Supplementary material

Samples and extraction of ancient DNA

The samples used in this paper are dental pieces, which compared to bones, are less liable to external contamination. Thus, to prevent the contamination of the endogenous DNA, we carefully selected those teeth that did not show any signs of caries or deep fissures that might extend into the pulp, our source of DNA.

The samples were processed according to a series of previously detailed criteria [39;40]. Thus, the extraction of DNA and set-up of the PCR were done in a positive-pressure, sterile chamber, which was physically separated from the laboratory where the post-PCR processes were carried out. All of the working surfaces were regularly cleaned with sodium hypochlorite and were, in addition, regularly irradiated with UV light. If possible, PCR reagents were exposed to intense UV light before use. Suitable disposable clothing was worn (lab coat, mask, gloves and cap). Contamination controls were applied during both the extraction and amplification processes. In parallel, we analyzed a second tooth from each of five samples in an independent laboratory.

In order to eliminate surface contamination, the teeth were washed with acids to depurinate possible contaminating external DNA. Besides, the entire tooth surface was irradiated with ultraviolet light. Then, after cutting the root of the tooth, it was incubated at 37ºC and with agitation overnight in a lysis buffer (5 ml) (0.5M EDTA pH 8.0–8.5; 0.5% SDS; 50mM Tris HCl pH 8.0; 0.01mg/ml proteinase K). Then, DNA was extracted using the conventional phenol-chloroform procedure. After the extraction, the DNA was concentrated and purified by means of Centricon-30 Amicon spin columns, (Millipore, Billerica, MA, USA). Each extraction session involved two contamination controls, i.e., blank tubes that were processed as normal samples.

DNA Quantification

We used our standard procedure to quantify the extracted DNA, which includes measuring the number of molecules of a segment of 100bp of HVR-I of mtDNA (Table S2).by means of RT-PCR (Step-One, Applied Biosystems). For this, we used as forward primer the oligo 5'-CACCATTAGCACCCAAAGCT-3' and as reverse primer the oligo 5'-ACATAGCGGTTGTTGATGGG-3'. These primers define a 113bp fragment. The Taqman probe sequence was: VIC-5'-GAAGCAGATTTGGGTAC-3' (Applied Biosystems). Real-time PCR amplification was performed in 30µl containing 1X TaqMan Universal PCR Master Mix (Applied Biosystems), 5 µM each primer, 10 µM probe, and 10µl DNA extract (diluted 1/10 with BSA). The cycling conditions were 50°C for 2min, 95°C for 10min, 45 cycles of 95°C for 15s and 60°C for 1min. in a StepOne Real-Time PCR System. Serial dilutions of a fragment of 450 bp (synthesized by Eurofins MWG/Operon) (from $1{,}4\times10^{6}$ to 140 molecules) were included in each experiment to generate the standard curve. At least three "no-template-control" were included with each experiment.

Reproducibility of the results

In parallel, we assessed the reproducibility of the results obtained in the University of the Basque Country by replicating the analysis of 6 overlapping fragments of mtDNA HVR-I of second tooth from each of 5 samples in additional independent laboratories, either in The Netherlands (Radboud University Nijmegen Medical Center), or in Spain (University La Laguna). MtDNA was chosen because its high variability increases the chances to individualize genetically each sample, and therefore to reduce the possibility of a false positive match (see Table S2). To further corroborate the results, PCR products corresponding to these 6 fragments of mtDNA HVR-I of 5 different samples were cloned (TOPO TA Cloning® Kit, Invitrogen) and from each cloning reaction 10 colonies were picked up, sequenced and checked for coincident results. In this case all clones showed the same sequence as the original PCR product

as expected. Finally, the mtDNA types of most of the personnel that was in contact with the samples was also obtained (Table S3) in order to exclude possible contamination from the manipulators.

