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Distribution of Mitochondrial DNA Intergenic COII/tRNALYS 9 bp Deletion in Iranian Populations

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Abstract
A 9 bp deletion between cytochrome oxidase subunit II and tRNA Lys genes in mitochondrial DNA, has proven to be an extremely informative marker for tracing population history. Using the PCR-PAGE and PCR-SSCP techniques in this study, 152 DNA samples collected from Iranian populations were screened for the 9 bp deletion. No deletion was observed. A length polymorphism, most probably a 9 bp triplication, was detected in one case. This observation provides further evidence for an ancient migration from Africa to India through Saudi Arabia, Iraq, Iran and Pakistan. It also supports the hypothesis, which suggested diffusion of Iranian farmers to India after agricultural revolution. The existence of 9 bp deletions in a background of haplogroup I in Mediterranean and its absence in Iran, suggests that there is no eastward diffusion of this marker and therefore this haplotype would not be too old. The presence of 9 bp triplication supports its multiple independent origins.

Keywords: *Mitochondrial DNA, 9 bp Deletion, Iranian populations*

Introduction
Mitochondrial DNA (mtDNA) has been used in human population genetics for more than 20 years. This molecule has unique features: mtDNA is maternally inherited (1, 2); has no recombination (3-7); shows high rate of mutations (8) and has high copy number in cells (9). These features make mtDNA as a good tool for tracing population history. Analysis of different human mtDNAs led to "Out of Africa" hypothesis (10). According to this hypothesis, modern humans appeared first in Africa in 150,000 years ago and then have migrated to Europe and Asia (11).

Many studies have taken place to clarify the migration routes of early humans. These studies are based on polymorphic mtDNA markers (12). One of these markers is a 9 bp deletion between genes for cytochrom oxidase subunit II (COII) and tRNALYS (13). In this region, there are two copies of CCCCCTCAC in most populations (14). However, in certain populations one of the two copies is deleted and this deletion, which leads to length polymorphism of mtDNA, has been used for many yeas as a strong marker for tracing population history (11, 15).

The 9 bp deletion is frequent in Polynesia and Southeast Asia. It seems that this deletion has been fixed in some areas of Asia and Pacific. The frequency in America is remarkable and is rare in Europe. In Africa, a different type of 9 bp deletion has been reported which has no correlation with Asian type of deletion (15, 16). The existence of this marker in Mediterranean region and recently in north India, have motivated geneticists to screen this deletion in Middle East. In present study, distribution of this marker was investigated among different Iranian populations.

Materials and Methods
Iran was divided to different parts based on ethnic groups (Arabs, Balooches, Kords, Lors, Persians and Turks). Blood sampling of these groups was carried out randomly. DNA was extracted from blood using non-enzymatic salting out method. The interest region was amplified with PCR using appropriate primers and temperatures published elsewhere (16). PCR products with 9 bp deletion have 119 bp and without the deletion have 121 bp in length. PCR products were subjected to agarose gel electrophoresis to see how PCR worked. Then the products were run on polyacrylamide gel electrophoresis (PAGE) along with positive control and size markers. Those PCR products suspected to have polymorphisms other than 9 bp deletion were subjected to single-strand conformational polymorphism (SSCP) analysis (16).

Results
The total of 152 collected DNA sample, fit well into sample sizes range shown in table 1. No deletion was detected in all of the 152 samples. In one case, the length of PCR product was more than 121bp (the maximum expected length), which indicates another length polymorphism (Fig.1). Based on the size of this marker, it could be due to 9 bp triplication or 9 bp expansion which will be discussed later.

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Table1. Published reports on distribution of 9 bp deletion in different countries

