



Genetic structure of autochthonous Basques through analysis of the HVI and HVII regions of mitochondrial DNA

C. Martínez-Bouzas^{a,*}, A. Castro^{b,c}, I. Fernández-Fernández^{b,c},
J.L. Rodríguez-Filgueira^a, M.M. de Pancorbo^{a,c}

^a*Dpto. de Z. Dinámica Celular A., F. Farmacia, U. País Vasco, Vitoria-Gasteiz, Spain*

^b*DataGene, Sondika (Bizkaia) Spain*

^c*Instituto Vasco de Criminología, U. País Vasco, Donostia-San Sebastián, Spain*

1. Introduction

Mitochondrial DNA (mtDNA) analysis has proven to be of great interest in human population studies to study phylogenetic relationships and differentiation among individuals [1]. MtDNA differs from nuclear DNA in the absence of recombination, maternal inheritance, large number of copies per cell and a mutation rate that is 5–10 times higher than that of nuclear DNA [2,3]. The non-coding region of mtDNA, known as the D-loop, accumulates more mutations than the rest of the molecule, making it a useful tool for studying short-term evolutionary phenomena [4], and the tool of choice for diagnosing matrilineal relationships.

A number of scientists have studied the mtDNA of Basque populations. Bertranpetit [5] and Corte-Real [6] have analyzed the base sequences of the hypervariable segment I (HVI). All of these studies were based on an underlying assumption of homogeneous population structure in the autochthonous Basque group. The D-loop regions analyzed in this study were HVI and HVII in two autochthonous population samples Arratia and Goierri, linguistically and geographically differentiated. Since the HVII segment has not been previously studied in samples from the Basque Country, the analysis in these populations will provide better information on the variability of mtDNA in the autochthonous Basque population and its usefulness in forensic casework.

Objectives: (a) to determine the homogeneity or heterogeneity of mtDNA in the autochthonous population of the Basque Country and to use the information contained in the sequence variations of the mtDNA of these fragments to analyze their phylogenetic

* Corresponding author.

relationships with other population groups; (b) to find the power of discrimination between maternal lineages using the combined analysis of HVI and HVII segments in the autochthonous population of the Basque Country.

2. Materials and methods

The HVI and HVII segments were studied in 55 autochthonous Basques from Arratia and Goierri regions. The DNA was extracted from peripheral blood leukocytes using the phenol–chloroform method. The HVI and HVII segments were amplified using the primers L15996 and H16401 and L29 and H408, respectively [7]. The M13 universal primers were added to these primers to sequence the PCR products using the BigDye primer kit (Applied Biosystems). Nucleotide substitutions and insertions/deletions were found using Anderson's reference sequence, followed by computation of match probability and discrimination power values. The genetic structure of the population samples was analyzed by calculating nucleotide diversity, gene diversity and mean number of pairwise differences.

The phylogenetic analysis was made following classification into haplogroups previously established for the HVI segment and after making a new classification to group the haplotypes of the HVII segment. Genetic distance D was calculated [8,9] yielding phylogenetic trees.

3. Results

HVI and HVII sequences of mtDNA were studied between 16024 and 16383 bp and 69 to 383 bp in 30 individuals from Arratia and 25 individuals from Goierri. Table 1 shows the sequence polymorphisms and diversity parameters in these samples of population.

The nucleotide and sequence diversities of Arratia and Goierri are similar, and lower than in other European populations. The mismatch observed means are also low; accordingly to this, the probability random match is a little higher than in other European populations.

The F_{st} and gene differentiation test between Arratia and Goierri were calculated (Table 1). No statistically significant P_{Fst} and P of gene differentiation exact test were found. These results indicate no gene substructure of mtDNA in these Basque population samples. Considering that Arratia and Goierri are linguistic and geographically very different Basque samples, the absence of mtDNA substructure between them may mean the lack of gene substructure in matrilineal lineages from the Basque population.

The Arratia and Goierri HVI combined haplotypes were classified into haplogroups as Ref. [10] in order to perform phylogenetic analysis of this Basque sample and other worldwide populations. The increase of the phylogenetic information provided by the HVII haplotypes was carried out by the subclassification of the HVI haplogroups, using the HVII nucleotide sequences. This procedure has allowed a new classification of the largest HVI haplogroups such as H, M, I, C, T and U. The distinct topology of the trees was obtained using the two systems of classification: (a) HVI haplogroups and (b) HVI–

Table 1
 Sequence polymorphisms, gene diversity, Fst test and gene differentiation test obtained for Arratia and Goierri Basque population samples

