Similarly, haplogroup J is uniformly distributed across Europe, with notable frequencies in regions such as Cornwall (20%), Wales (15%), and Iceland (14%), and is also common in the Middle East-particularly in Saudi Arabia (21%), Kuwait (16%), Kurdistan (15%), and Iraq (13%) (Dato et al., 2004, Clark et al., 2011).

The Kurdish population in Iraq exhibits a diverse mixture of mitochondrial haplogroups derived from European, Asian, and Indian ancestries, as shown by sample sequence analyses from MITOMAP (http://www.mitomap.org/). According to the data presented in (Tables 2 and 3), haplogroup U is the most prevalent among Kurds (22%), followed by H (19.2%) and J. Other haplogroups, such as N, R, L, M, K, and W, occur at lower frequencies within the Erbil population. The presence of these haplogroups suggests maternal genetic influences from both South Asia and Europe.

Supporting these findings, a study of various Kurdish subgroups- including Sorani, Hawrami, Kurmanji, and Kalhori- also demonstrated a high prevalence of haplogroups U, H, and J (Zarei and Rajabi-Maham, 2016). This genetic pattern implies significant recent gene flow from Europe into the Kurdish population. Notably, haplogroup U, which has an estimated age of 51,000 - 67,000 years, is substantially older than other haplogroups and appears widely across Europe and the Near East (Richards et al., 2000, Roostalu et al., 2007). Additionally, prehistoric European remains dating back up to 13,400 years have been linked to haplogroup U, further supporting its ancient presence in the region.

Comparative analyses of mtDNA haplogroup frequencies between the Kurdish population and neighboring populations revealed similar genetic patterns. As shown in (Table 4), the Kurdish population’s haplogroup frequencies closely resemble those of populations in Iran and Iraq. Similar frequencies were also observed in Anatolia (Turkey), Syria, Saudi Arabia, and Kuwait (Triki-Fendri et al., 2016).

Moreover, our findings indicated a low presence of the African haplogroup L in Iraqi Kurdistan (3.3%) and Iran (2.2%), in contrast to a significantly higher frequency in Saudi Arabia (10.5%). The occurrence of haplogroup L in Iraqi Kurdistan points to historical African gene flow into the Kurdish population. Additionally, the presence of haplogroups M, N, and R in the Kurdish population serves as markers of gene flow from Asian regions, as demonstrated in (Table 4) (Figure 1).

Table 1. Shows the mtDNA HVS1 sequence data and summary statistics for the Kurdish population, include the number of samples (N), gene diversity (H), nucleotide diversity, and Tajima's and Fu's FS.

Population	No.	Haplotype diversity	Nucleotide diversity	Tajima's diversity	Fu's FS	Reference
Kurdish	120	0.9986(0.0013)	0.06901(0.00855)	-1.76378	-96.565	Current study
Iraq	116	0.9918(0.0036)	0.019862(0.010659	−2.10200	−25.27102	(Al-Zahery et al., 2011)
Iran	92	0.9895(0.0049)	0.020453(0.010967)	−2.10033	−25.30270	(Nasidze et al., 2006)
Turkey	290	0.9851(0.0039)	0.017378(0.009419)	−2.22379	−25.06957	(Mergen, 2004)
Syria	69	0.9881(0.007)	0.019229(0.010419)	−2.14505	−25.44560	(Richards et al., 2000)
England	242	0.9651(0.0078)	0.014654(0.008122)	−2.24347	−25.48480	(Piercy et al., 1993)
Saudi Arabia	15	0.9905(0.0281)	0.022234(0.012566)	−1.32083	−7.63765	(Abu-Amero et al., 2007)
Kuwait	94	0.9863(0.0051)	0.0239(0.012661)	−1.87839	−25.40242	(Theyab, 2010)
Kurmanje -t	51	0.988		-2.05		(Nasidze et al., 2005)
Zazaki -t	27	0.986		-1.81		(Nasidze et al., 2005)

3.3. Phylogenetic analysis

To assess the genetic diversity of the Kurdish population in comparison to the revised rCRS, a neighbor-joining phylogenetic tree was constructed using MEGA version 11. As illustrated in (Figure 2), the tree was generated based on mtDNA HVS1 sequences from all samples relative to the rCRS. The analysis revealed a noticeable genetic divergence between the Kurdish population and the rCRS, which may be attributed to population-specific mutations. These findings align with a recent study that also reported a genetic gap between the Kurdish and rCRS populations, potentially due to region-specific mutations observed in the Basrah population (Ohied and Al-Badran, 2020).

