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Investigation of the Mitochondrial Haplogroups in A Selective Population of Isfahan Province

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Abstract

In order to investigate the mitochondrial haplogroups in a selective population of Isfahan province, 96 unrelated men in Isfahan province with at least two generations in this province were token blood. Then DNA was extracted and quality assessed. 25 coding SNPs defining the major haplo groups that occur in Africa, Western Eurasia and Eastern Eurasia were selected and combined into two multiplex genotyping assays. Each one consisting of a PCR step and a SNaPShot step. Then for support of our detective haplo groups by SNaPShot system, D-Loop region was sequenced in some samples. : In this province Western Eurasian haplo groups were predominant. Haplo group U and H (22%, 21%) and then T, J and U8b (11%, 10% and 7%) had the most frequency. Eastern Eurasian haplo groups (C, D, R9, R11, M and N) were present at a lower frequency and a North African haplo group (M1) at frequency of 1%.

Keywords: mtDNA; SNP; Haplo group; Isfahan province

Introduction

Human mitochondrion has a DNA independent of nucleus DNA called mtDNA. mtDNA is a dabble strand circular molecular that has some special feathers that make it useful in human evolution studies. It has maternal inheritance [1], higher rate of mutation than nuclear DNA [2], high copy numbers and lack or recombination[3]. Due to these special feathers, mtDNA are widely used as a useful tool in studies like population history, medical genetics, genetic genealogy, and genetic forensic. Analyzing mtDNA patterns in modern human use for tracing genetics journey of early women. By mtDNA studies researchers can trace a targeted maternal ancestry along time and defining maternal ancestor in a selective population.

A haplogroup is a genetically group of population that has common ancestors in maternal/paternal lines. Mutated nucleotides in mitochondrial genome are transferred as polymorphisms from mother to boy and girl children. The whole polymorphisms in an individual mtDNA is called mitochondrial haplotype. Varies haplo types in different ancestries create branches of mtDNA phylogenic tree and consequently aggregation of haplo types in this tree creates clusters as haplo group [4]. mtDNA phylogenic tree is classified in four macro haplo groups named L, M, N and R. each one has some haplo groups and each haplo group has some sub haplo groups.

mtDNA and Y chromosome haplo groups studies are useful in cases like investigation on human colonization patterns from different continents and understanding origin and genetic structure of different populations.

The Purpose of this study is defining of each haplo group frequency in a selective population of Isfahan province, a central province in Iran.

Material and Methods

96 unrelated men inhabited in Isfahan province with at least two generations in this province were token blood, based on the number of population of each city. Then DNA was extracted by RGDE method [5] and quality assessed by nano photometer and electrophoresis. We selected 25 coding SNPs [6] defining 32 of the major haplo groups that occur in Africa, Western Eurasia and Eastern Eurasia (Figure 1). SNPs combined into two multiplex genotyping assays. Each one consisting of a PCR step and a SNaPShot step.

