J Genet Resour2025; 11(1): 1-11 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2025.28072.1410*

J|G|R

Tracing Hypervariable Region I (HVR-I) of Mitochondrial DNA in Iranian Ethnic Groups

Misagh Moridi[1], Mostafa Ghaderi-Zefrehei[2], Anousheh Shasttiri[3] and Mustafa Muhaghegh Dolatabady*[2]

[1] Department of Animal Science, Faculty of Agriculture, Guilan University, Rasht, Guilan, Iran
[2] Department of Animal Science, Faculty of Agriculture, Yasouj University, Yasouj, Iran
[3] Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

ARTICLE INFO

Article history:
Received 20 November 2024
Accepted 19 December 2024
Available 14 January 2025

Keywords:
Genetic variation
Haplogroups
Mitochondrial DNA

Corresponding authors:
✉ M. Muhaghegh dolatabady
mmuhaghegh@yu.ac.ir

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

Genetic markers can confirm the role of ethnicity in forensic investigations. Mitochondrial DNA (mtDNA) can be applied as a useful marker in forensic casework due to its maternal inheritance, high copy number, and strong persistence, even in degraded samples. Therefore, the present study aimed to investigate the mtDNA's hypervariable region I (HVR-I) in individuals from different Iranian ethnic groups/subpopulations. For this purpose, haplogroup frequencies of mtDNA and the population genetic parameters in the HVR-I region of mtDNA were evaluated in 357 individuals from different ethnic groups (Fars, Lur, Arab, Kurd, Azeri, Gilak, Bandari, Jewish, Armani) and a group of patients with glucose-6-phosphate dehydrogenase (G6PD) disease using population genetics tools. For this purpose, haplotype and nucleotide diversity were determined using the DNASP 5.10 software. In addition, allelic variants and elucidating subpopulation haplogroups were investigated with the help of PhyloTree and HaploGrep 2 software, respectively. Overall, this study identified 316 different haplotypes. The Fars group had the highest number of samples and haplotypes, with 114 samples and 97 haplotypes, respectively. In addition, the lowest number of samples and haplotypes belonged to the Baluch group, with 13 samples and 11 haplotypes, respectively. In total, 139 unique haplogroups were detected in the HVR-I region of mtDNA in the Iranian population, where H2a2a1, J1b, and H2 were the most frequent across all subpopulations. The detected haplogroups belonged to 20 macro-haplogroups. The majority of known haplogroups were of European origin. Pairwise comparisons between Iranian subpopulations indicated that 23, 17, 17, and 21 of the comparisons had very high, high, medium, and low levels of genetic differentiation. The maximum genetic differentiations were observed between the G6PD and other ethnic groups. In general, this study indicated great genetic diversity in Iranian subpopulations. Further surveys can evaluate the relationship between this genetic diversity and important traits.

Please cite this paper as Moridi, M., Ghaderi-Zefrehei, M., Shasttiri, A., & Muhaghegh dolatabady, M.M. (2025). Tracing Hypervariable Region I (HVR-I) of mitochondrial DNA in Iranian ethnic groups. *Journal of Genetic Resources,* 11(1), 1-11. doi: 10.22080/jgr.2025.28072.1410

Introduction

Human mtDNA is a double-stranded and circular molecule. It has a length of 16,569 bp and encodes 13 essential polypeptides for the oxidative phosphorylation system, 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs). In addition, mtDNA contains a noncoding part (control region) that regulates the expression of the mitochondrial genome (Boulet *et al.*, 1992). There are hypervariable regions in the control region of human mtDNA that not only represent mutational hotspots but also

reflect the distribution of ancient mutations among mtDNA lineages through recombination (Hasegawa *et al.*, 1993). Therefore, the control region of mtDNA is frequently used in population, phylogeographic, and phylogenetic studies (Samehsalari *et al.*, 2023; Vanichanukulyakit *et al.*, 2023). Generally, the mutation rate in mtDNA is about 5-15 times higher than nuclear DNA (Bai *et al.*, 2021; Bircan *et al.*, 2019), and each new mutation in mtDNA creates a new branch on the maternal family tree (Stewart and Chinnery; 2021; Wallace, 2016). Thus, family differentiation in mtDNA and variants in mtDNA are established and maintained in the population (Connel *et al.*, 2024; Samehsalari *et al.*, 2023).

Haplogroups are classes of specific genotypes that contain neutral polymorphisms throughout the mtDNA. Each mtDNA haplogroup contains specific polymorphisms that evolved from the same maternal ancestor (Ienco *et al.*, 2011; Arjmand *et al.*, 2017). The main application of mtDNA haplogroups is investigating populations' historical origins and migration (Arjmand *et al.*, 2017). Migration, population, bottlenecks, and genetic drift affect the mtDNA haplogroup distribution in the human population. The environment and selection also contributed to mtDNA genetic diversity distribution (Stewart and Chinnery, 2021). Recently, potential relationships have been reported in several studies between the mtDNA haplogroups and different phenotypes and diseases, such as osteoporosis (Catheline *et al.*, 2023), Parkinson's (Sena-dos-Santos *et al.*, 2024), and neuromuscular phenotype (Gao *et al.*, 2024). Accordingly, the highly informative tool of mtDNA has been used to investigate population relationships and track past demographics.

Generally, Iran can be seen as a staging area for the Neolithic Agricultural Revolution and the home to some of the earliest world empires. The Iranian population consists of different ethnic groups. The main ethnic groups in Iran are Persians, Lurs, Arabs, Turkish, Kurds, Turkmen, Baluchis, Gilak, Mazandaranis, Armenians, Assyrians, and Zoroastrians (Shasttiri *et al.*, 2022). These ethnic groups are great sources for genetic studies, especially in mtDNA. Previous studies reported that Iranian populations show high mtDNA diversity, comparable to mtDNA diversity in the Caucasus, Anatolia, and Europe (Derenko *et al.*, 2013). In this way, it is also reported that the Iranian Plateau is an important source and recipient of mitochondrial gene flow between culturally, linguistically, and genetically distinct populations (Derenko *et al.*, 2013). In addition, another study reported high haplogroup diversity and mixing by exotic groups in mtDNA HVR-II of the Iranian population (Shasttiri *et al.*, 2022). Since there is still no comprehensive report on the HVR-I mtDNA region in Iranian populations, the objective of the present study was to investigate the frequency of mtDNA haplogroups and the population genetic parameters in this region of mtDNA in the Iranian subpopulation, including Fars, Lur, Arab, Kurd, Azeri, Gilak, Bandari, Jewish, Armani, and one G6PD group.