3.4. Sequence diversity of HV1

Table 5 summarizes all types of base mutations identified in the HVS1 of mtDNA, including both transitions and transversions. Among these, transition mutations were found to be the most prevalent. The most frequent transition was a thymine-to-cytosine (T→C) substitution, accounting for 11.170% of mutations, followed by a cytosine-to-thymine (C→T) transition at 3.217%, and a third, unspecified transition at 2.668%. In contrast, transversion mutations were comparatively rare, with a total frequency of only 0.921%. This observation aligns with previous literature, which consistently reports a predominance of transitions over transversions in human mtDNA variation (Hameed et al., 2015, Yang and Yoder, 1999).

Specifically, transitions were observed at positions 16100, 16122, and 16283, totaling 17.055%, while transversions were primarily detected at position 16292, accounting for the 0.921% frequency. Among all polymorphic sites, only two positions exhibited notably high levels of variation: position 16100, where the reference nucleotide T was substituted by C in 11.170% of cases, and position 16123, where a reference C was replaced by T in 3.217%. Despite these polymorphisms, the most frequent nucleotide at each site remained consistent with that of the revised rCRS. As shown in (Figure 3), the transition from T to C was the most frequent base substitution, comprising 62% of observed mutations, followed by C to T transitions at 18%. The least common mutation type was the A to T transversion, which constituted only 5% of the total.

Previous studies have shown that the mtDNA coding region exhibits lower levels of genetic variability compared to the non-coding (regulatory) region (Walsh et al., 1991, Tang, 2002). To enhance the discriminatory power of forensic mtDNA analysis, the use of highly polymorphic sites is essential (Hadi et al., 2014). Particular attention should be given to selecting polymorphisms that are phenotypically neutral, especially within the mtDNA coding region, to avoid potential ethical concerns related to medical genetics and ethnic profiling.

Looking forward, comprehensive sequencing of the entire mitochondrial genome- including both coding and non-coding regions- combined with the use of informative single nucleotide polymorphisms (SNPs) from the regulatory region, may significantly improve the resolution and forensic utility of mtDNA profiling (Warshauer et al., 2013, Hadi et al., 2014).

Table 3. Shows the comparison of haplogroup frequencies in the Kurdish population with different Regions in Iraq.

Population region	Sample size	Haplogroups frequency %											Ref.
		U	H	J	HV	T	N	R	L	M	K	W	
Iraqi Kurdistan	120	22.5	19.2	14.5	12.9	8	6.4	3.3	3.3	3.3	3.3	3.3	This study
Iraq-Kurd	15	26.64	6.66	20	-	13.32	-	13.32	-	6.66-	-	6.66	(Al-Zahery et al., 2013)
Sorani	79	10	35	10	5	-	15	10	-	-	-	-	(Zarei and Rajabi-Maham, 2016)
Hawrami	79	-	71.4	-	4.76	4.76	5.26	-	-	-	-	-	
Kurmanji	79	21.04	31.56	5.26	-	-	-	-	-	-	-	-	
Kalhori	79	15.78	31.56	10.52	5.26	10.52	-	5.26	-	-	-	10.52	
Iraq	100	18	14	10	10	7	7	-	1	9	2	-	(Al-Rashedi et al.,

													2015)
Thi-Qar	216	19	19.9	9.3	10.6	8.8	-	-	1.4	1.4	3.2	1.9	(Al-Zahery et al., 2013)
Iraq	153	15.3	16.9	8.06	6.4	-	-	-	4	8.1	12	8.87	(Azzawi et al., 2013)
Basrah	164	9	17	11	5	-	6	-	5	9	5	2	(Ohied and Al-Badran, 2020)

Table 4. Shows the comparison of Haplogroup frequencies in the Kurdish population with other Neighboring populations.

Population	**Sample size**	**Haplogroups frequency %**											
		U	**H**	**J**	**HV**	**T**	**N**	**R**	**L**	**M**	**K**	**W**	**Ref.**
Iraqi Kurdsiatn	120	22.5	19.2	14.5	12.9	8	6.4	3.3	3.3	3.3	3.3	3.3	This study
Iraq	100	18	14	10	10	7	7	-	1	9	2	-	(Al-Rashedi et al., 2015)
Iran	451	21.5	17.1	13.5	5.5	8.4	6.1	-	2.2	-	7.5	2	(Kivisild et al., 2003)
Anatolia	388	19.3	25	10.9	3.6	11.9	1.8	-	0.3	4.4	5.9	3.9	(Tajima, 1989)
Syria	69	15.9	24.6	10.1	4.3	10.1	-	-	5.8	1.4	4.3	2.9	(Ohied and Al-Badran, 2020)
Saudi Arabia	389	10.5	12.9	20.8	3.6	4.6	2.6	-	10.5	-	3.6	1.8	(Kivisild et al., 2003)
Kuwait	117	12	9	-	5	-	9.8	-	1.6	7	-	-	(Theyab, 2013)

