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MtDNA analysis of ancient samples from Castellón (Spain): Diachronic variation and genetic relationships

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Abstract. Thirty-seven bone and teeth samples from Chalcolithic and Iberian ages from several sites located in Castellón (Spain) were analyzed for mtDNA HVRI polymorphisms. Despite of the presence of *PCR* inhibitors, we recovered 150 bp fragments in nine cases. Lineages suggest a close relationship among individuals from the same archaeological site, due to a possible familiar relationship or to a common ethnic origin. Chalcolithic haplotypes differed from those recovered from Iberian samples. This indicates a possible genetic replacement between both periods in the Spanish Levant. © 2005 Published by Elsevier B.V.

Keywords: Ancient DNA; Mitochondrial DNA; Chalcolithic; Iberia

1. Introduction

Genetic studies suggest a Palaeolithic origin of European mtDNA diversity [1–3]. However, recent ancient mtDNA studies show that mtDNA composition of European populations may have changed substantially since Neolithic ages and even more recently [4]. We present results from a diachronic study of the Spanish Levant. This region, opened to the sea, has received several documented historical and prehistoric immigratory events. This work contains preliminary results of a wider project aimed to determine the genetic origins of this population from Palaeolithic to present times.

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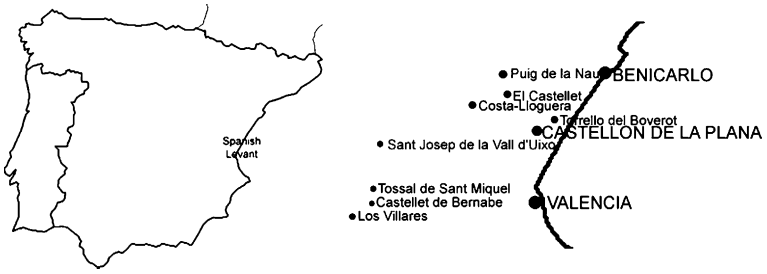

Fig. 1. Location of archaeological sites.

2. Materials and methods

Thirty-nine bone and teeth samples from Chalcolithic and Iberian ages from several archaeological sites from Castellón de La Plana and Valencia (Spain) were studied archaeological sites and samples are listed in Fig. 1 and Table 1, respectively. Extraction process was carried out into a separate isolated room, sterilized through UV radiation. Samples were cleaned superficially with a dentist grill (*Sand-Blaster Base 1 Plus, Dentalfarm*) and then ground in a cryogenic impact grinder (*Freezer Mill, Spex 6700*). Powder (600 mg) was first washed with 0.5 M EDTA pH 8.0 and then incubated o.n. at 37 °C into lysis buffer (5 mM EDTA, 10 mM TRIS, 0.5% SDS, 50 mg/ml proteinase-K). DNA was extracted by a phenol/chloroform protocol. Aqueous phase was concentrated with *Centriplus 30.000 (Mlilipore)*. Extraction controls without powdered sample were processed in parallel to test for contamination during the extraction process. Amplification was performed in another UV sterile room. HVR-I amplicons of two 150 bp overlapping fragments each (positions 16126–16369) were obtained through a Nested-PCR assay [5]. An inhibition test consisting on the addition of different aDNA volumes to a *PCR*-mix with "fresh DNA" was performed. Absence or diminishing of amplicon in a 2% agarose gel was interpreted as evidence of inhibition.

3. Results

Results are shown in Table 2. Low recovery efficiency (23.07%) probably reflects the high amount of ancient DNA extracts containing *PCR* inhibitors (51.28%). The highest probability of an haplotype, given by a mtDNA database [5], is indicated in Table 2. Frequencies of partial haplotypes are also included.

Table 1

Archaeological site	Location	No.	Sample	Age
El Castellet	Castellón	2	Bone	Chalcolithic
Costa-Lloguera	Castellón	8	Tooth	Chalcolithic
		2	Bone	Chalcolithic
Puig de la Nau	Benicarló (Castellón)	18	Bone	Iberian
Sant Josep	Vall d'Uixó (Castellón)	1	Bone	Iberian
Torrelló del Boverot	Castellón	2	Bone	Iberian
Los Villares	Caudete de las Fuentes (Castellón)	1	Bone	Iberian
Tossal de Sant Miquel	Líria (Valencia)	3	Bone	Iberian
Castellet de Bernabé	Líria (Valencia)	2	Bone	Iberian
Total		39		

Table 2
MtDNA partial haplotypes (positions 16258–16369) obtained in the studied samples

Sample	Archaeological site	Period	Haplotype	Frequency	Haplogroup
COST1	Costa-Lloguera-2	Chalcolithic	*CRS*	26.32%	H/U (43.6%)
COST3	Costa-Lloguera-2	Chalcolithic	*CRS*	26.32%	H/U (43.6%)
COST4	Costa-Lloguera-2	Chalcolithic	*CRS*	26.32%	H/U (43.6%)
COST5	Costa-Lloguera-2	Chalcolithic	*CRS*	26.32%	H/U (43.6%)
3COST1	Costa-Lloguera-3	Chalcolithic	16362C	1.98%	H (51.78%), pre-HV (23.21%)
3COST3	Costa-Lloguera-3	Chalcolithic	16362C	1.98%	H (51.78%), pre-HV (23.21%)
3COST4	Costa-Lloguera-3	Chalcolithic	*CRS*	26.32%	H/U (43.6%)
PB14	Puig de la Nau	Iberian	16293T, 16298C	$<3.55\times10^{-4}$	V
PB20	Puig de la Nau	Iberian	16293T, 16298C	$<3.55\times10^{-4}$	V

4. Discussion

Results show a low variability among individuals. Since mtDNA is a lineage marker, maternal relationships can not be inferred from individuals sharing the same mtDNA haplotype. Thus, our results can reflect matrilineal genetic relationships among these individuals but also low diversity in the population. Most of the samples belong to the common European motif *CRS*. This is a poor evidence of kinship. However, the other two samples present less common haplotypes, this suggesting a more strongly relationship between, for instance, PB14 and PB20. Haplotypes from Iberian samples differed so much from those from Chalcolithic ones. Iberian sequences can be classified as belonging to European haplogroup V. In Spain, this haplogroup shows the highest frequency in the Basque Country (12.4%) [5]. In Eurasia, it is virtually absent in Middle Eastern populations while reaches the highest frequency among the Saami [5]. However, haplogroup V has not been found in Neolithic and Bronze Age samples of the Basque Country [6]. Given that the amount of samples is still scarce and the presence of factors like poor preserved DNA or the possible existence of kinship among the individuals of the same burial, definitive populational conclusions still cannot be achieved. The results, however, seem to suggest a possible genetic replacement between both periods in the Spanish Levant. If this conclusion was confirmed, the origin of the Spanish Levant population would be traced back to a post-Neolithic age.

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