An. R. Acad. Nac. Farm., 2006, 72: 37-47

Artículo original

Mitochondrial lineages distribution in the Spanish population: anticipating association studies

Recibido el 6 de febrero de 2006

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SUMMARY

The genetic variation in mtDNA has been widely used to give a maternal genetic perspective of the human demographic history. Here, we have studied this variability in 686 samples coming from the Centre and North of Spain. These results showed that haplogroup frequencies were similar to other Spanish studies and European populations. Haplogroups from the HV lineage were over-represented in the Spanish population. A deeper analysis of the mitochondrial haplogroup U showed differences with Northern Europe populations. The frequencies of haplogroups found give them high valour when experimental design for mitochondrial disorder studies in population is planted. In addition, the use of these data is also important for forensic studies.

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Keywords: Mitochondrial DNA.—Human.—Variability.—Haplogroups.—Oxidative phosphorylation.

RESUMEN

Distribución de líneas mitocondriales en la población española: anticipándonos a los estudios de asociación

La variación genética en el mtDNA ha sido ampliamente utilizada para dar una perspectiva de la historia demográfica humana. En este estudio, nosotros hemos analizado esta variabilidad en 686 muestras del Centro y Norte de España. La frecuencia de los haplogrupos en la población española es muy similar a la observada en otros estudios sobre esta población y a las frecuencias en las poblaciones europeas. Un análisis más profundo del haplogrupo mitocondrial U mostró diferencias con las poblaciones del norte de Europa. El conocimiento de la distribución de frecuencias de los haplogrupos en nuestra población supone un resultado importante para el diseño de estudios sobre enfemedades mitocondriales. Además, nuestros resultados son también importantes en los estudios forenses.

Palabras clave: DNA mitocondrial.—Humanos.—Variabilidad.—Haplogrupos.—Fosforilación oxidativa.

INTRODUCTION

In 1997, different laboratories published an association between the mitochondrial genetic background and the Leber's Hereditary Optic Neuropathy (LHON) in patients with any of two different pathologic mutations, 11778A and 14484C (1-4). Mitochondrial haplogroup J was overrepresented in patients with any of these mutations and it was postulated that this haplogroup increased the penetrance of the pathologic mutation. Since then, other haplogroups have been associated with different phenotypes (5, 6), particularly with aging (7-10) and aging-related diseases, such as Parkinson disease (11-13) and Alzheimer disease (14, 15).

A mitochondrial haplogroup is a cluster of phylogenetically related mitochondrial genotypes (haplotypes). These haplogroups are defined by ancient mutations. These changes appeared and survived, therefore, they could not be deleterious mutations. Most of them probably had not phenotypic effect and they were neutral. Some of them had a beneficent effect and were positively selected. However,

this positive effect was related with a particular environment and nowadays, in other environmental conditions, they may have different effects on the phenotype (16-18).

Several major haplogroups (H, V, J, T, U, I, W, X) were described in Western Eurasian individuals (19) and their frequencies were very similar all around Europe (20). The increased interest in mitochondrial population genetics has made possible to subdivide these haplogroups in smaller clusters, called subhaplogroups. The frequency and distribution of these subhaplogroups is not the same in different European populations (6, 21-23). Similarly to the haplogroups, some of the mutations defining these subhaplogroups might have a phenotypic effect and then, they could contribute to the distinct prevalence of these diseases in different populations (24).

In advance of next epidemiologic studies in the Spanish population trying to associate phenotypes to mitochondrial genetic background, we decided to characterize the Spanish population according to the mitochondrial haplogroups.

MATERIAL AND METHODS

Peripheral blood was collected from 686 unrelated individuals from Northern and Centre of Spain (Zaragoza and Madrid).

Genomic DNA was extracted by conventional methods (25) and the samples were haplogrouped by PCR amplification of short mtDNA fragments, followed by restriction enzyme analysis (RFLP analysis). We used the haplogrouping strategy from (5) (Table 1). Hypervariable region I (HVR-I) was sequenced in order to confirm haplogroups and determine subhaplogroups U. Full description of oligodeoxynucleotides utilized and PCR amplification conditions are available upon request.

Results and differences in diverse mitochondrial variants among populations were assessed by the Chi square independence test from contingency tables and post hoc analysis. Significant differences were assumed when $P \leq 0.05$.