Materials and Methods

Ethical considerations

This study was approved and monitored by the National Institute of Genetic Engineering and Biotechnology Ethics Committee (IR.NIGEB.EC.1398.12.3.E.). All methods follow the instructions and guidelines. All participants, none of whom was under 18 years of age, gave written informed consent to participate in the study and to use their anonymous data for further analysis and reporting.

Samples collection

In this study, 12 different Iranian ethnic groups and a group with glucose-6-phosphate dehydrogenase (G6PD) deficiency were studied. A total of 357 people were surveyed. The number of samples by ethnic group is presented in Table 1. This research was similar to previous studies in terms of sample collection, extraction of DNA, primers used, PCR amplification, and DNA sequencing of the HVRI region of mtDNA (Najmabadi *et al.*, 2001; Akbari *et al.*, 2008; Rezaee *et al.*, 2012).

DNA extraction and amplification of HVR-I

According to the manufacturer's instructions, total DNA was isolated from blood samples by QIAamp DNA mini kit (Qiagen, Hilden, Germany). The extracted DNA was evaluated for its quality and purity using a NanoDrop 2000

spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The forward primer of PF, 5′-ATC ATT GGA CAA GTA GCA TC-3′ (791-810 bp) and reverse primer of PR, 5′-GAG CTG CAT TGC TGC GTG CT-3′ (780- 761 bp) (Accession No NC_0122920.1) were used to amplify mtDNA. TEMPase Hot Start 2× Master Mix A BLUE (Ampliqon, Odense, Denmark) was used as reaction space to amplify mtDNA. The final reaction volume (50 µL) included 100 ng of DNA, 10 pmol of each primer, 23.2 µL RNase-free water, and 25 µL of TEMPase 2 × Master Mix. The amplification cycle included pre-PCR incubation at 95 °C for 15 min (primary denaturation), 35 cycles of denaturation at 95 °C for 20 s, annealing at 60 °C for 45 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. Confirmation of the specific amplification fragment was done using 1.5% agarose gel electrophoresis. Then, the amplified fragment was sequenced by the chain termination method (Bioneer, South Korea). The Codon Code Aligner 6.0.2 program (Codon Code, Centerville, MA, USA, https://www.codoncode.com/aligner/new60.htm) was used to analyze sequencing results. The BLAST sequence analysis tool compared the sequences to the revised Cambridge Reference Sequence (Accession No NC_012920.1).

Genetic analysis

The present study used BioEdit software 7.0.9.0 to edit 400 bp of the HVRI region (https://bioedit.software.informer.com/7.2/) (Hal, 1999). To assess under study population sequences GenBank mtDNA data from the East Asian (DQ826448, DQ834253-DQ834261, EF446784, EF488201, DQ418488, DQ437577, DQ462232-DQ462234, and DQ519035) and African (EF184580-EF184641 and FJ236978-FJ236983), and European (DQ523619-DQ523681 and AY339402-AY339593) populations were investigated. The CLUSTALW package was used to sequence alignment with a reference sequence (Thompson *et al.*, 1994). The haplogroups assignment included the mtDNA sequence alignment with reference sequence that contains variant sets classified into corresponding worldwide consensus-based haplogroups. This resulting alignment was then cross-referenced with an open-access phylogenetic tree. For this purpose, the DNASP 5.10 software was applied to determine haplotype and nucleotide diversity in subpopulations and excluded polymorphic sites in sequences from the subsequent analyses (Librado *et al.*, 2009). In addition, similar sequences were treated as the same haplotype. The obtained haplotypes were deposited at the GenBank under accession numbers MZ416977 to MZ417227. PhyloTree (http://www.phylotree.org) (Van, 2015) and HaploGrep 2 (v2.1.19) (https://haplogrep.uibk.ac.at) (Weissensteiner *et al.*, 2016) were used to evaluate the allelic variants and elucidate the subpopulation haplogroups, respectively.

Results

Haplogroups and genetic diversity

The descriptive statistics of detected haplogroups, haplotype diversity, and nucleotide diversity in different ethnic groups are presented in Table 1. A total of 357 samples were examined, and 316 haplotypes were identified. The Fars group had the highest number of samples (114) and haplotypes (97). In addition, the lowest number of samples and haplotypes belonged to the Baluch group, with 13 samples and 11 haplotypes.

In total, 139 special haplogroups were detected in the HVR-I region of mtDNA in the Iranian population. H2a2a1 was the most frequent haplogroup. The J1b and H2 (T152C, T16311C) haplogroups were next with 23 and 16 replicates, respectively. Haplogroup H2a2a1 was observed in all subpopulations. Furthermore, haplogroup J1b was observed in all subpopulations except Azari. Seventy haplogroups were observed in only one individual, and 24 haplogroups in only two individuals. Haplotype diversity was 1 for all ethnic groups. The highest observed nucleotide diversity belonged to the Gilak group (0.0228), and the lowest observed nucleotide diversity belonged to the Balouch group (0.0121).

Macro-haplogroups identification

The identified macro-haplogroups and their origins are presented in Table 2. This study identified 20 macro-haplogroups, of which 4, 6, and 10 macro-haplogroups originated in Africa,

Asia, and Europe, respectively. Most macro-haplogroups originated from Europe in all subgroups and the overall population. The identified macro-haplogroups have the least similarity to the African population. The pie chart for identified macro-haplogroups is shown in Fig. 1. The most observed macro-haplogroups in the whole population were H (151), HV (60), T (42), N (29), and U (27), respectively.

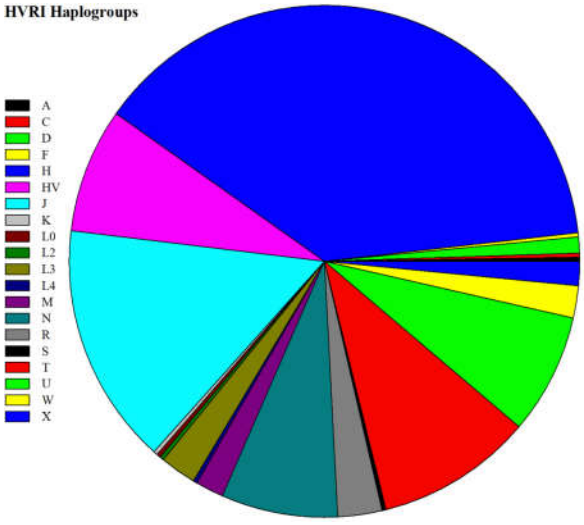

Fig. 1. Pie chart for identified macro-haplogroups.

