

Investigation of the mitochondrial haplogroups M, BM, N, J, K and their frequencies in five regions in Iran

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Abstract

The frequencies of the Asian (M, BM) and European (N, J, K) mtDNA haplogroups in five major regions of Iran was investigated. Unexpectedly, the frequencies of the Asian haplogroups M and BM were low in Iran (2.34% for haplogroup M; 17.6% for haplogroup BM and 80.06% for haplogroup N). Almost identical frequencies for haplogroups J and K were found in the present study (10.81% and 10.14% for haplogroups J and K, respectively). On the other hand, the frequencies of haplogroups M and BM in Eastern regions were more than their frequencies in Western regions of the country. In contrast, the frequencies of haplogroups J and K in Western regions were more than their frequencies in Eastern regions of Iran. As a result, this study gives evidence for similarity between Iranian population ethnic groups and people from Northwest Asia and Southeast Europe. Our data suggest that Iranian tribes probably played a remarkable role in the formation of these ethnic groups. It gives the indication that the haplogroup J may be older than 6000-10000 years, and probably developed in Iran, and then expanded to different regions in Europe and Northwest Asia. On the other hand, it seems that the super-haplogroup M has developed after the inhabitants of Iran moved to Eastern Asia or this group migrated from Southern Iran/North of Arabian halve O to Pakistan and then to Asia.

Keywords: mtDNA, Mitochondrial, Haplogroup, Iran.

INTRODUCTION

Analysis of mitochondrial DNA (mtDNA) variations has permitted the reconstruction of the ancient migration of women. This has provided evidence that our

species arose in Africa about 150000 years before present (YBP), migrated out of Africa into Asia about 60000 to 70000 YBP and into Europe about 40000 to 50000 YBP, and migrated from Asia and possibly Europe to Americas about 20000 to 30000 YBP (Wallace *et al.*, 1999). Two aspects of the mitochondrial DNA make it particularly useful in human evolutionary studies. Firstly, contrast to the nuclear encoded gene, which shows Mendelian inheritance mtDNA has been shown to be maternally inherited (Giles *et al.*, 1980) and secondly, the mtDNA sequence evolution rate is much higher than that of average nuclear gene (Miyata *et al.*, 1982; Wallace *et al.*, 1987). Consequently, a substantial number of mtDNA mutations have accumulated sequentially along radiating maternal lineage that have diverged as human populations colonized different geographical regions of the world. Restriction fragment length polymorphism (RFLP) studies of mtDNAs from a wide range of human populations have revealed a number of stable polymorphic sites in the mtDNA coding regions. These define related groups of mtDNAs are called haplogroups. Most of the mutations observed in both mtDNA coding and control regions in modern human populations have occurred on these pre-existing haplogroups and define the individual mtDNA types or haplotypes (Torroni *et al.*, 1993a and Graven *et al.*, 1995).

The majority of haplogroups have been shown to be continent-specific. In Asia about 55% of East Asian and Siberian mtDNAs are members of super haplogroup M (Ballinger *et al.*, 1992; Torroni *et al.*, 1993b,c; Chen *et al.*, 1995 and Wallace, 1995). About 77% of Asian mtDNAs are encompassed within a super-haplogroup M defined by a *DdeI* site gain at 10394 bp and an *AluI* site gain at 10397 bp (Ballinger

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et al., 1992; Torroni *et al.*, 1993a, b; Chen *et al.*, 1995; Wallace, 1995). Essentially, in ancient time, the haplogroup has originated from haplogroup BM (*DdeI* +10394, *AluI* -10397) and N (*DdeI* -10394, and *AluI* -/+ 10397). Hence, the haplogroups BM are older than haplogroup M. In this study, we report the frequencies of Asian M, BM and European J, K haplogroups in different groups of Iranian populations.

MATERIALS AND METHODS

Samples: In present study, 256 individuals were analyzed for haplogroups M, BM and N; and 148 individuals for haplogroup J and K. According to their place of birth these samples were divided into five major regions; Northern provinces (Guilan, Mazandaran, Golestan, Ghazvin and Tehran), Western provinces (Western and Eastern Azerbaijan, Ardebil, Zanjan, Lorestan, Kordestan and Hamedan), Eastern provinces (Khorasan, Sistan, Baloochestan, Semnan and Kerman), Central provinces (Isfahan, Qom and Markazi) and Southern provinces (Khuzestan, Bushehr, Hormozgan and Fars). Informed consent forms were signed by all individuals selected for this study. All individuals were unrelated to each other according to their information's records and because of haplogroup J and K were studied by one of the student as part of his theses (Babrzadeh, 2003), the number individual in haplogroup J, K and M, BM, N were not equal.

Haplogroups M, BM and N: Altogether, 256 individuals from different regions of Iran Western (96), Eastern (28), Northern (81), Southern (19) and Central (32) were studied on the haplogroups M, BM and N (Table 1).