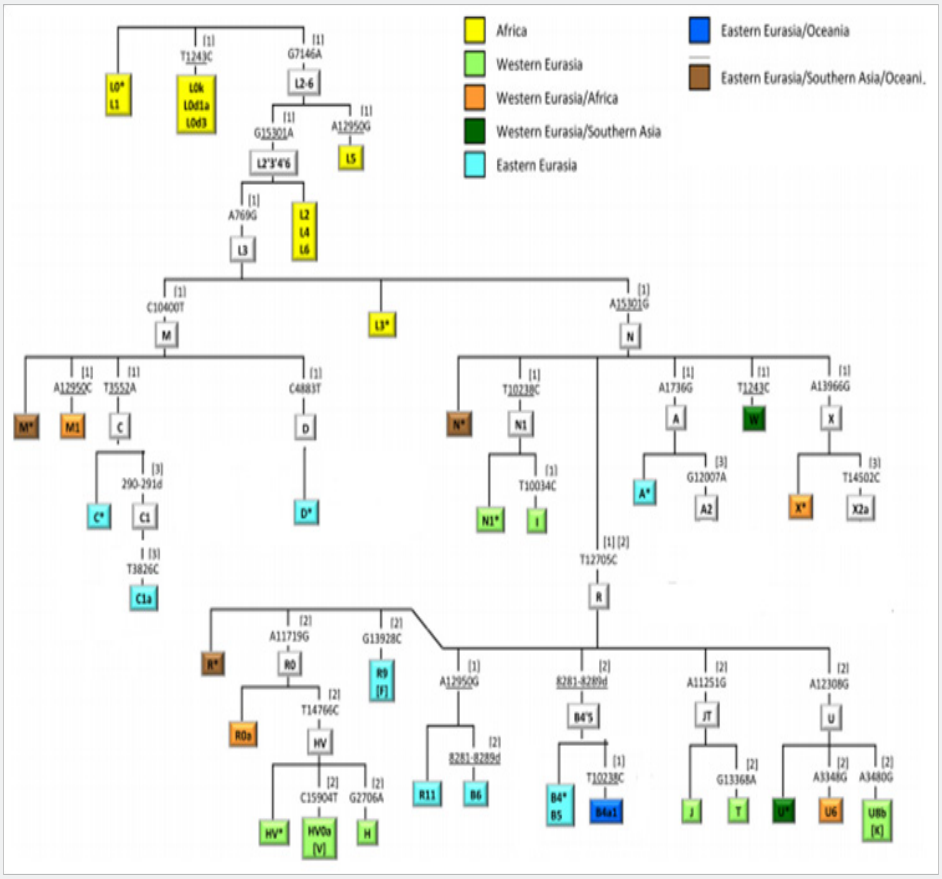

Figure 1: Targeted mtSNPs with geographic haplogroup classification

Table 1: Multiplex 1 Primers details

		PCR amplification			Single-base extension				
Site		**Primer sequences (5'-3')**	**Concentration**	**Amplicon size (bp)**	**Primer sequence (5'-3') (5' aspecific tail in lowercase italics)**	**Concentration**	**Length**	**Orientation**	**Alleles (dye)**
		(pM)			**(pM)**	**(nt)**			
769	F	ACATC ACCCCAT AAACAA ATAGG	1	158	act(gact)l0 CGTTTT GAGCTG CATTG	2	60	R	A (red), G (yellow)
	R	AGCGT TTTGAGC TGCATTG	1						
1243	F	AATCGAT AAACCC CGATCAA	0.04	93	actgact CGATC AACC TCACC ACC	0.15	24	F	C (yellow), T (red)
	R	TGCGCT TACTT TGTAG CCTTC	0.04						

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1736	F	GCTAAA CCTAGC CCCAA ACC	0.2	106	t(gact)2gac TCAAT TTCTA TCGCC TATAC TTTAT	0.15	37	R	A (red), G (yellow)
	R	CTAT TGCGC CAGGTT TCAAT	0.2						
3552	F	CGCTGA CGCCA TAAAA CTCT	4	128	actgact AGGGGG GTTCATA GTAGAAG	4	27	R	A (red), C (blue), T (green)
	R	GTATG GGGAG GGGG GTTC	4						
4883	F	TGAC ATCC GGCC TGCTT	0.075	114	(gact)4g CATGA CAAAAA CTAGC CCC	0.3	36	F	C (yellow), T (red)
	R	TGGAT AAGAT TGAGA GAGT GAGGA	0.075						
7146	F	AGACC AAACC TACGC CAAAA	0.15	130	ct(gact)cga TACGC CAAAA TCCA TTTC	0.3	58	F	A (green), G (blue)
	R	GGTGTA TGCATC GGGGTAGT	0.15						
10034	F	TCTCC ATCTA TTGATG AGGGTCT	0.3	108	ct(gact)4g GTACC GTTAAC TTCCA ATTAA CTAG	0.15	44	F	C (yellow), T (red)
	R	TTAAG GCGAA GTTTAT TACTC TTTTT	0.3						
10238	F	GCGTCC CTTTCT CCATAAAA	0.25	80	ct(gact)4gac CTCCA TAAAAT TCTTCT TAGTA GCTAT	0.3	48	F	C (yellow), T (red)