Amplification and sequencing of TLR4

PCR amplification was performed with the incorporation of the extraction controls obtained during the DNA extraction process. Negative controls for the PCR reaction were included. For the detection of each *TLR4* polymorphism one primer pair was used to amplify the region of interest. Both regions are located in exon 3 of the *TLR4* gene. PCR products were obtained by cycling 96ºC 1min for 1 cycle, followed by 45 cycles of 95ºC 15s, anneal temperature 30s, 72ºC 30s and a final cycle of 72ºC 10min, using the primers listed in Table S4, accompanied by the corresponding annealing temperatures. After the amplification PCR products were purified by ExoSAP-IT (USB Corporation, OH, USA). Both forward and reverse sequences were obtained using the listed primers and Rhodamine chemistry in an ABI310 (Applied Biosystems, CA, USA).

Table S1. Number of studied individuals from each population and allele frequencies for *TLR4* SNPs Asp299Gly and Thr399Ile.

Population (number)	WT/WT	Asp299Gly/WT	WT/Thr399Ile	Asp299Gly/Thr399Ile
Trio-Indians, Surinam (N = 99)	1.00	-	-	-
Papua New Guinea (N = 49)	1.00	-	-	-
Han Chinese (N = 100)	1.00	-	-	-
Indonesia (N = 98)	0.985	0.005	0.01	
Netherlands (N = 209)	0.928	-	-	0.072
Germany (N = 632)	0.943	0.002	-	0.055
Basque, Spain (N = 107)	0.907	-	-	0.093
Greece (N = 162)	0.963	0.003	0.006	0.028
Romania (N = 102)	0.917	-	0.005	0.078
Sudan (N = 101)	0.901	0.094	-	0.005
Cameroon (N = 142)	0.933	0.063	-	0.004
Tanzania (N = 121)	0.926	0.058	-	0.017
Dogon tribe, Mali (N = 241)	0.911	0.083	-	0.004
Fulani tribe, Mali (N = 243)	0.975	0.025	-	-

Table S2. Results of quantification, replication and cloning of a subset of samples.

Sample	site	molec/µl	HVR-I[a, b. c]	replicated by lab
SJ-105	SJAPL	-	CRS	Netherlands
SJ-234	SJAPL	7348	224-311	Netherlands
SJ-242	SJAPL	9238	069-126-278	Netherlands
SJ-246	SJAPL	30344	270	Netherlands
SJ-266	SJAPL	1241	311	Netherlands
AIZ	Aizpea	12797	051-093-189-192-270	La Laguna, Spain

[a]HVR-I: Hypervariable Region I of mtDNA

[b]CRS: Cambridge Reference Sequence.

[c]The figures correspond to the position in region I of HVR of mtDNA that change with respect to the CRS. Precise mitochondrial coordinates can be obtained by adding 16,000.

Table S3. Mitochondrial haplotypes (HVR-I) of researchers in this study.

Lab	researcher	HVR-I haplotype[a]
Univ. Basque Country (Spain)		
	Researcher #1	CRS
	Researcher #2	CRS
	Researcher #3	189
	Researcher #4	319
	Researcher #5	092-224-311
Univ. La Laguna (Spain)		
	Researcher #1	129-316
	Researcher #2	227-278-311-362
Radboud Univ. (The Netherlands)		
	Researcher #1	CRS
Archaeologists		
	Researcher #1	304
	Researcher #2	291
	Researcher #3	220
	Researcher #4	051-162-264
	Researcher #5	298

[a]HVR-I: Hypervariable Region I of mtDNA. CRS: Cambridge Reference Sequence. The figures correspond to the position in region I of HVR of mtDNA that change with respect to the CRS. Precise mitochondrial coordinates can be obtained by adding 16,000.

Table S4. Primer sequences and annealing temperatures used to amplify the two regions of interest of exon 3 of the *TLR4* gene.

Target	Forward primer	Reverse primer	Anneal temp. (°C)
D299G	5'-CCATTGAAGAATTCCGATTAGC-3'	5'-TCACCAGGGAAAATGAAGAAA-3'	55
T399I	5'-TCAAAGGTTGCTGTTCTCAAA-3'	5'-CCAAGAAGTTTGAACTCATGGTA-3'	59.5