TABLE 1. *RFLP polymorphisms used for mtDNA Haplogroups determination. Individuals of non-European origin as haplogroups L (African), M (Asian) and those that we could not ascribe to any of the known Caucasian haplogroups were grouped as others (O) as it has been previously reported (5).*
** Indicate non synonymous polymorphism*

Haplogroups	Characteristic restriction site(s) Coding-region mutations	Hipervariable Region I
H	- T14766C* *MseI, Tru9I* - T7028C *AluI*	
V	- T14766C* *MseI, Tru9I* - G4580A *NlaIII*	T16298C
HV*	- T14766C* *MseI, Tru9I*	
J	+ T4216C* *Afl III* - G13708A* *Mva I*	C16069T-T16126C
T	+ T4216C* *Afl III* + A4917G* *Mae I*	T16126C-C16294T
U	+ A12308G *Hinf I*	
I	- A4529T *Hae II*	G16129C-C16223T G16391A
W	- G8994A *Hae III*	C16223T-C16292T
X	+ T14470A *Acc I*	T16189C-C16223T C16278T

RESULTS

The analysis of 686 Spaniard individuals from North and center of Spain gave us a picture of the haplogroup frequencies in the Spanish population (Table 2). Then, we compared our results with those from a collection of 718 Spaniard samples from the literature coming from the whole country (Table 2). The individual haplogroup frequencies were very similar, although we found significant differences (χ = 29.8, df = 9, P < 0.001). These differences were due to an excess of HV* [P (post-hoc cell contribution-phcc) < 0.001] and a defect of W [P (phcc) = 0.009] individuals in our samples versus the other studies {HV*, [P (phcc) < 0.001]; W, [P (phcc) = 0.012]}. However, there was no difference in the major haplogroups. As we were interested in the haplogroup distribution in the whole country because epidemiologic studies require large populations and because the differences were only found in minor haplogroups, we decided to combine all the individuals in a big Spanish sample (1404 individuals).

Next, we compared the whole Spanish sample with a collection of 2648 European individuals from the literature (Table 2). We found significant differences in the haplogroup distribution (χ = 136.0, df = 9, P < 0.001). H, V and HV* were overrepresented in the Spanish population but T, I, and O lineages were underrepresented [P (phcc) $\leq$ 0.009, for all of them]. The opposite tendency was found in the European samples [P (phcc) $\leq$ 0.001, for H, HV*, I and O].

Mitochondrial haplogroup U is an ancient cluster widely distributed in Western Eurasian and very well genetically defined. Therefore, we subdivided our 155 U samples in subhaplogroups and compared them to 1802 U European (6) and 455 Italian individuals (24) (Table 3). Despite some classification problems, we were able to confirm the previously observed tendency in the subhaplogroups U distribution. Those subhaplogroups defined by changes in very well evolutionary conserved positions in the cytochrome b were overrepresented in northern latitudes, the rest were prevalent in southern latitudes (Table 3). An exception was mitochondrial subhaplogroup K. Its prevalence was clearly higher in the South in both Spanish and Italian populations.

TABLE 2. *Mitochondrial haplogroups distribution (number of individuals and percentages values between bracket) in North and Centre of Spain. The results obtained in this study are compared with those found in other studies of Spain: Andalusia (158), Basque country (173), Catalonia (78), Centre Spain (50), Galicia (103), Valencia (30) [34] and Asturias (126) [35]; and European population [27]*

European Haplogroups	This study (N = 686)	Spain (N = 718)	Europe (N = 2648)
H	323 (47.1)	373 (51.9)	1134 (42.8)
V	34 (5.0)	45 (6.3)	111 (4.2)
HV*	31 (4.5)	8 (1.1)	—
J	61 (8.9)	53 (7.4)	260 (9.8)
T	47 (6.9)	40 (5.6)	211 (7.9)
U	155 (22.6)	137 (19.1)	579 (21.9)
I	4 (0.6)	4 (0.6)	59 (2.2)
W	4 (0.6)	14 (1.9)	54 (2.0)
X	8 (1.2)	13 (1.8)	40 (1.5)
O	19 (2.8)	31 (4.3)	200 (7.6)

DISCUSSION

Mitochondrial DNA only encodes proteins involved in the oxidative phosphorylation (OXPHOS) system and the tRNAs and rRNAs necessaries to their expression. This system is the common final pathway in the cell energy production and it is ubiquitous. Therefore, it is a key component in the cell function. By affecting energy production, mitochondrial genetic variants might play a role in the resistance-susceptibility to different phenotypes. As previously mentioned, more and more diseases are being related to the mitochondrial genetic background. However, the molecular bases for these associations are far away to be completely understood yet. It is still unknown which are the haplogroup or subhaplogroup defining polymorphisms causing the phenotypic effects.