Genetic differentiation

The differences between subpopulations and pairwise fixation indices (Fst) for comparison of subpopulations are presented in Table 3. A total of 78 comparisons were made between subpopulations, of which 21 comparisons showed significant differences between subpopulations. The Gilak and G6PD subpopulations were not significantly different from other subpopulations. The Pars subpopulation showed the most significant difference from other subpopulations. F_{ST} is a criterion for genetic differentiation. Genetic differentiation is very high for F_{ST}> 0.25, high for 0.15< F_{ST} <0.25, moderate for 0.05< F_{ST} <0.15, and low for F_{ST}<0.05 (Govindaraju, 1989).

In the present study, Fst values for pairwise comparison between subpopulations were obtained as 23, 17, 17, and 21 comparisons, which showed very high, high, medium, and low genetic differentiation, respectively. Table 4 presents the exact matching between the Iranian HVR-I haplogroup in the present study and ancient DNA sequences.

Discussion

In the present study, the Gilak and Balouch groups showed the highest and the lowest observed nucleotide diversity, respectively. The highest and lowest number of haplotypes were identified in the Pars and Baluch, respectively. The number of haplotypes was high in all studied groups. This indicates that there is a high genetic diversity in these ethnic groups. Previous studies have also reported high genetic diversity in the mtDNA of the Iranian population (Amjadi *et al.*, 2024; Derenko *et al.*, 2013; Shasttiri *et al.*, 2022). The range of nucleotide diversity was observed from a high (0.0292) in the Iranian Azeris to a low (0.0037) in the Dravidian group (Amjadi *et al.*, 2024). In addition, from 3840 Eurasian individuals, 2505 distinct haplotypes were identified, from which 1119 haplotypes were observed within the Iranian populations. Low haplotype diversity was found for Iranian Zoroastrians, Jews, and Baloch groups (Amjadi *et al.*, 2024).

In this study, the H2a2a1 haplogroup was the most abundant in the Iranian population. The H2a2a1 haplogroup has been reported to show a potential protective effect against Leber's hereditary optic neuropathy (LHON) (Qiao *et al.*, 2015; Peverelli *et al.*, 2021). LHON is a mitochondrial dysfunction, often in a homoplasmic state, caused by missense point mutations in mtDNA (Yu *et al.*, 2011). In addition, another study reported that variants in high-altitude pulmonary edema (HAPE) subjects were mainly associated with H2a2a1 (Sharma *et al.*, 2021). It has been suggested that mutations in the mitochondrial NADH-ubiquinone oxidoreductase chain 1 (ND1) gene play an important role in the development of HAPE (Engebretsen *et al.*, 2007).

Haplogroup J1b is another frequent haplogroup in the Iranian population. The haplogroup J1b is a branch of the human maternal tree that originated between 12,800 and 1,700 years ago (Behar *et al.*, 2012).

The haplogroup J1b is abundant in the Mediterranean and southern Atlantic regions. In the present study, haplogroup J1b was found in

most ethnic groups of Iran in different geographical regions, and it seems that the J1b haplogroup is widespread throughout Iran. A previous study reported that the frequency of this haplogroup is higher in northern Iran (Terreros *et al.*, 2011), which does not correspond to the current study's findings. Haplogroup H2 was the third most abundant haplogroup in this study. This haplogroup originated about 10,500 to 13,000 years ago (Behar *et al.*, 2012).

Haplogroup H2 is one of the most abundant haplogroups of macro-haplogroup H. It most likely originated in the Caucasus or Eastern Europe (Pereira *et al.*, 2005). Several studies have reported the association of the H1 mitochondrial haplogroup with proliferative diabetic retinopathy (PDR) (Estopinal *et al.,* 2014; Sarhangi *et al.*, 2017). However, in another study, the possibility of association between them was ruled out (Liu *et al.*, 2019). Mutations in the mitochondrial genes such as UUR, UCP2, and Mn-SOD genes play an important role in the PDR (Sarhangi *et al.*, 2017).

In this study, the identified haplogroups belonged to 20 macro-haplogroups. Macro-haplogroups H and J were the only haplogroups observed in all studied subpopulations. Among different subpopulations, 73 to 100% of macro-haplogroups were of West Eurasian origin. The Balouch and Bandari subpopulations revealed West Eurasian-origin macro-haplogroups' highest and lowest percentages, respectively. Asian macro-haplogroups were observed in all Iranian subpopulations except Arab, Baluch, and Lur. However, the frequency of Asian macro-haplogroups was low in different Iranian subpopulations. N haplogroup showed the highest frequency among Asian macro-haplogroups. In a similar study in Iranian populations, most haplogroups identified in HVR-I were predominantly of West Eurasian origin, namely H, HV, I, J, K, N, T, U, V, and W. In contrast, haplogroups associated with East Eurasia (A, C, D, F, G, and Z), South Asia (M), and sub-Saharan Africa (L1, L2, and L3) were restricted to specific and smaller ethnic subsets (Farjadian *et al.*, 2011). An approximately similar result was found for the frequency distribution of macro-haplotypes in Iranian subpopulations (Merheb *et al.*, 2019). In all subpopulations, the greatest and lowest number of macro-haplogroups revealed European and African origins (Merheb *et al.*, 2019; Bahmanimehr *et al.,* 2015) observed the highest frequencies of West Eurasian lineages, such as H, J, and U, within the Iranian populations. However, this prominence was not revealed in the Baloch and Zoroastrian ethnic groups. Furthermore, mtDNA haplogroups H, N, HV, J1, T, U7, and M were the most abundant haplogroups in Iranian communities, except for Zoroastrian ethnicity (Amjadi *et al.*, 2024). Haplogroup H is the most dominant mtDNA lineage in Europe and the Near East. Still, coalescent age estimates showed that this haplogroup in the Near East is significantly older than in Europe (23–28 vs. 19–21 kilo years ago, respectively) (Richards *et al.*, 2000). Given the presence of the oldest haplogroup H clade in the southern Caucasus and the northern part of the Near East, it has been suggested that the initial expansion of haplogroup H may have occurred in these regions (Roostalu *et al.*, 2007).

