Moroccan J. Biol. 2-3 (2006) 68-73

Mitochondrial genetic variability of North Morocco population

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Abstract

The population of North Morocco is demographically different of the rest of the country due to the presence of Rif Berber population. However, the genetic variability of this population has not been well studied. To check out this diversity and to compare it with the population of Southern Spain, mitochondrial DNA (mtDNA) hipervariable region (HVR-I) was sequenced and RFLP analysis performed in a sample of 200 men coming from Oujda (130 individuals) and Tetouan (70 individuals). The results obtained show a high heterogeneity containing Caucasian, African, and Asian mtDNA variants. Comparing these results with others previously reported from Agadir and Andalucia (Spain), we can conclude that Berber population is slightly more frequent in Tetouan than Oujda, whereas Subsaharian presence was higher in Agadir. Remarkably, the presence of Asian variant was increased in Oujda respect to Tetouan in agreement with historical data reporting a higher Arabic penetrance in Oujda than in Tetouan.

Keywords: Mitochondrial DNA; Haplogroups; North Morocco; Berbers

Introduction

Modern Morocco territory has been inhabited by a series of human groups. In particular, the Berbers are considered to be the descendents of the Palaeolithic early inhabitants of North Africa. Later, Moroccan population has experienced a long history in which Africans, Arabs, and Caucasians have been frequently involved. Thus, the expansion of the arabisation along the Maghrib gave rise to an important Near East cultural influence. Besides that, the Subsaharian population pressure in North Africa also contributes to the important demographic complexity of Morocco. In the case of North Morocco, Berbers still remain as the most important demographic pool, but no systematic studies on their demographic heterogeneity have been performed.

Mitochondrial DNA (mtDNA) variability is the most commun genetic marker used for the study of population heterogeneity and human phylogenetic studies. Human mtDNA is a circular molecule of approximately 16.6 kb coding for 13 polypeptides of the respiratory complexes, 2 ribosomal RNAs and 22 transfer RNAs. It also contains a non-coding region named displacement loop (D-loop) (Fernandez-Silva *et al*., 2003). Some characteristics of this DNA are useful to studies of human phylogeny.

Thus, high frequency of mutations (10 to 100 times more than nuclear DNA) (Howell *et al*., 1996, Polyak *et al*., 1998, Wallace *et al*., 1998), the absence of genetic recombination and its unidirectional (maternal) inheritance makes it a very adapted tool for this kind of studies. It is well known that the D-loop

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possesses a higher degree of variation than the coding region becoming very useful for evolutionary studies. Therefore, two different analytical approaches are commonly used to detect mtDNA polymorphisms; one is the use of Restriction Fragment Lengh Polymorphism (RFLP) analysis of amplified fragment of the coding region where polymorphisms are less frequent, and second, sequence of amplified D-loop fragment where mutations are very frequent. In addition, D-loop sequence may eventually be of interest to identify individual haplotypes.

mtDNA point mutations (single nucleotide polymorphisms or SNPs) are linked in genetic lineages named haplogroups, which can be further associated in larger genetic groups (clades). Human mtDNA clades are confined in discrete geographical regions. Thus, Caucasian populations are mostly integrated by four clades; HV, JT, KU, and IWX. In the case of Moroccan population, native Berbers are well known to be of Caucasian origin.

The most important African lineage consists of the L clade which in the case of Morocco defines the population of Subsaharian origin. On the other hand, haplogroup M is mostly completely integrated by Asian population. Therefore, mitochondrial genetic variability could be potentially used to check out the population heterogeinety of Morocco.

Two previous studies reported the mtDNA variability of Berbers (Plaza *et al*., 2003, Rando *et al*., 1998) but the precise geographical origin was omitted. Besides this report, only other work describes this variability in a population coming from Agadir (Brakez *et al*., 2001). A deeper knowledge of the mitochondrial genetic variabilty of the North Morocco should be however of interest to establish the ethnical differences of its demographic distribution. Furthermore, it will provide the basis to further investigate the phylogenetic link of the Berber population with Caucasian mtDNA variants.