TABLE 3. *Subhaplogroups U distribution. Number of individuals and percentage values (in brackets) are given. NE and SE encode for North and South of Europe, respectively. 8 U5 samples from Spain were not subdivided. Urest from Italy includes U subhaplogroups belonging to U1811rest (24)*

			Europe	
Subhaplogroup U	**Spain**	**Italy**	**SE**	**NE**
	(N =155)	(N = 455)		
			(N = 588)	(N =1214)
Urest	21 (13.6)	69 (15.2)	78 (13.3)	52 (4.8)
U4	9 (5.8)	41 (9.0)	59 (10.0)	235 (19.4)
U1811rest	18 (11.6)	72 (15.8)	63 (10.7)	130 (10.7)
Uk	48 (31.0)	154 (33.8)	156 (26.5)	280 (23.1)
U5a	21 (13.6)	72 (15.8)	79 (13.4)	309 (25.5)
U5b	30 (19.4)	47 (10.3)	153 (26.0)	208 (17.1)

A phylogenetic approach to the association studies has the benefit of being an external criterium. However, and due to the high mutational rate of the mtDNA, subhaplogroups from different haplogroups might have similar effects, therefore masking potential associations. In this sense, the lack of association in LHON patients with the haplogroup J in the Iranian population (26) has been recently solved by a finest haplogroup analysis (24). In this population, the major subhaplogroup J is different from that of the European population and it is not defined by those polymorphisms candidate to affect the OXPHOS functionality.

Haplogroups from the HV cluster (H, V and HV*) are more prevalent in the Spanish population. Haplogroup H originated in the Near East but its frequency in this region (20-30%) is lower than the European frequency (40-50%) (27). The Spanish frequency of this haplogroup is in the upper site of this range (49.6%). However, H subhaplogroups have highly distinctive geographical distributions (6, 21-23). The knowledge of these spatial patterns can have important implications for disease studies. Thus, Spanish families affected by nonsyndromic sensorineural deafness due to the mtDNA

1555G mutation in the 12S rRNA gene were overrepresented in the mitochondrial haplogroup H (28). Two alternatives could explain this fact. In the first one, the haplogroup might increase the penetrance of the mutation, similar to the LHON/haplogroup J phenomenon. In the second alternative, a founder event was proposed. The analysis of the H subhaplogroups allowed to chose the second one as the more probable explanation (21).

Haplogroup U has also been associated to different phenotypes (6, 29-31). Particularly interesting is its association with the MELAS syndrome. One study showed an excess of individuals from this haplogroup in patients with MELAS (30). However, another more extensive study was unable to reproduce this association (32). Interestingly, the first study was based in patients from northern Europe and the second one from the south and we have recently shown significant differences in the U subhaplogroup distribution in Europe (6). In the north, subhaplogroups U defined by polymorphisms in very well conserved positions of the cytochrome b and therefore with a potential phenotypic effect are overrepresented. It has been hypothesized that these genetic variants would affect the balance between the energy and heat production (6, 17, 18). Northern haplogroups would be biased to a higher heat production but lower energy production efficiency. The contrary situation would be found in Southern haplogroups. Being this true, then our results would suggest that the Spanish population, poorer in U subhaplogroups defined by cytb mutations, would be less susceptible to develop MELAS syndrome, as observed in one of the previous studies (32).

The finest characterization of the genetic background from different populations is a necessary first step to a rational approach in the epidemiologic association studies as previously proposed in the Human Genome Diversity Project (33).

ACKNOWLEDGMENTS

We would like to thank S. Morales for his technical assistance. This work was supported by the Aragon Government Consolidated Groups (B33), the Spanish Network for Mitochondria Disorders

(G03-011/G03-056), FIS (PI050639, PI050647 and PI050648) and the European Commission (QLG-CT-2001-0096).

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