The global distribution of macro-haplogroups N and M suggests that both N and M macro-haplogroups may have evolved outside Africa after the mass migration of prehistoric humans out of Africa (Olivieri *et al.*, 2006). The oldest types of macro-haplogroups N are observed in Australia and Asia (Gounder Palanichamy *et al.*, 2004).

The various lineages of macro-haplogroup N in India show the least association with language, geographical region, and ethnicity (Maji *et al.*, 2008). The Bandari subpopulation had the highest percentage of African macro-haplogroups (12%). African haplogroups were found only in Arab, Bandari, Johoud, Lur and Pars subpopulations with low frequency. The L haplogroup is known as the African macro-haplogroup.

Table 1. The descriptive statistics of detected haplogroups, haplotype diversity and nucleotide diversity in different ethnic groups.

Ethnic groups	Geographical location	N*	K**	Haplogroups (Number of Samples)	Haplotype diversity	Nucleotide diversity
Arab	Khuzestan (Ahvaz)	19	15	H2(T152C T16311C) (1), H2a2a1 (3), H2a2a1d (2), H2a3 (1), H6 (1), J1b (3), J1b2a (1), L3e1b (1), R0a1a (1), R0a2c (1), U7a4a (3), X2f1 (1)	1 ± 0.024	0.02202 ± 0.00300
Armani	Tehran (Aramaneh center)	17	15	H103 (1), H2 (T152C T16311C) (2), H20a (1), H21 (1), H2a2a1 (1), H2a2a1d (1), H2a2a1g (1), H2a2a1 (1), J1b (1), J1b5 (1), J1d1 (1), N1a1a1 (1), N1b1 (2), U5a1d2b (1), X (1)	1 ± 0.024	0.01964 ± 0.00252
Ashouri	Tehran (Ashouri center)	20	17	H1z1(1), H2a2a1 (4), H6a1b4 (1), H7c4 (1), H7f (1), HV1 (1), HV12a1 (1), J1b (2), J1b4a (1), J1c1c (2), N (1), T1(2), T2b (1), X (1)	1 ± 0.020	0.02023 ± 0.00202
Azari	East Azerbaijan (Tabriz)	23	21	H2(T152C T16311C) (1), H2a2(A16235G) (1), H2a2a1 (6), H3h1 (1), H5(T16311C) (1), HV2a1(1), HV4b (1), HV9b (1), J1d1 (1), M3 (1), N7 (1), R0a (1), T1(1), T2b2b (2), U7(1), U7a4a (1), W6 (1)	1 ± 0.015	0.02069 ± 0.00261
Balouch	Sistan and Baluchestan (Balochistan)	13	11	H20 (1), H2a2a1 (6), H2a3 (1), HV0 (1), HV2 (1), HV2a1 (1), J1b (1), T1(1)	1 ± 0.039	0.01209 ± 0.00264
Bandari	Bushehr (Bushehr)	33	30	A2((C64T) T16111C) (1), H(G16129A) (1), H1cf (1), H2a1i (1), H2a2a1 (2), H2a2a1c (2), H2a2a1g (4), H6 (1), H76a (1), HV0 (1), J1b (1), J1b3 (1), J1c3j (1), L0a2 (1), L2a1a3c (1), L3'4 (1), L3f1b1a (1), M3 (1), N7 (3), R7 (1), T1 (3), U2a (2), W6 (1)	1 ± 0.009	0.02226 ± 0.00178
Gilak	Gilan (Rasht)	23	20	C4a1 (1), H(G16129A) (1), H1t1a1 (1), H2(T152C T16311C) (1), H2a2a1(3), H6(1), HV2(2), HV6(1), HV9b (1), J1(C16193T) (1), J1b (3), J1b5 (1), N1a3a (1), N1b1 (1), N7 (1), U5a (1), U7a4a (2)	1 ± 0.016	0.02279 ± 0.00488
Johoud	Tehran (Johoud center)	40	35	H1(C16278T) (1), H101 (1), H13a1d (1), H14a (2), H2(T152C T16311C) (2), H2a2a1 (7), H2a2a1c (2), H42a (1), H6a1b1 (1), H76a (1), H7a1 (1), HV1 (1), HV1a1 (1), HV2 (1), HV9b (1), J (2), J1b (1), J1b9 (2), J1c(C16261T) (1), L3'4 (1), L3a (1), M4a (1), N1b1a8a (1), N2a (1), T2g1a1 (3), U5a1d2b (1), X2i (1)	1 ± 0.007	0.02030 ± 0.00179
Kord	Kurdistan (Sanandij)	25	25	D4c (1), D4o2a (1), H13a1c (1), H2(T152C T16311C) (3), H2a2a1 (3), H5e (1), HV1a1 (1), HV1a3 (1), HV2a1 (1), J1b (3), J1b3 (1), J1c2m (1), K1a24a (1), T1 (1), T1b (1), T2b (1), U5a1(T16192C) (1), U7a4a (1), W4d (1)	1 ± 0.011	0.01979 ± 0.00166
Lur	Lorestan (Khorramabad) and Chaharmahal and Bakhtiari (Shahr-e Kord)	22	19	H(G16129A) (3), H2(T152C T16311C) (2), H2a2a1 (1), H8(C114T) (1), HV12b1 (1), HV1a3 (1), HV22 (1), J1b (2), J1b1b1c (2), J1b3 (1), J1b9 (1), J1c(C16261T) (1), L4 (1), R6(G16129A) (1), U1b2 (1), U5a1(T16192C) (1), U7a4a1b (1)	1 ± 0.017	0.02332 ± 0.00307
Mazani	Mazandaran (Sari)	24	23	H(G16129A) (1), H21 (1), H2a2a1 (1), H2a2a1c (1), H2a3 (1), H7h (1), HV12b1 (1), J1b (2), J1b1a1 (2), J1c2m (3), N2a (1), N7 (1), T2a1b (1), T2b (1), T2b2b (3), T2c1 (1), W3a2 (1), X (1)	1 ± 0.013	0.01770 ± 0.00160
Pars	Esfahan (Isfahan), Kerman (Kerman), Razavi Khorasan (Mashhad), Fars (Shiraz), Yazd (Yazd)	114	97	D4j8 (2), F1a1 (1), H1(C16239T) (1), H1(C16278T) (3), H1(C16355T) (1), H10(T16093C) (1), H101 (2), H107 (1), H10a1 (1), H11a (1), H13a1d (2), H14a (1), H1ab1 (1), H2(T152C T16311C) (3), H2a2a1 (13), H2a2a1g (1), H42a (2), H5 (1), H6 (1), H76a (1), H7a2 (1), H7h (1), H8(C114T) (1), HV0 (1), HV12b1 (1), HV15 (1), HV6 (1), HV9b (3), J1(C16193T) (2), J1b (2), J1b1a1 (1), J1b4a (1), J1b7a (1), J1d2a (1), L3'4 (1), L3a (1), L3d1a1'2 (1), L3f (1), M3 (1), M33a2 (1), M4a (1), M81 (1), N (2), N1a1a (1), N1a3a (1), N1b1 (6), N1b1a8a (1), N7 (1), R0a (2), R0a2n (2), R6 (1), R6b (1), S1 (1), T1 (4), T2b (1), T2b2b (4), T2g1a1 (1), T2h2 (1), T2i2 (4), U5a1(T16192C) (1), U5a1d2b (2), U5b1(T16189C T16192C) (1), U7 (1), U7a4 (1), U7a4a (3), W1c1 (1), W4d (1), W6 (2), X2i (1)	1 ± 0.001	0.02034 ± 0.00107
G6PD	Random samples ***	20	18	H101 (1), H2(T152C T16311C) (1), H2a2a1 (2), H2a2a1d (1), H2a3 (1), H7f (1), J (1), J1b (2), J1b1a1 (1), J1b5 (1), N10 (1), N2a (1), T1 (1), T2b2b (1), U5 (2), U7a4 (2)	1 ± 0.019	0.02024 ± 0.00193
Total		357	316		0.9941 ± 0.0015	0.01906 ± 0.00058