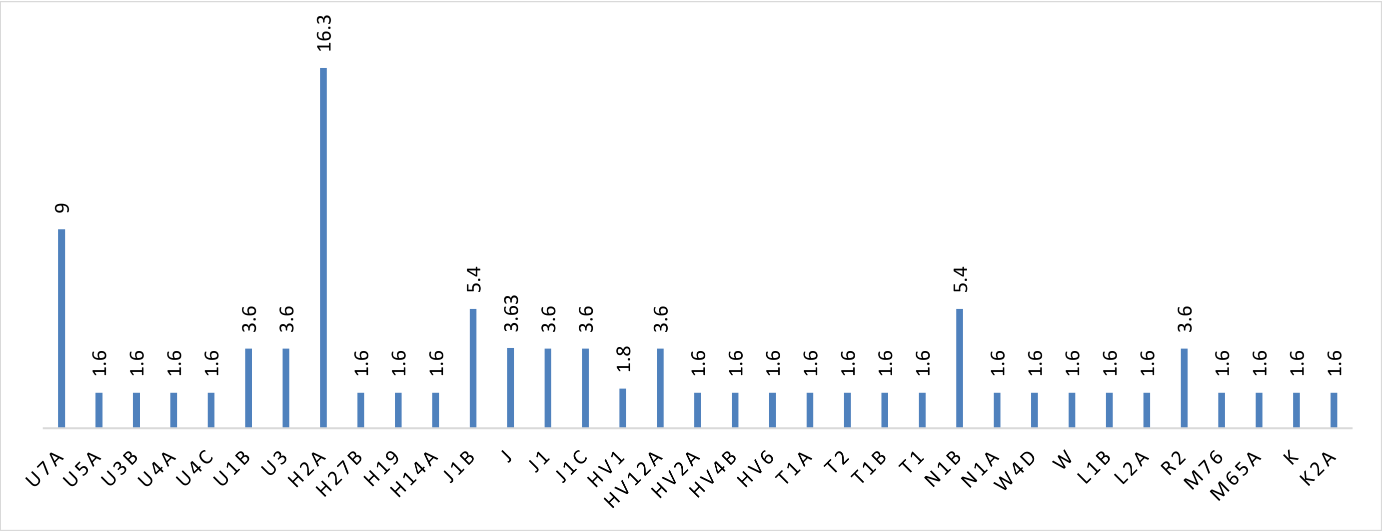

Figure 1. Shows the frequency of haplogroup subclade on HVSI of mtDNA in the Kurdish population.

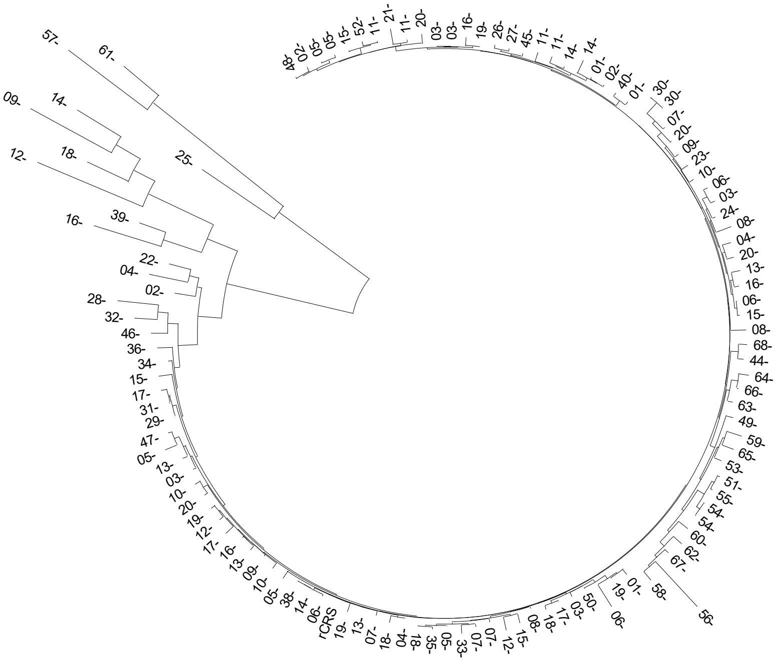

Figure 2. Shows the neighbor-joining tree of HV1 segment in Erbil/Kurdish population.