	Constant sites	Transitions	Transversions	Deletions	Insertions	Nucleotide diversity	Sequency diversity	Mismatch observed mean	Random match	P_{Fst}	Gene differentiation test
<i>HVI</i>											
Arratia	317 93%	22	1	–	1	0.008 ± 0.005	0.890 ± 0.052	2.892	0.14		
Goierri	315 92.4%	20	3	1	3	0.011 ± 0.007	0.946 ± 0.031	3.953	0.088		
Arratia + Goierri	306 89.7%	29	3	1	3	0.010 ± 0.006	0.917 ± 0.030	3.353	0.336	0.423 ± 0.045	0.487
<i>HVII</i>											
Arratia	297 92.2%	22	–	–	3	0.013 ± 0.007	0.986 ± 0.013	4.078	0.062		
Goierri	304 94.4%	15	–	–	4	0.012 ± 0.007	0.970 ± 0.022	3.773	0.075		
Arratia + Goierri	292 90.7%	24	–	–	3	0.012 ± 0.007	0.971 ± 0.011	3.908	0.113	0.117 ± 0.009	0.052
<i>HVI+ HVII</i>											
Arratia	613 92.5%	44	1	–	4	0.010 ± 0.006	0.982 ± 0.013	7.910	0.043		
Goierri	619 93.4%	35	3	1	6	0.012 ± 0.006	0.990 ± 0.014	7.100	0.067		
Arratia + Goierri	597 90%	53	3	1	6	0.011 ± 0.006	0.989 ± 0.006	7.189	0.05	0.901 ± 0.009	0.096

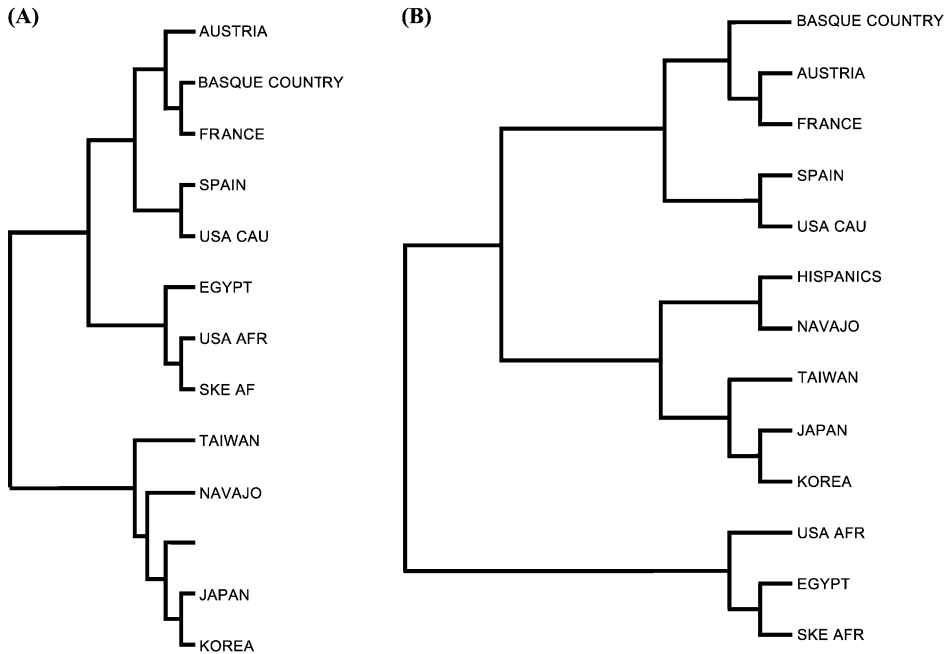


Fig. 1. UPGMA tree of the genetic distance D : (A) tree based on HVI haplogroups, (B) tree based on HVI+HVII haplogroups. Austria, France, Korea, Japan, Spain, Taiwan, Egypt, SKE Afr., USA Afr., USA CAU, Hispanics and Navajo populations were studied by Budowle et al. [11].

HVII haplogroups. They are shown in Fig. 1. The combined HVI–HVII haplogroup has more phylogenetic information than HVI so this combination of HVI and HVII gives a tree with the best separation of racial groups. The Basque population appears in this tree integrated into the Caucasian cluster and without differentiation from the European populations. Furthermore, this tree proves to be more specific and it discriminates between Amerindian–Hispanic populations and Asian populations.

In summary, no genetic substructure was observed in the two Basque population samples studied. These results are sufficiently indicative of the lack of heterogeneity in the maternal lineages of the autochthonous Basque population. The sequence diversity observed is similar to that of other Caucasoid populations, meaning that the power of discrimination value is also similar. Finally, phylogenetic analyses place the Basque populations among the Caucasoids, without providing evidence for differential characteristics in the mtDNA of this population.

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