The target of this work consisted in cheking out the mitochondrial genetic diversity of the population of North Morocco and to compare it with data known with the population of Southern Spain and the rest of Morocco. For that, we have chosen Tetouan and Oujda, two cities situated respectively in the West and East part of the Rifain region, known to be enriched in Berber population (Figure1).

Figure 1. Geographical situation of Tetouan and Oujda in Morocco

Material and methods

Samples and DNA extraction

Blood was obtained from 200 unrelated healthy volunteers between 20 and 45 years old, including 70 from Tetuan and 130 from Oujda. Total DNA was extracted from blood (3-5 ml in EDTA tube) using Proteinasa K, followed by standard phenol-chloroform method, and the DNA was precipitated using absolute ethanol, as was previously described (Marcuello *et al*., 2005).

mtDNA amplification and RFLPs

For the genetic characterization of the individuals, all the samples were analized by RFLP and by sequencing the hipervariable region I (HVR-I).

Amplifications were carried out in 50µl of reaction mixture and PCR products were loaded in 2% agarose gel. Positive amplifications were digested at 37˚C in 10µl reaction volumes. The polymorphisms used and PCR conditions are shown in (Table 1).

mtDNA sequencing fragments

A fragment of 468 bp (HVS-I) was amplified using the primers L15977 (5'-CCACCATTA GCACCCAAAGC-3') and H16455 (5'-CGAGGAGAGTAGCACTCT TG-3'), where "L" and "H" refer respectively to "Light" and "Heavy" strands of mtDNA. The sequences were aligned to the revised Cambridge Reference Sequence (rCRS) (Anderson *et al*., 1981, Andrews *et al*., 1999) using BLAST program.

Table1. Conditions used for identification of polymorphisms. mtDNA fragments amplified under the PCR conditions here defined, were digested for RFLP analysis. One of the primers used for the detection of 12308 polymorphism have a mismatch.

Amplicon	Restriction enzyme	Haplogroup identified	Primers 5'- - - - - - - - - - - - - - - - 3'	Annealing temperature (C)	Digested PCR product -- (bp) +
3350-3680	HpaI	L	TCGCAATGGCATTCCTAATG GAGTTTGATGCTCACCCTGA	62	331 243/88
4096-4407	Afl III	J-T	CTACTTCTAACCTCCCTGTT CTTACTTTAGGATGGGGTGT	60	312 121/191
4308-4739	Nla III	V	GGAGCTTAAACCCCCTTA GGAGCTTAAACCCCCTTA	60	432 273/159
6874-7134	Alu I	H	TCGCCACACTCCACGGAAG TGGCGTAGGTTTGGTCTAGG	65	183/78 152/31/78
10270-10579	Alu I	M	TCCTTTTACCCCTACCATGAG ATTATTCCTTCTAGGCATAGTAG	62	310 128/182
12101-12338	Hinf I	K-U	TCCCTCAACCCCGACATCATTACCG CTTTTATTTGGAGTTGCACCAAGATT	64	67/168 67/138/30
14430-14580	Acc I	X	ATGCCTCAGGATACTCCTCAATAGCCGTC TTGATTGTTAGCGGTGTGGT	60	151 36/115

Table 2. Frequences of mtDNA genetic lineages found in Tetouan and Oujda populations. The number of individuals found in each lineage is indicated between brackets.

Haplogroups	**Tetouan** n=70	**Oujda** n=130	**North of Morocco** *n=200*
H	31,4 (22)	26,2(34)	28 (56)
V	7,1(5)	3,1(4)	4,5 (9)
J	1,4(1)	7,7(10)	5,5 (11)
T	8,6(6)	6,2(8)	7,0 (14)
U_{rest}	5,7(4)	3,8(5)	4,5 (9)
U_5	4,3(3)	3,1(4)	3,5 (7)
U_6	12,9(9)	10,8(14)	11,5 (23)
K	4,3(3)	5,4(7)	5,0 (10)
X	0,0(0)	2,3 (3)	1,5 (3)
L	12,9(9)	17,7(23)	16 (32)
M	1,4(1)	4,6(6)	3,5 (7)
Others	10,0(7)	9,2(12)	9,5 (19)