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	R	GGGTA AAAGG AGGG CAATTT	0.25						
10400	F	GCCCTA AGTCT GGCCT ATGA	0.15	90	ct(gact)5gac CGTTT TGTT TAAACT ATATACC AATTC	0.3	52	R	C (blue), T (green)
	R	TGAGTC GAAATC ATTCG TTTT	0.15						
12705	F	CCCAA ACATTA ATCAGT TCTT CAA	0.18	102	t(gact)6g TTAATC AGTT CTTCAA ATATCT ACTCAT	0.15	54	F	C (yellow), T (red)
	R	TCTCA GCCGA TGAAC AGTTG	0.18						
12950	F	TCCTC GCCTTA GCATG ATTT	0.2	101	act(gact)n TGAGG CTTGGAT TAGCG	0.5	64	R	A (red), C (blue), G (yellow)
	R	GAGGC CTAGT AGTGG GGTGA	0.2						
13966	F	ACCGC ACAATCC CCTA TCTA	0.25	132	ct(gact) 13 GCAGGT TTTGG CTCG	0.5	69	R	A (red), G (yellow)
	R	AGGTG ATGATG GAGGT GGAG	0.25						
15301	F	CCACCCTC ACACGA TTC TTT	0.08	119	ct(gact)4 ATTCTT TACCT TTCACT TCATCTT	0.15	42	F	A (green), G (blue)
	R	GGTG ATTCCTA GGGGG TTGT	0.08						

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Table 2: Multiplex 2Primers details

		PCR amplification			Single-base extension				
Site		Primer sequences (5'-3')	Concentration	Amplicon size (bp)	Primer sequence (5'-3') (5' aspecific tail in lowercase italics)	Concentration	Length	Orientation	Alleles (dye)
			(pM)			(pM)	(nt)		
2706	F	CGAGG GTTCAGC TGTCT CTT	0.04	88	(gact)w GTCTT CTCGT CTTGC TGTGT	0.1	60	R	A (red), G (yellow)
	R	AGGGT CTTCTC GTCTTG CTG	0.04						
3348	F	CAGTC AGAGGTTC AATTCC TCTT	0.2	142	act(gact),,g GGAA TGCCA TTGC GAT	0.2	64	R	A (red), G (yellow)
	R	GGGCC TTTGC GTAGT TGTAT	0.2						
3480	F	CGCT GACGC CATAA AACTCT	0.12	120	act(gact)2ga GCCATA AAACT CTTC ACCAA	0.15	33	F	A (green), G (blue)
	R	AGGGG GGTTC ATAGT AGAAG	0.12						
8281¬	F	GAAAT CTGTGG AGCA AACCAC	0.5	179/170	(gact)2g CCCTA TAGCAC CCCC TCTA	0.7	28	F	a (yellow), d (blue)*
8289									
	R	AGAG GTGTT GGTTC TCTTAA TCTTT	0.5						
11251	F	TGAA CGCAGG CACAT ACTTC	0.1	92	t(gact)5gac CCCCT ACTCA TCGCACT	0.05	41	F	A (green), G (blue)
	R	TGAGCC TAGGGTG TTGTGAG	0.1						

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11719	F	GGCGCA GTCAT TCTCAT AATC	0.1	85	(gact)6 GCAGA ATAGTA ATGAG GATG TAAG	0.15	48	R	A (red), G (yellow)
	R	TGTGAG TGCGTTC GTAGTTTG	0.1						
12308	F	CAGCT ATCCAT TGGTCT TAGGC	2	169	t(gact)9gac TGGTC TTAGG CCCCAA	3	56	F	A (green), G (blue)
	R	GATTTT ACATA ATGGG GGTAT GAGT	2						
12705	F	ACTTC TCCAT AATATT CATC CCTGT	1.3	184	act(gact)5g TTAAT CAGTT CTTCAA ATATC TACTCAT	0.8	52	F	C (yellow), T (red)
	R	TCTCAGC CGATGA ACAGTTG	1.3						
13368	F	CGCCTT CTTCA AAGCC ATAC	0.25	127	ct(gact)2gac TAAGG TTGTGG ATGAT GGA	0.3	32	R	A (red), G (yellow)
	R	GGTGAG GGAGGT TGAA GTGA	0.25						
13928	F	CAGCC CTAGA CCTCAA CTACCT	0.04	119	ct(gact)5ga AACAT ACTCG GATTCT ACCCTA	0.04	46	F	C (yellow), G (blue)
	R	ATAGGG GATTGTG CGGTGT	0.04						
14766	F	TCAACT ACAAGA ACACCA ATGACC	0.05	109	c GACC CCAA TACG CAAAA	0.15	18	F	C (yellow), T (red)
	R	ATCAT GCGGAG ATGTT GGAT	0.05						

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15904	F	CATCC GTACTA TACTTC ACAAC AATC	1	184	act(gact)4 GGCCTG TCCTT GTAGTA TAAA	0.6	40	F	C (yellow), T (red)
	R	GGTGCT AATGGTG GAGTT AAAGA	1						

Table 3: Mitochondrial haplogroups frequency in Isfahan province and some population