*N= Number of samples, **K= Number of haplotypes, *** Random samples= Random samples from different populations in the National Institute of Genetic Engineering and Biotechnology Clinic in Tehran

Table 2. The identified macro-haplogroups and their origins.

Ethnic groups	Africa					Asia							Europe										
	L0	L2	L3	L4	Total (%)	A	C	D	F	M	N	Total (%)	H	HV	J	K	R	S	T	U	W	X	Total (%)
Arab			1		1 (5)								8		4		2			3		1	18 (95)
Armani											3	3 (18)	9		3					1		1	14 (82)
Ashouri											1	1 (5)	8	2	5				3			1	19 (95)
Azari										1	1	2 (9)	10	3	1		1		3	2	1		21 (91)
Balouch													8	3	1				1				13 (100)
Bandari	1	1	2		4 (12)	1				1	3	5 (15)	13	1	3		1		3	2	1		24 (73)
Gilak							1				3	4 (17)	7	4	5					3			19 (83)
Johoud			2		2 (5)					1	2	3 (7.5)	20	4	6				3	1	1		35 (87.5)
Kord								2				2 (8)	8	3	5	1			3	2	1		23 (92)
Lur				1	1 (4.5)								7	3	7		1		3				21 (95.5)
Mazani											2	2 (8)	6	1	7				6		1	1	22 (92)
Pars			4		4 (3.5)			2	1	4	12	19 (16.7)	40	7	8		6	1	15	9	4	1	91 (79.8)
G6pd											2	2 (10)	7		5				2	4			18 (90)
Overall	1	1	9	1	12 (3)	1	1	4	1	7	29	43 (11)	151	31	60	1	11	1	42	27	9	5	338 (86)

Table 3. Difference (above diagonal) and Fst values (below diagonal) for subpopulation comparisons.

	Arab	Armani	Ashouri	Azari	Balouch	Bandari	Gilak	Johoud	Kord	Lur	Mazani	Pars	G6PD
Arab		-	-	-	+	-	-	-	-	-	-	-	-
Armani	0.180		-	+	+	-	-	-	-	-	+	-	-
Ashouri	0.180	0.081		-	-	+	-	-	-	-	-	+	-
Azari	0.081	0.036	0.198		-	+	-	-	-	+	+	-	-
Balouch	0.009	0.009	0.207	0.126		+	-	-	+	+	+	+	-
Bandari	0.153	0.522	0.009	0.000	0.000		-	+	-	+	-	+	-
Gilak	0.486	0.324	0.432	0.540	0.207	0.054		-	-	-	-	-	-
Johoud	0.144	0.090	0.189	0.117	0.081	0.000	0.757		-	-	-	+	-
Kord	0.261	0.315	0.631	0.595	0.027	0.072	0.568	0.477		-	-	+	-
Lur	0.495	0.162	0.081	0.009	0.000	0.027	0.027	0.162	0.297		-	+	-
Mazani	0.342	0.045	0.180	0.027	0.018	0.189	0.243	0.135	0.459	0.099		+	-
Pars	0.198	0.063	0.009	0.099	0.009	0.009	0.081	0.000	0.027	0.018	0.045		-
G6PD	0.414	0.180	0.802	0.450	0.180	0.063	0.910	0.270	0.694	0.162	0.685	0.189	-

Pluses (+) and minuses (−) display the existence and non-existence of significant differences amongst subpopulations (above diagonal) (P value < 0.05).

Table 4. Exact matching between Iranian HVRI haplogroup in the present study and ancient DNA sequences.