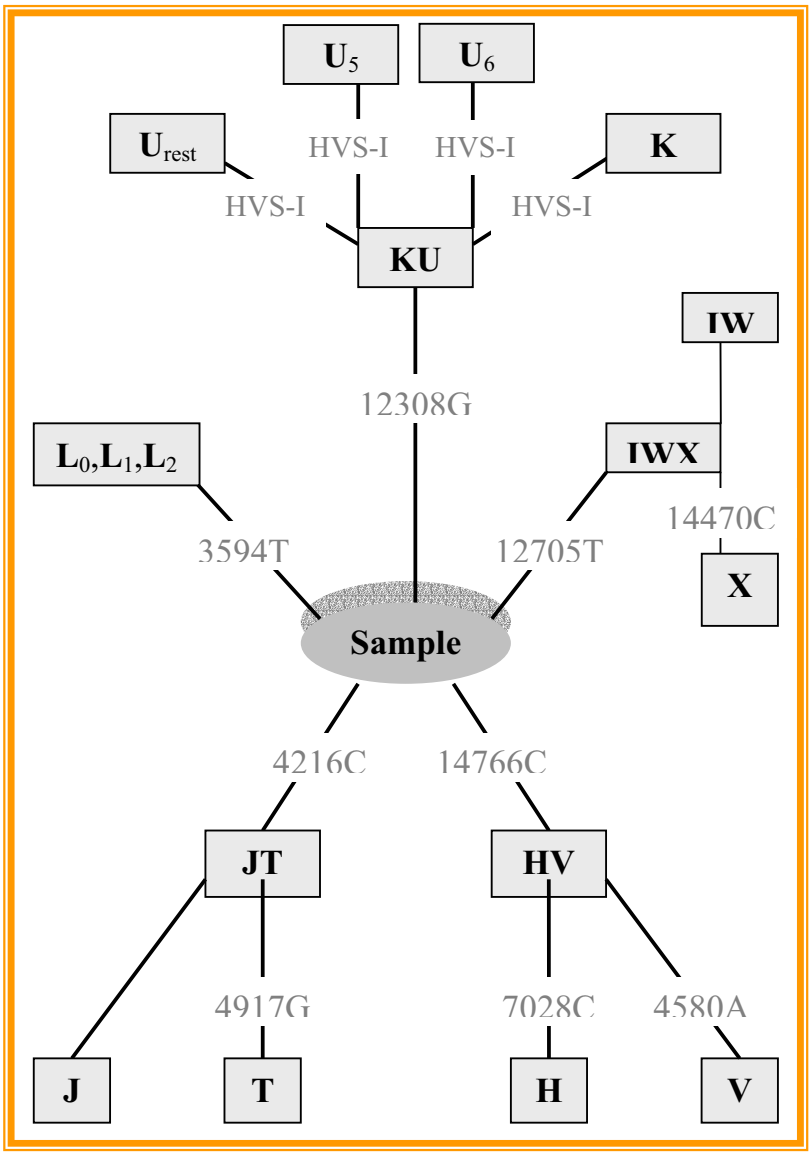
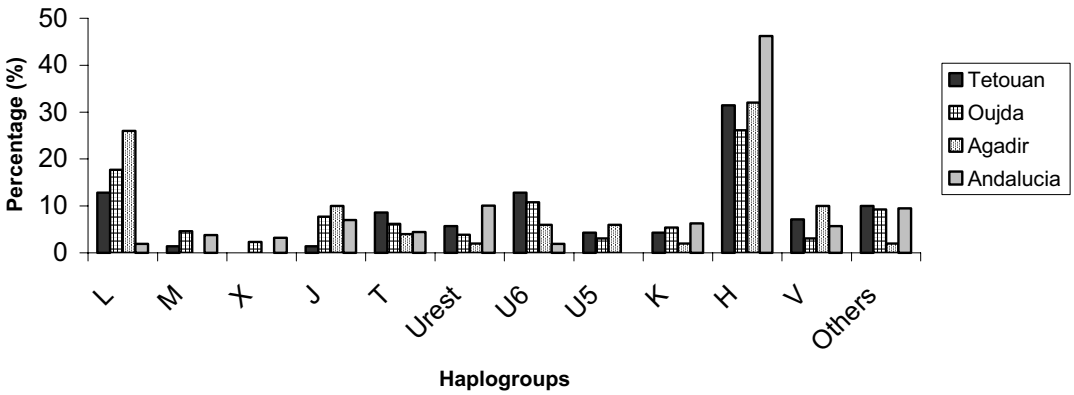


Figure 2. Polymorphisms used for haplogroups identification in the mtDNA coding region. KU sublineages and L3 were identified by polymorphims found in HVS-I according to (Richards *et al*,. 2000).