Population /region	Sample size	Haplogroupsfrequency(%)																	
		Pre-HV	HV	H	. U	J	M	T	I	K	B	L1	L3	W	X	V	N	D	others
Isfahan	96	-	4	21	22	10	3	11	‘2	7	-	-	-	5	-	-	2	1	11	This study
Iraq	100	1	10	14	18	10	9	7	4	2	2	1	3	-	-	-	7	7	8	Nihad., 2013
Iraq	153	-	6.4	16.9	15.3	8.06	8.1	3.22	1.61	12	3.22	-	4	8.87	-	-	-	-	11.52	Basheer et al, 2013
Iraq	216	4.2	10.6	19.9	19	9.3	1.4	8.8	1.9	3.2	0.9	1.4	-	1.9	2.8	0.5	-	-	11.5	Al-Zahery et al, 2003
Iran	415	5.5	5.5	17.1	21.5	13.5	NR	8.4	2	7.5	NR	NR	2.2	2	2.9	-	-	-	8.6	Kivisild et al., 2003
Arabia	389	3.6	3.6	12.9	10.5	20.8	NR	4.6	0.8	3.6	NR	NR	10.5	1.8	1.8	-	-	-	6.2	Kivisild et al., 2003
Syria	69	4.3	4.3	24.6	15.9	10.1	1.4	10.1	-	4.3	‘-	2.9	-	2.9	-	2.9	-	-	11.6	Richards & Macaulay, 2000

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Palestinian	117	1.7	1.7	30.8	7.6	9.4	1.7	12.8	-	6.8	-	0.9	-	2.6	3.4	'-	-	-	15.4	Richards & Macaulay, 2000
Georgian	139	7.2	7.2	17.3	21.6	3.6	2.9	12.9	2.2	10.1	-	-	-	1.4	10.1	0.7	-	-	9.3	Tambets et al, 2000
American	192	7.3	7.3	30.9	22.5	8.9	- ^	11.5	1.6	7.9	0.5	-	-	1	2.1	-	-	-	5.3	Tambets et al, 2000
Anatolia	388	3.6	3.6	25	19.3	10.9	4.4	11.9	2.3	5.9		NR	0.3	3.9	4.4	-	-	-	4.5	Kivisild et al., 2003; Tambets et al, 2000
Italian	99	2	2	33.3	22.2	7.1	'-	9.1	4	8.1	- J	-	-	2	3	5.1	-	-	4.1	Toroni et al, 2003
Slav	324	NR	NR	41.4	19.4	10.5	0.9	12.3	2.8	3.7	' -	-	-	0.9	0.6	3.1	-	-	4.4	Kivisild et al., 1999
Finno-Ugri	149	NR	NR	45.6	22.8	12.1	0.7	6	1.4	3.3	-	-	-	2.7	2	2	-	-	1.4	Kivisild et al., 1999
German	200	NR	NR	50	13.5	7.5	-	8.5	2.5	6.5	-5	-	' -	1	0.5	2.5	-	-	7.5	Lutz et al., 1998
C-Asian	205	NR	NR	14	8	2.5	38.5	3.5	1	0.5	6.8	-	-	1	-	-	-	-	24.2	Comas et al., 1998
Indian	1300	0.6	0.6	2.4	12	0.8	NR	1.1	0.6	0.2	NR	-	-	1.5	0.2	-	-	-	13.8	Kivisild et al., 2003

Multiplex PCRs were performed in a reaction volume of 6µl, which contained 1X Gene Amp PCR buffer, 100µM of each dNTPs 0.35units of Taq DNA polymerase, 1.2µl watery genomic DNA in 5mg/ml and 15 pairs PCR primers for multiplex1 (Table1) and 12