	Country	Sample size	Frequency (%)	Reference	
1	New Zealand	30	100	Hertzberg M. *et al* 1989	16
2	Nepal	107	8	Passarino G. *et al* 1993	17
3	Hawaii	25	92	Lum JK. *et al* 1994	18
4	Italy	56	1.7	Torroni A. *et al* 1995	19
5	Pakistan	76	0	Melton T. *et al* 1995	20
6	Polynesia	1178	94	Sykes B. *et al* 1995	21
		150	93	Hertzberg M. *et al* 1989	16
7	Philippine	176	40	Melton T. *et al* 1995	20
8	Malaysia	81	25.9	Melton T. *et al* 1995	20
9	South Africa	257	16.3	Soodyall H. et al 1996	22
10	Central Africa	169	14.2	Soodyall H. *et al* 1996	22
11	America (Whites)	147	0	Merriwether DA. *et al* 1996	23
12	Australia (Natives)	290	1.37	Betty D. *et al* 1996	24
		30	0	Hertzberg M. *et al* 1989	16
13	Bangladesh	31	0	Melton T. *et al* 1995	20
14	Botswana	25	0	Soodyall H. *et al* 1996	22
15	Canada	42	2.4	Merriwether DA *et al* 1996	23
16	Congo Kinshasa	75	25	Soodyall H. *et al* 1996	22
17	Kenya	60	1.6	Soodyall H. *et al* 1996	22
18	Gambia	48	0	Soodyall H. *et al* 1996	22
19	Malawi	45	26.7	Soodyall H. *et al* 1996	22
20	Mongolia	42	2.4	Merriwether DA *et al* 1996	23
21	Namibia	361	0.8	Soodyall H. *et al* 1996	22
22	Java	98	26	Melton T. *et al* 1995	20
23	Russia	176 Bering	1.70	Shields GF. *et al* 1992	25
		411 (Siberia)	0	Torroni A. *et al* 1993	26
		50 (Siberia)	0	Merriwether DA. *et al* 1996	23
		145	3	Derenko MV. *et al* 1998	27
24	Brazil	245	8.6	Alves-Silva J. *et al* 1999	28
25	Brunei	24	33	Hagelberg E. *et al* 1999	29
26	Portugal	96	2.1	Alves-Silva J. *et al*, 1999	28
27	Algeria	50	0	Ivanova R. *et al* 1999	30
28	Fiji	24	66	Hagelberg E. *et al* 1999	29
29	Vietnam	50	20	Ivanova R. 1999	31
30	China	1218	32	Yao YG. *et al* 2000	32
31	India	75 (South)	8	Melton T. *et al* 1995	20
		47 (North)	0	Melton T. *et al* 1995	20
		646	0.6	Watkins WS. *et al* 1999	33
		898	0.6	Clark VJ. *et al* 2000	34
		644	0	Roychoudhury S. *et al* 2000	35
32	Indonesia	143	21	Melton T. *et al* 1995	20
		1091	14.3-35.4	Handoko HY. *et al* 2001	36
33	Taiwan	88	32	Sykes B. *et al* 1995	21
		215	18-40	Fucharoen G. *et al* 2001	37
34	Nicobar	33	15	Prasad BV. *et al* 2001	38
35	New Guinea	202	0	Tommaseo-Ponzetta M. *et al* 2002	39
36	Iran	152	0	Peresnt Study	

S1 M S2

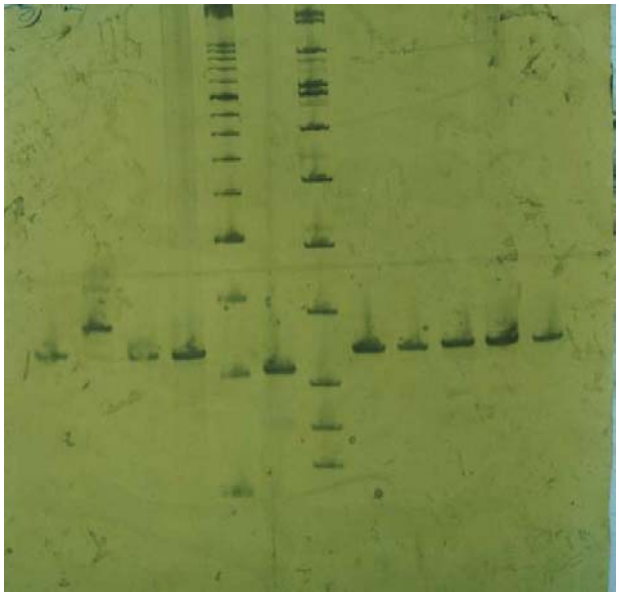

Fig.1: PAGE of PCR products. Unexpected band shown by arrow. S1and S2 is size markers and C is positive control (DNA with the 9 bp deletion)

Discussion

The first studies on 9 bp deletion suggested that this deletion defines haplogroup B. However, later studies showed that this deletion also exists in haplogroup I. Nine bp deletion in a background of haplogroup I have reported from Mediterranean, Italy and Liberia so far (40). Since Iran is geographically close to Mediterranean, the possibility of diffusion of this marker to Iran could be considered. Detection of no 9 bp deletion in this study, suggests that this haplotype has no eastward diffusion and therefore this haplotype is young.