Figure 3. Distribution of mtDNA in Tetouan, Oujda, Agadir and Andalucia



Results

Figure 2 shows the phylogenetic relationships between the mitochondrial haplogroups and the defining polymorphisms used for characterizing lineages KU, IWX, X, I+W (by exclusion), V, JT, T, J (by exclusion), and L (L_0+L_1+L_2; L_3 not included). KU sublineages (U_{rest}, U_5, U_6 and K) were defined by specific polymorphims of HVS-I (Richards *et al*., 2000). Samples that could not be included in one of these groups were grouped as Others.

Table 2 shows the distribution of the different haplogroups found in the whole Morocco, and en Tetouan and Oujda populations. None invidual harbouring haplogroup W or I was found and therefore these haplogroups were not included in the table. The most frequent haplogroup was H either in Tetouan or Oujda, followed by L and U_6. In any case this is the largest sample ever tested in Morocco for populational genetic studies.

Figure 3 shows a comparative representation of the results found by us in Tetouan and Oujda with those previously reported from Agadir (Brakez *et al*., 2001) and Andalucia (Plaza *et al*., 2003). It can be observed that H haplogroup is more represented in Andalucia, being less frequent in Oujda. U_6 was almost absent in Andalucia and more frequent in Tetouan. L was also absent in Andalucia but particulary frequent in Agadir. Interesently M was more represented in Oujda and Andalucia than in Tetouan being almost absent in Agadir.

Discussion

The results obtained show that the highest proportion of the population of Tetouan and Oujda belongs to Caucasian mtDNA lineages. However, the high presence of U_6 identifies this population as Berber. Subsaharian influence is highly important in South Morocco, as previously reported by the study of Agadir (Brakez *et al*., 2001) in this last case the frequence of L haplogroup was slightly lesser than H. However L haplogroup was much lower than H in Tetouan and Oujda. This fact points out for a lesser Subsaharan influence in these last cities.

Remarkably, Arabic influence indicated by M haplogroup (Richards *et al*., 2003) was highest in the case of Oujda followed by Andalucia. This observation suggests a higher Arabic influence in the population of Eastern Morocco (Oujda) that in Western country (Tetouan). Thus, it even appears that Arabic influence is higher in Southern Spain (Andalucia) than in Western Morocco (Tetouan and Agadir).

These results are in agrement with previous studies that have shown a high genetic diversity of North-West African population (Plaza *et al*., 2003, Rando *et al*,. 1998, Fadhlaoui-Zid *et al*,. 2004). In the case of Morocco, Phoanicians, Carthagians, Romans, Vandals and Byzantines arrived and lived there. However, aboriginal population was Berber although the most important influence was the Arabic one.

Tetouan is set upon the Mediterranean sea. Founded in the 3rd century B.C was under Andalucian influence from the South Spain, particulary at the end of 15th century. In the other hand, Oujda situated in North Eastern Morocco was founded by Berbers in 944. The city underwent numerous invasions, and it is well known its strong Arabic influence.

The obsevation here presented opens interesting questions. U_6 is a haplogroup present in North Africa and Near East. A deep analysis of U_6 sublineages could be of interest to define the aboriginal nature of Berbers and its influence in Southern Spain. Likewise, further studies on M sublineages will also allow to distingish the Arabic influence in Southern Spain and Northern Morocco. And finally the high frequence of H haplogroup will make possible and more detailed characterization of the population heterogeinity of Morocco as compared with Southern Spain.