Place	Haplogroup	Macro-haplogroup	Culture_Grouping	Location	Reference
South and Central Asia	H	H	Ganj_Dareh_N	Ganj Dareh	Achille *et al.*, 2204
South and Central Asia	H2a	H	Ganj_Dareh_N	Ganj Dareh	Achille *et al.*, 2204
Near East	H29, H40a	H	Ganj_Dareh_Iran_Neolithic	Ganj Dareh	Akbari *et al.*, 2008
Near East	HV	HV	Ganj_Dareh_Iran_recent	Ganj Dareh	Akbari *et al.*, 2008
South and Central Asia	HV	HV	Ganj_Dareh_N	Ganj Dareh	Achille *et al.*, 2204
Mazandaran, Iran	HV, HV2	HV	Achaemenid Period	Gohar tepeh, Mazandaran	Kivisild, 2019
Mazandaran, Iran	I1	I	Achaemenid Period	Gohar tepeh, Mazandaran	Kivisild, 2019
South and Central Asia	J1, J1c10, J1d, J1d6	J	Hajji Firuz_C, Hajji Firuz_IA	Hajji Firuz	Achille *et al.*, 2204
South and Central Asia	K1a12a, K1a17a	K	Hajji Firuz_C, Hajji Firuz_BA	Hajji Firuz	Achille *et al.*, 2204
Near East	K1a20	K	Iran_HotuIIb	Hotu Cave	Achille *et al.*, 2204
Near East	K1a20, K1a3, K1b1a, K2a	K	Iran_Late_Neolithic, Iran_Chalcolithic	Seh Gabi	Achille *et al.*, 2204
Near East	L3f2b	L	Iran_Chalcolithic	Seh Gabi	Akbari *et al.*, 2008
Near East	M30b, M33a2a	M	Iran_Chalcolithic	Seh Gabi	Akbari *et al.*, 2008
South and Central Asia	N1a3a	N	Shahr_I_Sokhta_BA	Seistan, Shahr-i Sokhta	Achille *et al.*, 2204
South and Central Asia	R, R2, R30b, R5a2b, R7	R	Shahr_I_Sokhta_BA	Seistan, Shahr-i Sokhta	Achille *et al.*, 2204
South and Central Asia	T1a3, T2c, T2d2, T2h2	T	Shahr_I_Sokhta_BA	Seistan, Shahr-i Sokhta	Achille *et al.*, 2204
South and Central Asia	U, U1a1, U1a1a, U1a1c1, U1a1c1d, U1a4, U1a'c	U	Shahr_I_Sokhta_BA	Seistan, Shahr-i Sokhta	Achille *et al.*, 2204
Eastern Fertile Crescent	U2c1, U3a'c, U5b2	U		Central Zagros	Arjmand *et al.*, 2017
Eastern Fertile Crescent	U7	U		Northen Zagros	Arjmand *et al.*, 2017
South and Central Asia	U7	U	Tepe_Hissar_C	Tepe Hissar	Achille *et al.*, 2204
South and Central Asia	W, W3, W6	W	Tepe_Hissar_C	Tepe Hissar	Achille *et al.*, 2204
South and Central Asia	X2	X	Tepe_Hissar_C	Tepe Hissar	Achille *et al.*, 2204

The frequency of this macro-haplogroup is high in the African population. In some South African countries, the frequency of L haplogroups reaches 100% (Rosa *et al.*, 2004). African macro-haplogroups also have low to moderate frequencies in West Asia. The relative frequency of H haplogroups was 3% in the total population of Iran. Previous studies have reported similar lineage composition of mtDNA in Iranian subpopulations, consisting mainly of the Western Eurasia component, which accounts for about 90% of the total sample, and very limited contribution to Eastern Eurasia, South Asia, and Africa (Amjadi *et al.*, 2024; Derenko *et al.*, 2013; Terreros *et al.*, 2011; Metspalu *et al.*, 2004; Quintana-Murci *et al.*, 2004). The pairwise genetic distances (F_{ST}) were estimated using the mtDNA sequence of HVR-I. The Kurd and Gilak subpopulations showed very high genetic differentiation compared to seven others. It can be concluded that these two subpopulations differ from others in terms of mitochondrial DNA, which shows that the maternal line of these two subpopulations is very different from other Iranian races. High genetic differentiation was observed between group G6PD and Iranian subpopulations. The DNA sequence under investigation may be related to the development of this disease. (Amjadi *et al.* 2024) reported a low and no significant F_{ST} among the Iranian urban populations with the Persian language (Tehran, Isfahan, and Shiraz). In addition, negligible genetic distance was observed between the ethnic groups of Lur and Bakhtiari (Amjadi *et al.*, 2024). While mtDNA is a valuable tool for population genetic studies, researchers should be aware of its limitations and consider using other genetic markers, such as nuclear or Y-chromosome DNA, to complement their analyses. The HVR-I data alone often does not contain sufficient information to ensure the confident assignment of haplogroup affiliation. By combining different genetic markers, researchers can better understand the population's genetic history and diversity.

Conclusion

This study investigated the haplogroups in the HVR-I region of mitochondrial DNA in the Iranian population and population genetics and archaeological studies in these populations. The majority of identified haplogroups were of European origin. High diversity of mitochondrial DNA was observed among Iranian subpopulations. The high diversity of haplogroups and HVR-I DNA indicates that Iran has been a destination for people of different races and has undergone several mutations. Further studies can identify the probable association between the DNA of the HVR-I region and diseases or important traits used for the specific prevention and treatment of diseases.

Conflict of Interest

The authors declare no conflict of interest.

References

Akbari, M. T., Izadi, P., Izadyar, M., Kyriacou, K., & Kleanthous, M. (2008). Molecular basis of thalassemia intermedia in Iran. *Hemoglobin*, 32(5), 462-470. https://doi.org/10.1080/03630260802341851

Amjadi, M., Hayatmehr, Z., Egyed, B., Tavallaei, M., & Szécsényi-Nagy, A. (2024). A comprehensive review of HVS-I mitochondrial DNA variation of 19 Iranian populations. *Annals of Human Genetics*, 88(3), 259-277. https://doi.org/10.1111/ahg.12544

Arjmand, S., Khaledi, N., Fayazmilani, R., Lotfi, A. S., & Tavana, H. (2017). Association of mitochondrial DNA haplogroups with elite athletic status in Iranian population. *Meta Gene*, 11, 81-84. https://doi.org/10.1016/j.mgene.2016.11.009

Bahmanimehr, A., Eskandari, G., & Nikmanesh, F. (2015). The congruence between matrilineal genetic (mtDNA) and geographic diversity of Iranians and the territorial populations. *Iranian Journal of Basic Medical Sciences*, 18(1), 30-37. https://doi.org/10.22038/IJBMS.2015.3885

Bai, R., Cui, H., Devaney, J. M., Allis, K. M., Balog, A. M., Liu, X., ... & Suchy, S. F. (2021). Interference of nuclear mitochondrial DNA segments in mitochondrial DNA testing resembles biparental transmission of mitochondrial DNA in humans. *Genetics in Medicine*, 23(8), 1514-1521. https://doi.org/10.1038/s41436-021-01166-1

Behar, D. M., Van Oven, M., Rosset, S., Metspalu, M., Loogväli, E. L., Silva, N. M.,

... & Villems, R. (2012). A "Copernican" reassessment of the human mitochondrial DNA tree from its root. *The American Journal of Human Genetics*, 90(4), 675-684. https://doi.org/10.1016/j.ajhg.2012.03.002

