



Haplogroup H in prehistoric osseous remains from the Basque Country as a genetic marker to study the resettlement of Europe

S. Cardoso ^{a,*}, S. Amory ^b, M. Álvarez ^a, A. Gómez ^c,
C. Keyser-Tracqui ^b, B. Ludes ^b,
J. Fernández Eraso ^c, M. de Pancorbo ^a

^a *Servicio de Genómica, Banco de ADN, University of The Basque Country, Basque Country, Spain*

^b *Institut de Médecine Légale, Strasbourg Cedex, France*

^c *Faculty of Geography and History, University of the Basque Country, Basque Country, Spain*

Abstract. Working with ancient DNA is an extremely useful approach in prehistoric population genetics. In this work we observed that all the samples already analysed belonged to haplogroup H of the mtDNA. Thus, we aimed to check the frequency of haplogroup H in samples from the Basque Country to assess the possibility of using it as a genetic marker of the postglacial recolonization that occurred in Europe after the LGM, approximately 15,000 years ago. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

Wide studies have been done on how the resettlement of Europe took place after the end of the Last Glacial Maximum (LGM), approximately 15,000 years before the present. The Franco-Cantabrian coast is said to have played a major role as a refuge during the LGM and as an expansion focus during the resettlement of the European continent [1]. These studies have been carried out using mainly mitochondrial DNA data from modern populations. However, these data could be influenced by some aspects such as the

* Corresponding author. Servicio de Genómica, Banco de ADN, Facultad de Farmacia, Paseo de la Universidad, 7 01006, Vitoria-Gasteiz, Spain. Tel.: +34 945 013849; fax: +34 945 013014.

E-mail address: zobcamas@vc.ehu.es (S. Cardoso).

Table 1

Complete profiles obtained with *AmpFISTR*[®] Profiler Plus[®] Kit for two of the samples analysed in this study

	Amel	D13	D18	D21	D3	D5	D7	D8	FGA	vWA
LY010	X, Y	8, 10	12, 17	28, 29	18, 18	11, 13	10, 12	12, 14	21, 23	17, 19
LY013	X, X	8, 12	12, 20	29, 29	16, 18	12, 13	6, 12	11, 14	20, 24	17, 19

Contamination was discarded by comparing them with the profiles obtained for all the members of the researcher group.

variation generated along the generations by prehistoric and historic migrations or genetic drift. To overcome these problems it is of great value to use ancient DNA. Working with ancient osseous remains to obtain DNA requires extremely careful manipulation and is not always successful [2]. However, the possibility of analysing ancient mitochondrial DNA is of great interest in this kind of studies.

2. Materials and methods

Right ulna bones and upper right canines, collected in the site of Las Yurdinas II (Álava, Spain), were analysed. These samples yielded a radiocarbon date of 4350 ± 50 years.

Samples were processed following a described protocol [3]. The surface of the bones was removed with a sanding machine (Dremel) to eliminate contaminants. The surface of the teeth was deeply cleaned with distilled water, DNA Away[™] and absolute alcohol.

Powdered bone was generated by grinding bone fragments under liquid nitrogen in a 6800 Freezer Mill (Fischer Bioblock). For the canine LYd13, it was directly opened in two halves following a longitudinal axis with a sandpaper disc attached to a sanding machine. The powdered bone was incubated overnight in buffer solution (proteinase K 20 mg/ml) at 55 °C. After phenol/chloroform/isoamyl alcohol extraction, the DNA was purified by affinity chromatography (Cleanmix ,Talent) and concentrated by Microcon YM-30 (Millipore).

Six bone samples were analysed for microsatellite DNA with *AmpFISTR*[®] Profiler Plus[®] Kit.

PCR was performed for the region HVI of mtDNA in 14 samples and the product of amplification tested by agarose gel.

Amplified DNA was analysed in an ABI Prism 3100 DNA sequencer. Genotyper was used to analyse the results for nuclear DNA. Sequences for mtDNA were edited

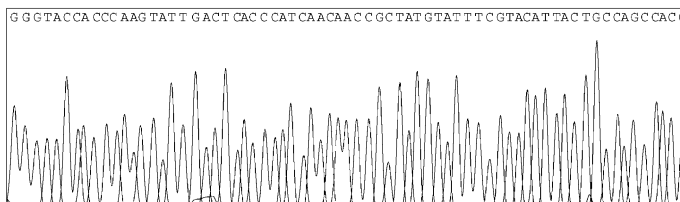


Fig. 1. Only high quality sequences were considered as positive results. This figure shows part of the HVI region for the sample LYd4 as an example of the kind of sequences analysed in this work.

MitomapRCRS Historical LYd4-hv1a-F	TCACCCATCAACAACCGCTATGTATTTGCGTACATTACTGCCAGCCACCAT TCACCCATCAACAACCGCTATGTATTTGCGTACATTACTGCCAGCCACCAT *****
MitomapRCRS Historical LYd4-hv1a-F	GAATATTGTACGGTACCATAAACTACTTGACCACCTGTAGTACATAAAAAC GAATATTGTACGGTACCATAAACTACTTGACCACCTGTAGTACATAAAAAC *****
MitomapRCRS Historical LYd4-hv1a-F	CCAATCCACATCAAACCCCTCCCCATGCTTACAAGCAAGTACAGCAAT CCAATCCACATCAAACCCCTCCCCATGCTTACAAGCAAGTACAGCAAT *****

Fig. 2. After editing the sequences by means of the program Chromas Pro, they were aligned, as shown here for the sample LYd4, with the revised Cambridge Reference Sequence using Clustal W.

using Chromas Pro and compared with the reference sequence (rCRS) by means of Clustal W.

3. Results and conclusions

In order to prevent contamination samples were processed in specific laboratory for ancient DNA and negative controls for all the steps were included. Moreover, all the people involved in the processing of the samples were typed. Thus it was possible to discard any false positive result caused by external contamination.

Two of the six samples analysed for microsatellite DNA yielded a complete profile (Table 1). These results show that it would be possible and very interesting to get results of nuclear DNA for more samples as a way to study the familiar structure in Basque prehistoric populations.

Fourteen samples were analysed for the HVI region of mtDNA and 7 of them, 2 teeth and 5 bone samples, yielded high quality sequences (Fig. 1).

The 7 samples compared with the rCRS belonged to haplogroup H (Fig. 2). Our results probably reflect the fact that this haplogroup was the most frequent in prehistoric populations from the Basque Country. Thus, it could be possible to consider this haplogroup as a genetic marker for the Palaeolithic expansion, as stated from modern populations data. The next step would be to study a greater number of samples with the aim of establishing a theory about the importance of haplogroup H and its subhaplogroups in the contribution to the gene pool in Europe after the LGM [4].

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