Acknowledgments

We are grateful to the original donors of samples. We thank Dr Hussein Imlahi, Dr Noureddine Boukhatem and Dr Mohamed-Amine Afilal for supplying us with the blood samples. This research was supported by grants from Agencia Española de Cooperación Internacional (AECI 145-03-P), the Diputación General de Aragón (Grupos Consolidados B33), Spanish Fondo de Investigación Sanitaria (FIS-04-0009), and Networks for Mitochondrial Disorders and Ataxias (G03-011/G03-056).

References

Anderson.S, Bankier A.T, Barrell B.G, De Bruijn M.H., Coulson A.R. and Drouin J, *et al*., (1981). Sequence and organization of the human mitochondrial genome, *Nature* **290**, 457–465.

Andrews R.M, Kubacka I, Chinnery P.F, Lightowlers R.N, Turnbull D.M and Howell N. (1999). Reanalysis and revision of the Cambridge Reference Sequence for human mitochondrial DNA. *Nat. Genet.* **23**, p. 147.

Brakez Z., Bosch E., Izaabel H., Akhayat O, Comas D, Bertranpetit J & Calafell F. (2001) Human mitochondrial DNA sequence variation in the Moroccan population of the Souss area. *Ann Hum Biol* **28**, 295–307.

Fadhlaoui-Zid K, Plaza S, Calafell F, Ben Amor M, Comas D, Bennamar El gaaied A. (2004). Mitochondrial DNA heterogeneity in Tunisian Berbers. *Ann Hum Genet*; **68**(Pt 3):222-33.

Fernández-Silva P, Enriquez J.A, Montoya J. (2003). Replication and transcription of mammalian mitochondrial DNA. *Experimental Physiology*; **88.1**, 41-56

Howell N, Kubacka I, Mackey D.A. (1996). How rapidly does the human mitochondrial genome evolve?, *Am. J. Hum. Genet.* **59**, pp. 501–509

Marcuello A, Gonzalez-Alonso J, Calbet JA, Damsgaard R, Lopez-Perez MJ, Diez-Sanchez C. (2005). Skeletal muscle mitochondrial DNA content in exercising humans. *J Appl Physiol*.;**99**(4):1372-7.

Plaza S, Calafell F, Helal A, Bouzerna N, Lefranc G, Bertranpetit J, Comas D. (2003). Joining the pillars of Hercules: mtDNA sequences show multidirectional gene flow in the western Mediterranean.*Ann Hum Genet*; **67**(Pt 4):312-28.

Polyak K, Li Y, Zhu H, Lengauer C, Willson J.K, Markowitz S.D, *et al*.(1998). Somatic mutations of the mitochondrial genome in human colorectal tumours, *Nat. Genet.* **20**, pp. 291–293

Rando J.C., Pinto, F. Gonzalez, A.M. Hernandez, M. Larruga, J.M. Cabrera V.M. & Bandelt H.J. (1998). Mitochondrial DNA analysis of northwest African populations reveals genetic exchanges with European, near-eastern, and sub-Saharan populations. *Ann Hum Genet* **62**, 531−550.

Richards M, Rengo C, Cruciani F, Gratrix F, Wilson J.F, Scozzari R, Macaulay V, Torroni A. (2003). Extensive female-mediated gene flow from sub-Saharan Africa into near eastern Arab populations. *Am. J. Hum. Genet.* **72**, pp. 1058–1064

Richards M, Macaulay V, Hickey E, Vega E, Sykes B, Guida,V, Rengo C, Sellitto D, Cruciani F, Kivisild T, Villems R, Thomas M, Rychkov S, Rychkov O, Rychkov Y, Gölge M., Dimitrov D, Hill E, Bradley D, Romano V, Calì F, Vona G, Demaine A, Papiha S, Triantaphyllidis C, Stefanescu G, Hatina J, Belledi M, Di Rienzo A, Novelletto A, Oppenheim A, Nørby S, Santachiara-Benerecetti S, Scozzari R, Torroni A, Bandelt H.J. (2000). Tracing European founder lineages in the Near Eastern mtDNA pool. *American Journal of Human Genetics*, **67**, 1251-1276

Wallace D.C, Brown M.D, Melov S, Graham B, Lott M. (1998). Mitochondrial biology, degenerative diseases and aging, *Biofactors* **7**, pp. 187–190