Bircan, R., Gözü, H. I., Ulu, E., Sarıkaya, Ş., Gül, A. E., Şirin, D. Y., Özçelik, S., & Aral, C. (2019). The mitochondrial DNA control region might have useful diagnostic and prognostic biomarkers for thyroid tumors. *Experimental and Clinical Endocrinology & Diabetes*, 127(07), 423-436. https://doi.org/10.1055/a-0869-7355

Boulet, L., Karpati, G., & Shoubridge, E. A. (1992). Distribution and threshold expression of the tRNA (Lys) mutation in skeletal muscle of patients with myoclonic epilepsy and ragged-red fibers (MERRF). *American Journal of Human Genetics*, 51(6), 1187. https://www.cell.com/ajhg/home

Catheline, S. E., Kaiser, E., & Eliseev, R. A. (2023). Mitochondrial genetics and function as determinants of bone phenotype and aging. *Current Osteoporosis Reports*, 21(5), 540-551. https://doi.org/10.1007/s11914-023-00816-4

Derenko, M., Malyarchuk, B., Bahmanimehr, A., Denisova, G., Perkova, M., Farjadian, S., & Yepiskoposyan, L. (2013). Complete mitochondrial DNA diversity in Iranians. *PloS One*, 8(11), e80673. https://doi.org/10.1371/journal.pone.0080673

Engebretsen, B. J., Irwin, D., Valdez, M. E., O'Donovan, M. K., Tucker, A., & Van Patot, M. T. (2007). Acute hypobaric hypoxia (5486 m) induces greater pulmonary HIF-1 activation in hilltop compared to madison rats. *High Altitude Medicine & Biology*, 8(4), 312-321. https://doi.org/10.1089/ham.2007.1031

Estopinal, C. B., Chocron, I. M., Parks, M. B., Wade, E. A., Roberson, R. M., Burgess, L. G., ... & Samuels, D. C. (2014). Mitochondrial haplogroups are associated with severity of diabetic retinopathy. *Investigative Ophthalmology and Visual Science*, 55(9), 5589-5595. https://doi.org/10.1167/iovs.14-15149

Farjadian, S., Sazzini, M., Tofanelli, S., Castrì, L., Taglioli, L., Pettener, D., ... & Luiselli, D. (2011). Discordant patterns of mtDNA and ethno-linguistic variation in 14 Iranian Ethnic groups. *Human Heredity*, 72(2), 73-84. https://doi.org/10.1159/000330166

Gao, F., Schon, K. R., Vandrovcova, J., Wilson, L., Hanna, M. G., ICGNMD Consortium, ... & Horvath, R. (2024). Mitochondrial DNA disorders in neuromuscular diseases in diverse populations. *Annals of Clinical and Translational Neurology*. https://doi.org/10.1002/acn3.52141

Gounder Palanichamy, M., Sun, C., Agrawal, S., Bandelt, H. J., Kong, Q. P., Khan, F., ... & Zhang, Y. P. (2004). Phylogeny of mitochondrial DNA macrohaplogroup N in India, based on complete sequencing: implications for the peopling of South Asia. *The American Journal of Human Genetics*, 75(6), 966-978. https://doi.org/10.1086/425871

Govindaraju, D. R. (1989). Variation in gene flow levels among predominantly self-pollinated plants. *Journal of Evolutionary Biology*, 2(3), 173-181. https://doi.org/10.1046/j.1420-9101.1989.2030173.x

Hall, T. A. (1999, January). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *In Nucleic Acids Symposium Series,* 41, 95-98. https://doi.org/10.1002/acn3.52141

Hasegawa, M., Di Rienzo, A., Kocher, T. D., & Wilson, A. C. (1993). Toward a more accurate time scale for the human mitochondrial DNA tree. *Journal of Molecular Evolution*, 37, 347-354. https://doi.org/10.1007/BF00178865

Ienco, E. C., Simoncini, C., Orsucci, D., Petrucci, L., Filosto, M., Mancuso, M., & Siciliano, G. (2011). May "Mitochondrial Eve" and mitochondrial haplogroups play a role in neurodegeneration and Alzheimer′ s disease? *International Journal of Alzheimer's Disease*, 2011(1), 709061. https://doi.org/10.4061/2011/709061

Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451-1452. https://doi.org/10.1093/bioinformatics/btp187

Liu, E., Kaidonis, G., Gillies, M. C., Abhary, S., Essex, R. W., Chang, J. H., ... & Craig, J. E. (2019). Mitochondrial haplogroups are not associated with diabetic retinopathy in a large Australian and British Caucasian sample.

Scientific Reports, 9(1), 612. https://doi.org/10.1038/s41598-018-37388-8

Maji, S., Krithika, S., & Vasulu, T. S. (2008). Distribution of mitochondrial DNA macrohaplogroup N in India with special reference to haplogroup R and its sub-haplogroup U. *International Journal of Human Genetics*, 8(1-2), 85-96. https://doi.org/10.1080/09723757.2008.11886022

Merheb, M., Matar, R., Hodeify, R., Siddiqui, S. S., Vazhappilly, C. G., Marton, J., Azharuddin, S., & Al Zouabi, H. (2019). Mitochondrial DNA, a powerful tool to decipher ancient human civilization from domestication to music, and to uncover historical murder cases. *Cells*, 8(5), 433. https://doi.org/10.3390/cells8050433

Metspalu, M., Kivisild, T., Metspalu, E., Parik, J., Hudjashov, G., Kaldma, K., ... & Villems, R. (2004). Most of the extant mtDNA boundaries in south and southwest Asia were likely shaped during the initial settlement of Eurasia by anatomically modern humans. *BMC Genetics*, 5, 1-25. https://doi.org/10.1186/1471-2156-5-26

Najmabadi, H., Karimi-Nejad, R., Sahebjam, S., Pourfarzad, F., Teimourian, S., Sahebjam, F., Amirizadeh, N., & Karimi-Nejad, M. H. (2001). The β-thalassemia mutation spectrum in the Iranian population. *Hemoglobin*, 25(3), 285-296. https://doi.org/10.1081/HEM-100105221

Olivieri, A., Achilli, A., Pala, M., Battaglia, V., Fornarino, S., Al-Zahery, N., ... & Torroni, A. (2006). The mtDNA legacy of the Levantine early Upper Palaeolithic in Africa. *Science*, 314(5806), 1767-1770. https://doi.org/10.1126/science.1135566

Pereira, L., Richards, M., Goios, A., Alonso, A., Albarrán, C., Garcia, O., ... & Amorim, A. (2005). High-resolution mtDNA evidence for the late-glacial resettlement of Europe from an Iberian refugium. *Genome Research*, 15(1), 19-24. https://doi.org/10.1101/gr.3182305