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pairs PCR primers for multiplex 2 (Table 2). The reactions were performed in a ABI Bio system applied 2730 with conditions: an early denaturation at 95 ºC for 10minuet and 30 cycles of 94 ºC for 15 seconds, 60 ºC for 45seconds and a final extension at 60 ºC for 5minuet. Then PCR products were purified by adding 1.5µl ExoSAP to them and incubation at 37 ºC for15 minuet and 80 ºC for 15 minute. Multiplex SNaPShot were performed in a reaction volume of 5µl, which contained 1µl Ready Reaction Mix, 1µl purified PCR product and extensions primers (Table1 & 2). The reactions were performed in conditions: 96 ºC for 2minutes and 25 cycles of 96 ºC for 10 seconds, 50 ºC for 5seconds and 60 ºC for 30 seconds. The reaction products were purified by adding 1µl CIP to them and incubation at 37 ºC for 45 minutes and 75 ºC for 15 minutes. prepared a mixture of 1µl purified extension product, 8.8 µl Hi- Di for maimed and 0.2 µl GeneScan-120 LIZ size standard and ran samples in genetic analyzer ABI 1330XL with POP-7 polymer. Results were analyzed using Gene Mapper ID version 3.2 software. The results of SNP patterns in two assays was determining mitochondrial haplo groups in samples of the study. Then for support of our detective haplo groups by SNaPShot system, D-Loop region was sequenced in some samples by genetic analyzer ABI 1330XL.

Results

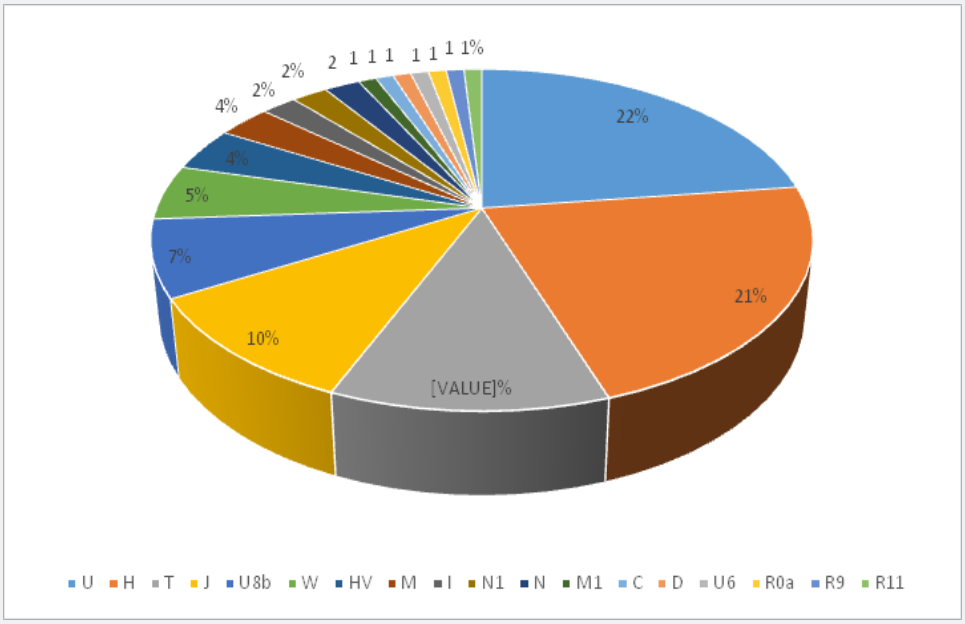

Figure 2: Mitochondrial DNA haplogroups frequency (%) in a selective population of Isfahan province..

96 mtDNA samples from Isfahan province were analyzed and 18 haplogroups were found (Figure 2). 94% of this population belonged to Western Eurasian haplo groups and only 5% belonged to Eastern Eurasian haplo groups (C, D, R9, R11, M and N). So Western Eurasian haplo groups were predominant. Haplo group U and H (22%, 21%) and then T, J and U8b (11%, 10% and 7%) had the most frequency and Eastern Eurasian haplo groups and a North African haplo group (M1) were present at a lower frequency.

Discussion

Previous studies indicated a high frequency of 77% of Asian mtDNA is belonged to haplogroup M. Since Iran is located in Asia it is expected that this haplo group has a high frequency in Iran. But results of Houshmand et al. 2004 study showed a low frequency of this haplo group (0-4%). This study found the same results and only three cases of persons who take part in this study had haplo group M.

In other side about 99% of mtDNA in European population belong to at least one of the nine haplogroups: H, U, J, T, K, I, V, W, and X. Six of them (H, J, T, K, I and w) are especial for European population and probably originated from Caucasoid and genetically separated from ancestors of African and Asian [7]. Studies about Iran country showed a high frequency of Haplo group U, also in this study had the most frequency (22%). This haplo group is much older than the others and estimated to originate about 51,000-67,000 years ago [7]. It seems that this haplo group originated from Africa and then expanded into Middle East and Europe. In the other hand haplo groups H, T, J and K had the most frequency after haplo group U in Isfahan province. It is estimated that originated about 8,000-30,000 years ago [7,8].

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