The 9 bp deletion can tell us about migration routes of early humans. It is believed that humans left Africa to India through Saudi Arabia, Iraq, Iran and Pakistan 60-85 kilo years ago (11, 42). Most of Africans have no deletion. Those having it also have African motifs, which are different from Asian ones (22). Therefore, if the above rout is correct, one will expect no deletion in populations located on the route (or if any deletion exists, it must have African motif). No published report was found on screening of this marker in Saudi Arabia and Iraq. But the frequency of this marker in Pakistan is zero (20). Roychodhury also found no deletion in India and suggested that the absence of this marker was an evidence for migrating from Africa to India (35). Since we did not detect any deletion in Iran either, the migration from Africa to India through Iran is further supported.

A number of other migrations occurred after agricultural revolution. Agriculture has begun in three regions of the world independently. The oldest one is Fertile Crescent in Middle East. This crescent contains Palestine, north of Syria and west of Iran. A few of historians believed that agriculture introduced to India as a result of diffusion of Iranian farmers to the east. However, India is closed to China, the other agricultural center. Therefore, other historians believed that agriculture in India originated in China. The average frequency of 9 bp deletion in China is 15%. The majority of haplotypes with the deletion have the following genotype: *Dde* I 10394/*Alu* I 10397 (-/-) 9 bp del. (24). As it was mentioned earlier, the 9 bp deletion was found neither in Iran nor in India. There is much evidence that China left its influence on South-East Asia. The existence of two completely different hoplotypes in this region, with the deletion and without it, suggests that the populations of this area have two origins. Based on different studies, two waves of migrations suggested for South-East Asia. First, is an ancient wave occurred 40,000 years ago and introducing haplotype +/+ 9 bp non-del, to this region. This wave is the continuation of migration from Africa to India, which leads to Papua New Guinea and Java (39). The second wave is a recent one, which originated from China and introduced haplotype -/- 9 bp del to South-East Asia (42). Based on the above information, Iran and India are similar to each other in terms of 9 bp deletion. Therefore, India was mainly under the influence of Iran.

While Roychoudhury reported no 9 bp deletion in India, other studies reported 9 bp deletion in north and south of India with frequency of less than 1% and 10% respectively (20, 33, 34). The existence of 9 bp deletion in north of India raises the possibility of existence of this marker in Middle East in low frequency.

The length of one of the PCR products in our study was longer than 121 bp, the expected maximum length. It could be as a result of the expansion of either first or second 9 bp repeat. Length expansion of first repeat is due to the insertion of a homopolymer sequence of C. This type of expansion is usually accompanied with 8277 T>C. The number of inserted C could be more than 12. Handoko et al have sequenced the control region of those mtDNAs, which have the first repeat expansion. While the numbers of inserted C were different, the control region sequence was similar among the samples. Therefore, one can conclude that the insertion of a homopolymer sequence occurs more rapidly than nucleotide substitution (36). Length expansion of second repeat is due to the insertion of a homopolymer sequence of C, accompanied with 8286 T>C. Length expansion of second repeat is less frequent than Length expansion of first repeat. The similarities among control regions of those mtDNA that have this expansion, suggest one more time that the insertion of a homopolymer sequence, occurs more rapidly than nucleotide substitution. The length of PCR product could also be a result of 9 bp triplication. The triplication of 9 bp has been reported from Italy, Brazil, Portugal, Scotland, Bering, Nepal, China, Indonesia, Fiji, Micronesia and Polynesia islands. The result of this study was supported by Y chromosome analysis.

Quintana-Murci et al have measured the frequency of hologroups HG3 and HG9 in Iran, Pakistan and India and compared them with the frequency of these two haplogroups in Middle East (Lebanon, Syria and Palestine), Europe (Turkey, Russia, Ukraine, Latvia, Poland, Greece, Italy and Spain) and Africa. They found that the frequency of these haplogroups show a gradient, which suggested population movement in south west of Asia. The HG9 frequency reduces by moving from Iran to India. This suggests migration in this direction. Since HG9 first appeared in Iran (6300 to 9500 years ago) and then in Pakistan and India, the migration from Iran to India is suggested again 43).

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