4. Conclusion

The present study highlights the high level of mtDNA genetic diversity within the Kurdish population, as revealed by the analysis of the HVS1. The Tajima's D test yielded a value of -1.76378, suggesting an excess of low-frequency polymorphisms, which may be indicative of population expansion or purifying selection. Similarly, Fu's Fs statistic, with a strongly negative value of -96.565, further supports the presence of population growth and demographic expansion. The nucleotide diversity was calculated at 0.06901, while the haplotype diversity reached 0.9986, indicating a remarkably high level of genetic variability in the population.

In terms of haplogroup distribution, the Kurdish population is predominantly represented by haplogroups U (22.5%), H (19.2%), and J (14.5%). These maternal lineages are associated with ancient human migrations across Europe, the Middle East, and Central Asia, providing valuable insights into the historical and evolutionary origins of the Kurdish people.

The genetic patterns observed in this study not only support the complex maternal ancestry of the Kurdish population but also offer a deeper understanding of their historical demographic processes. The combination of high haplotype diversity and signals of past population expansion underscores the genetic richness and historical mobility of Kurdish-speaking groups.

However, despite these important findings, the study has certain limitations that should be acknowledged. The sample size of 120 individuals may not adequately represent the genetic diversity of the entire Kurdish population, and the focus on individuals from Erbil City limits the geographic scope, potentially excluding variation present in other Kurdish regions such as Sulaimani and Duhok. Moreover, the exclusive analysis of the HVS1 region, while informative, may overlook additional variations found in other mtDNA segments like HVS2 and HVS3. The study also does not explore correlations between mtDNA variants and clinical conditions, which could be essential for understanding mitochondrial disorders in this population.

Consequently, continued genetic analyses of mtDNA particularly when integrated with data on nuclear DNA, Y-chromosomal markers, and archaeological evidence can significantly contribute to reconstructing the migration history and ancestral origins of Kurdish populations. These findings open avenues for more comprehensive research into the genetic legacy of populations residing in the broader Middle Eastern region.

Table 5. Type of base mutations on HVS1 of mDNA.

Mutation type		Base	Position	Frequency
Transition		T-C	16100	11.170%
Transition		C-T	16122	3.217%
Transition		A-G	16283	2.668%
Transversion		A-T	16292	0.921%

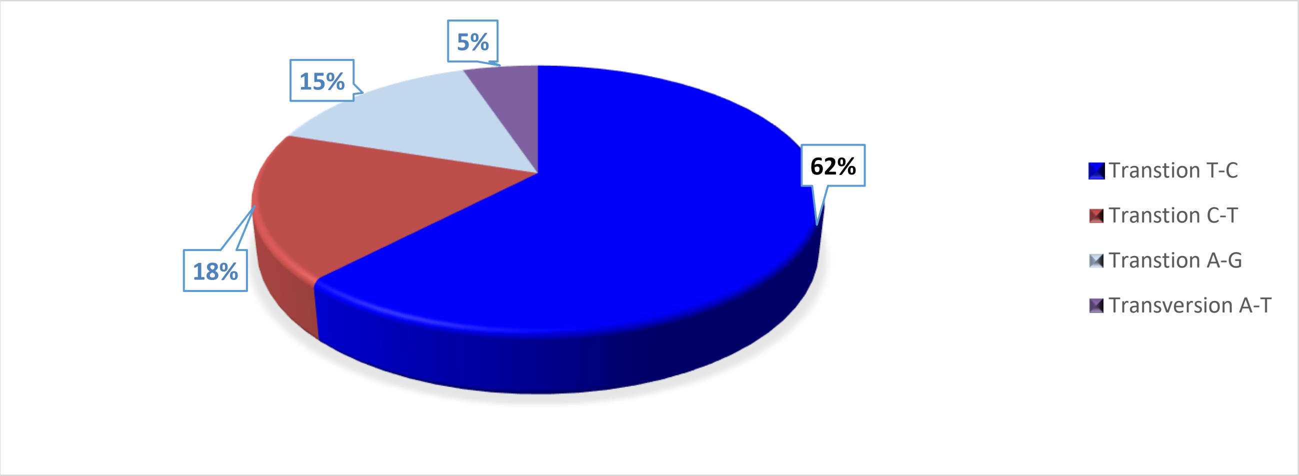

Figure 3. Shows the percentage of base mutation frequency on HVI of mtDNA.

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Data Availability Statement: Applicable.

Research

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