Peverelli, L., Catania, A., Marchet, S., Ciasca, P., Cammarata, G., Melzi, L., ... & Lamperti, C. (2021). Leber's hereditary optic neuropathy: a report on novel mtDNA pathogenic variants. *Frontiers in Neurology*, 12, 657317. https://doi.org/10.3389/fneur.2021.657317

Qiao, C., Wei, T., Hu, B., Peng, C., Qiu, X., Wei, L., & Yan, M. (2015). Two families with Leber's hereditary optic neuropathy carrying G11778A and T14502C mutations with haplogroup H2a2a1 in mitochondrial DNA. *Molecular Medicine Reports*, 12(2), 3067-3072. https://doi.org/10.3892/mmr.2015.3714

Quintana-Murci, L., Chaix, R., Wells, R. S., Behar, D. M., Sayar, H., Scozzari, R., ... & McElreavey, K. (2004). Where west meets east: the complex mtDNA landscape of the southwest and Central Asian corridor. *The American Journal of Human Genetics*, 74(5), 827-845. https://doi.org/10.1086/383236

Rezaee, A. R., Banoei, M. M., Khalili, E., & Houshmand, M. (2012). Beta-thalassemia in Iran: new insight into the role of genetic admixture and migration. *The Scientific World Journal*, 1, 635183. https://doi.org/10.1100/2012/635183

Richards, M., Macaulay, V., Hickey, E., Vega, E., Sykes, B., Guida, V., ... & Bandelt, H. J. (2000). Tracing European founder lineages in the Near Eastern mtDNA pool. *The American Journal of Human Genetics*, 67(5), 1251-1276. https://doi.org/10.1016/S0002-9297(07)62954-1

Roostalu, U., Kutuev, I., Loogväli, E. L., Metspalu, E., Tambets, K., Reidla, M., ... & Villems, R. (2007). Origin and expansion of haplogroup H, the dominant human mitochondrial DNA lineage in West Eurasia: the Near Eastern and Caucasian perspective. *Molecular Biology and Evolution*, 24(2), 436-448. https://doi.org/10.1093/molbev/msl173

Rosa, A., Brehm, A., Kivisild, T., Metspalu, E., & Villems, R. (2004). MtDNA profile of West Africa Guineans: towards a better understanding of the Senegambia region. Annals of Human Genetics. *Annals of Human Genetics*, 68(4), 340-352. https://doi.org/10.1046/j.1529-8817.2004.00100.x

Samehsalari, S., Mohsenpour, K., & Chandrasekar, A. (2023). Identification of South Indian muslims by sequencing the control region of mitochondrial DNA. *Journal of Genetic Resources*, 9(2), 215-221. https://doi.org/10.22080/jgr.2023.25572.1361

Sarhangi, N., Khatami, F., Keshtkar, A., Heshmat, R., Atlasi, R., & Mohammadamoli,

M. (2017). The role of mitochondrial DNA (mtDNA) in the development of diabetic retinopathy (DR): A systematic review. *Med Hypothesis Discov Innov Ophthalmol*, 6(2), 30-38. https://mehdijournal.com/index.php/mehdiophthalmol

Sena-dos-Santos, C., Moura, D. D., Epifane-de-Assunção, M. C., Ribeiro-dos-Santos, Â., & Santos-Lobato, B. L. (2024). Mitochondrial DNA variants, haplogroups and risk of Parkinson's disease: a systematic review and meta-analysis. *Parkinsonism & Related Disorders*, 107044. https://doi.org/10.1016/j.parkreldis.2024.107044

Sharma, S., Singh, Y., Sandhir, R., Singh, S., Ganju, L., Kumar, B., & Varshney, R. (2021). Mitochondrial DNA mutations contribute to high altitude pulmonary edema via increased oxidative stress and metabolic reprogramming during hypobaric hypoxia. *Biochimica et Biophysica Acta Bioenergetics*, 1862(8), 148431. https://doi.org/10.1016/j.bbabio.2021.148431

Shasttiri, A., Moridi, M., Safari, A., Raza, S. H. A., Ghaderi-Zefrehei, M., Houshmand, M., Oryan, A., Sanati, M.H., Smith, J. & Amjadi, M. (2022). Following the Trace of HVS II Mitochondrial region within the nine Iranian ethnic groups based on genetic population analysis. *Biochemical Genetics*, 1-20. https://doi.org/10.1007/s10528-021-10141-z

Stewart, J. B., & Chinnery, P. F. (2021). Extreme heterogeneity of human mitochondrial DNA from organelles to populations. *Nature Reviews Genetics*, 22(2), 106-118. https://doi.org/10.1038/s41576-020-00284-x

Terreros, M. C., Rowold, D. J., Mirabal, S., & Herrera, R. J. (2011). Mitochondrial DNA and Y-chromosomal stratification in Iran: relationship between Iran and the Arabian Peninsula. *Journal of Human Genetics*, 56(3), 235-246. https://doi.org/10.1038/jhg.2010.174

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Van Oven, M. (2015). PhyloTree Build 17: Growing the human mitochondrial DNA tree. *Forensic Science International: Genetics Supplement Series*, *5*, e392-e394. https://doi.org/10.1016/j.fsigss.2015.09.155

Vanichanukulyakit, J., Khacha-Ananda, S., Monum, T., Mahawong, P., Moophayak, K., Penkhrue, W., ... & Thongsahuan, S. (2023). The analysis of genetic polymorphism on Mitochondrial hypervariable Region III in Thai population. *Genes*, 14(3), 682. https://doi.org/10.3390/genes14030682

Wallace, D. C. (2016). Mitochondrial DNA in evolution and disease. *Nature*, 535(7613), 498-500. https://doi.org/10.1038/nature18902

Weissensteiner, H., Pacher, D., Kloss-Brandstätter, A., Forer, L., Specht, G., Bandelt, H. J., Kronenberg, F., Salas, A., & Schönherr, S. (2016). HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Research*, 44(W1), W58-W63. https://doi.org/10.1093/nar/gkw233

Yu-Wai-Man, P., Griffiths, P. G., & Chinnery, P. F. (2011). Mitochondrial optic neuropathies–disease mechanisms and therapeutic strategies. *Progress in Retinal and Eye Research*, 30(2), 81-114. https://doi.org/10.1016/j.preteyeres.2010.11.002