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## The mitochondrial lineage U8a reveals a Paleolithic settlement in the Basque country.

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TITLE: The mitochondrial lineage U8a reveals a Paleolithic settlement in the Basque country.

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## **Abstract**

### **Background**

It is customary, in population genetics studies, to consider Basques as the direct descendants of the Paleolithic Europeans. However, until now there is not an irrefutable genetic proof to support this supposition. Even studies based on mitochondrial DNA (mtDNA), an ideal

It has been demonstrated, for the first time, that Basques show the oldest lineages in Europe for subhaplogroup U8a. Coalescence times for these lineages suggest their presence in the Basque country since the Upper Paleolithic. The European U8 phylogeography is congruent with the supposition that Basques could have participated in demographic re-expansions to repopulate central Europe in the last interglacial periods.







quantitatively minor clade that partially overlaps with U8a in Europe. However, its presence





DNA isolated from bucal swabs or blood samples from 211 autochthonous, unrelated Basques from the Iberian provinces were analyzed. Appropriate informed consent to anonymously use their data was obtained from all the individuals sampled.

### **HVSI-II and RFLPs**

Total DNA was PCR amplified as in Pinto et al [34], and directly sequenced for both complementary strands as detailed in Rando et al [35]. A sequence of 978 bp of the HVSI-II of the mtDNA control region, from position 15997 to 00408 [36] was determined and sorted into defined haplogroups [24]. To confirm this HVS-based haplogroup classification, all individuals assigned to a specific haplogroup were additionally tested by restriction analysis of the diagnostic coding region mutations proposed to unambiguously classify sequences into haplogroups [24].

### **Complete mtDNA sequences**

Four Basques (three U8a, one K1) and one Jordan (U8b) rare lineages belonging to the U/K haplogroup were fully sequenced. The complete mitochondrial DNAs (mtDNA) were amplified by PCR using primer pairs already described [19]. Amplified products were sequenced for both complementary strands with the Big Dye Terminator Cycle sequencing kit (Applied Biosystems). Sequencing reactions were analyzed on an Applied Biosystems 3100 DNA analyzer.

### **Genetic analyses**

Genetic diversity ( $p \pm \sigma$ ) was estimated as the average number of nucleotide differences between two sequences [37], using the HVSI region in the range from 16070 to 16365 nucleotide positions.



- 5) Collection of data to assess the distribution of U8a/b haplogroups in Europe, Near-East, Asia and Africa
- 6) Phylogenetic reconstruction for HVSI, and complete mtDNA sequences
- 7) Discussion of the results obtained
- 8) To draft the manuscript (text, table, figures and additional files)

AMG participated in 1, 4, 5, 6, 7 and 8 steps. OG participated in 1,2, 3, 5, and 7 steps. JML participated in 1, 3, 4, 5, 7 and 8 steps. VMC participated in 1, 2, 4, 6, 7 and 8 steps. All authors read and approved the final manuscript.

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## Figures:

### Figure 1 - Phylogenetic tree based on complete U8 sequences.

Numbers along links refer to nucleotide positions. Open boxes are nodes from which other (not shown) sequences branch. A, C, indicate transversions; “d” deletions and “i” insertions. Star has the following mutations with respect to rCRS: 73, 263, 311i, 750, 1438, 2706, 4769, 7028, 8860, 11719, 14766 and 15326 and the following ones respect to L3\*: 8701, 9540, 10398, 10873, 12705, 15301, 16223, 16519. Subject origins are: Dutch (Dut86; [38]),

Austrian; BQ = Basque; BRI = British-australian; EST = Estonian; FIN = Finn; FRA = French; GER = German; KAR = Karelian; LIT = Lithuanian; NCE = North-central European; NEE = Northeast European; NOR = Norwegian; NSP = Northeast Spanish; POL = Polish; POR = Portuguese; RUS = Russian; SCT = Scotch; SIC = Sicilian; SLO = Slovenian; SPA = Spanish; SSP = South Spanish; SWZ = Swiss; TUK = Turk; VOL = Volga-Ural.

## **Tables**

**Table 1 - Gene diversity (p) and frequency of U8a/**

## **Additional files**

### **Additional file 1 - European distribution of subhaplogroup U8a/1.**

File González\_add1.xls have data about sample and percentage of U8a/1 haplogroup in different populations from Europe and North-Africa.

### **Additional file 2 - References cited in additional file 1.**

File González\_add2.doc shows a list of references cited in additional file 1.



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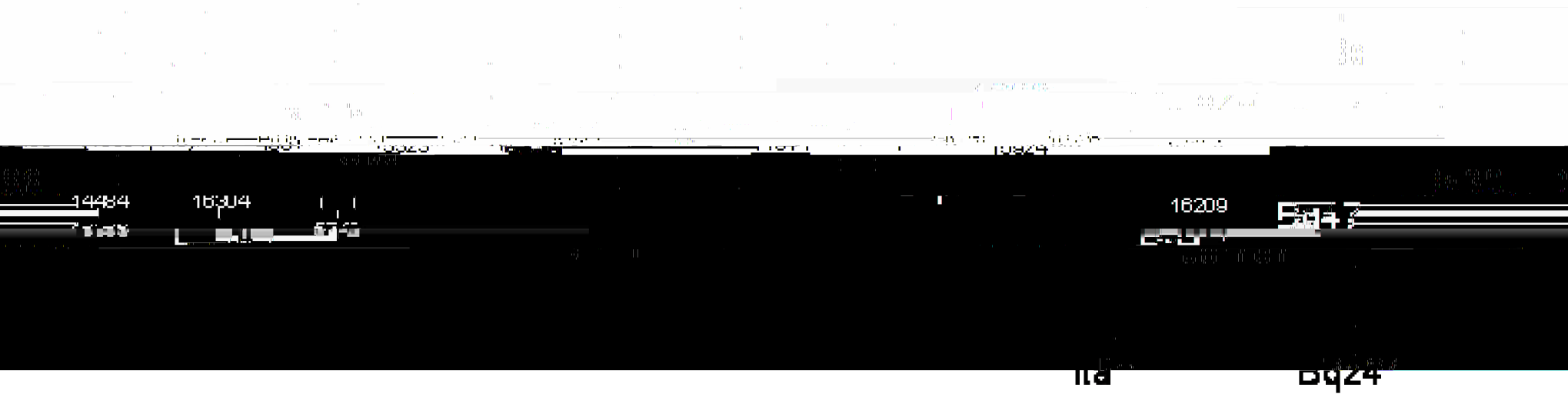


Figure 1



Figure 2



**Additional files provided with this submission:**

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