Haplogroups J and K: For haplogroups J and K 148 samples including 48, 20, 41, 17 and 22 individuals from West, East, North, South and Centre of Iran respectively, were analysed (Table 1).

MtDNA analysis: Total DNA from blood samples were extracted by using of standard protocols. Fresh samples were extracted as per salting out method and phenol-chloroform-isoamylalcohol extraction method (Sambrook-Russle, 2001).

The haplogroups J, M, BM, and N were typed using PCR-RFLP technique. To investigation of 16065 polymorphism, the primers ONPF98 (5'-ATC ATT GGA CAA GTA GCA TC-3'), and ONPR99 (5'-GTG GTC AAG TAT TTA TGG TA-3'), were used for amplification and *HinF* I restriction enzyme to identify the mutation. For 13704

polymorphism, primers ONPF204 (5'-CTC CAA AGA CCA CAT CAT CGA AAC-3'), and ONPR205 (5'-GCT GTG AGT TTT AGG TAG AGG GGG-3'), were used for amplification and *MvaI* restriction enzyme was used for identification of mutation. Primers ONPF93 (5'-TCT GGC CTA TGA GTG ACT AC-3') and ONPR94 (5'-GAC CGA TAT ACT AGT ATT CC-3') were used in conjunction with restriction enzyme *DdeI* and *AluI* to identify 10394 and 10937 polymorphism, respectively.

The total 50 µl of reaction mixture contained: 1X final concentration of PCR reaction buffer (Roche Molecular Biochemical Co. Ltd), 1.5 mM MgCl₂, 200 µM of each dNTP, 20 pmol of each primer, 1 unit of *Taq* polymerase (Roche Molecular Biochemical) and 500 ng of the DNA.

The reaction mixture underwent initial denaturation process at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 50-54°C for 60 sec, and 72°C for 60 sec. The final extension was performed at 72°C for 10 min. The PCR fragments were run in 2% agarose or 8-12% polyacrylamide gel and visualized by Etidium-bromide or silver staining.

RESULTS

The screening for Asian (M and BM) and European (N, J and K) mtDNA haplogroups in the five major regions viz., Eastern, Western, Northern, Southern, and Central of Iran was carried out. For analyzing haplogroups M, BM and N were selected 256 individuals from five major regions. Among these individuals, 2.34% belonged to haplogroup and 17.6% to BM, respectively. Among 148 individuals selected for screening of presence of European haplotypes, 80.06% belonged to N, 10.81% to J and 10.15% to K haplogroups.

We defined the frequency of haplogroups J, K, M, BM, and N in 5 major regions of Iran, including West, East, North, South and Centre of Iran (Table 1, Fig. 1). In the Eastern and Southern regions no appearance of M was found while a few number of the samples in the other regions found to have this haplogroup. The frequency of haplogroup J, K and N were very high, while the frequency of super-haplogroup M was very low as compared to other regions of Asia.

DISCUSSION

On the basis of previous study, about 77% of Asian mtDNAs come under a super haplogroup, called M (Ballinger *et al.*, 1992; Torroni *et al.*, 1993a, b; Chen *et al.*, 1995; Wallace, 1995). In ancient time, the hap-

Figure 1: Distribution of haplogroup M, BM, N, J and K in 5 Iranian regions.

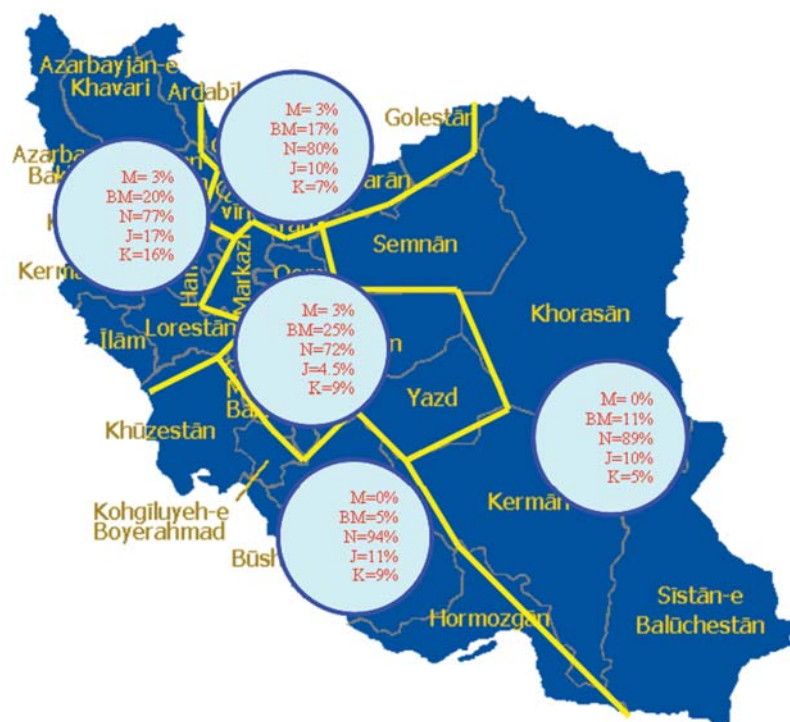


Table 1. Distribution of mtDNA haplogroups among the five different regions of Iran. S1= Number of sample for haplogroup M, BM, N; S2= Number of sample for haplogroup J, K; += Number of positive haplogroup; % = percentage of each haplogroups:

Iran Region	Haplogroup M, BM, N							Haplogroup J, K				
	S1	M+	M%	BM+	BM%	N+	N%	S2	J+	J%	K+	K%
Western	96	3	3.13	19	19.8	74	77.08	48	8	16.67	7	14.58
Eastern	28	0	0.00	3	10.7	25	89.28	20	2	10	1	5
Northern	81	2	2.47	14	17.3	65	80.24	41	4	9.8	3	7.32
Southern	19	0	0.00	1	5.26	18	94.73	17	1	5.9	2	11.76
Central	32	1	3.12	8	25.0	23	71.87	22	1	4.5	1	9.10
Total	256	6	2.34	45	17.6	205	80.06	148	16	10.81	14	9.46

logroup M is said to be originated from haplogroup BM, hence haplogroup BM being much older than haplogroup M. Since Iran is situated in Asian continent, we expect high frequency of Asian haplogroups M and BM before this research. But contradictory to that, our results represented low frequencies of these haplogroups.

On the other hand about 99% of European mtDNAs fall into one of nine haplogroups: H, I, J, K, T, U, V, W or X (Torroni *et al.*, 1994, 1996a and Hernstadt *et al.*, 2002). Six of the European haplogroups (H, I, J, K, T and W) are essentially confined to European population (Torroni *et al.*, 1994 and 1996a) and probably originated after Caucasoid become genetically separated

from the ancestors of the modern Africans and Asians. It is estimated that haplogroups H, J, K, T and V may be having relatively recent origin, i.e. 8000-30000 years ago (Torroni *et al.*, 1996a) and this supports the hypothesis that they originated after the genetic and geographical separation of the ancestral Caucasoid from the ancestors of modern Africans and Asians. On the other hand, haplogroup U appears to be much older than the others, with an estimated age of 51000-67000 years (Torroni *et al.*, 1996a), raising the possibility that it may have originated in Africa and subsequently expanded into Middle East and Europe.

Haplogroup J is found in about 11.3% of European mtDNAs and haplogroup K is observed in 9.1% of

European mtDNAs. Contrary to our initial assumption, the similar frequencies were found for these haplogroups in our samples (10.81% for haplogroup J and 9.46% for haplogroup K). Moreover, frequencies of J and K haplogroups in Western regions (16.67% for J and 14.58% for K haplogroup) were more than their frequencies in Eastern regions of Iran (10% for J and 5% for K haplogroup).

Haplogroup N shows the highest frequency all around Iran, whereas haplogroup M is found most in Western Iran with its minimum frequency in Eastern and Southern parts. According to Malyarchuk and his colleagues (2002), the frequency of haplogroup M from Western to Eastern region of Iran varies between 0-4% that means the overall frequency of M in the Iranian population is about 2%.

Historical documents approve these results. Since ancient times, the population living in Western regions of the Central Asia and Iranian upland played the key role in the inhabitation of Eastern European territories. It is suggested that one of the routes of the Homo sapiens penetration to Europe passed through the Caspian regions. Later during the Mesolithic, the Caspian regions and refuges at the South of Eastern Europe were the starting points for recolonization of the European territories (Stringer and Andrews, 1998; Soffer, 1987). During the Bronze age, cattle-breeder tribes from the Caspian regions settled in the European territories (Zvelebil *et al.*, 1980 and Alekseev and Gokhman, 1984). According to paleoanthropological data, during Scythian time (the early Iron age) Eastern European steppes were the territories of activity of tribes belonging to the Scythian confederation (Alekseev and Gokhman, 1984). Apparently, Iranian tribes played an important role at the first stages of Scythian ethnogeny. Later Scythians spread over wide territories of Europe, Central Asia and Southern Siberia, accumulating many ethnic components. The Iranian substrate also played a significant part in the formation of Eastern Slavs, specially, Ukrainians and Southern ethnic territorial groups of Russians. According to anthropological data, in early Middle ages Iranian-Slavic symbiosis (Chernyakhovskaya culture) was a typical feature of the population of East European steppes (Sedov *et al.*, 1995 and Alekseev, 1973). In view of this, it is likely that Iranian, or Iranianized tribes could have been affiliated with Eastern Slavs.

The data on biochemical and molecular genetics generally agree with the ideas of anthropologists, archaeologists, and linguists considered above. This is the pilot study for Iranian diversity in Iran and more studies with more haplogroups and more individuals

(1000-1500 individuals) need for correct pictures of Iranian diversity conjunction of Y chromosome polymorphisms.

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