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Human β-Globin Gene Polymorphisms Characterized in DNA Extracted from Ancient Bones 12,000 Years Old

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Summary

Analyzing the nuclear DNA from ancient human bones is an essential step to the understanding of genetic diversity in current populations, provided that such systematic studies are experimentally feasible. This article reports the successful extraction and amplification of nuclear DNA from the β-globin region from 5 of 10 bone specimens up to 12,000 years old. These have been typed for β-globin frameworks by sequencing through two variable positions and for a polymorphic $(AT)_x(T)_y$ microsatellite 500 bp upstream of the β-globin gene. These specimens of human remains are somewhat older than those analyzed in previous nuclear gene sequencing reports and considerably older than those used to study high-copy-number human mtDNA. These results show that the systematic study of nuclear DNA polymorphisms of ancient populations is feasible.

Introduction

Current efforts to expand our understanding of the migration patterns of human populations—and in the process, to learn more about our evolution—are based on two complementary strategies: macroscopic examination of ancient and fossil bone specimens and analysis of genetic polymorphism in living populations. The sequencing of ancient DNA preserved in ancient bones should form a highly informative stepping-stone, provided, of course, that such systematic studies are experimentally feasible.

The interest in ancient DNA has grown considerably since it was first demonstrated that mtDNA could be recovered from dried muscle of the quagga (Higuchi 1984). These pioneering studies demonstrated that at least some molecules of ancient DNA can still survive intact today. Later, DNA amplification by PCR (Saiki et al. 1985) made systematic studies possible. At the beginning, most research focused on DNA extracted from soft tissues (Pääbo 1985, 1989; Doran et al. 1986; Pääbo et al. 1988; Del Pozzo and Guardiola 1989; Thomas et al. 1989, 1990; Golenberg et al. 1990; Lawlor et al. 1991; DeSalle et al. 1992; Höss et al. 1994). If the DNA is preserved in optimum condition, startling results can be obtained, as illustrated by the sequencing of DNA extracted from a 120–135-million-year-old weevil entombed in Lebanese amber (Cano et al. 1993). This analysis, however, relied on very rare and exceptionally well-preserved archaeological remains. As bone remains are much more numerous than soft archaeological specimens, they form a much larger pool of ancient biological material, suitable for the systematic vertical study of genetic diversity. Recently, it has proved possible to amplify successfully and sequence mtDNA (Hagelberg et al. 1989, 1994; Horai et al. 1989; Hänni et al. 1990; Hagelberg and Clegg 1991) and even nuclear DNA (Hummel and Herrman 1991; Kurosaki et al. 1993; Gill et al. 1994) from such material. Since it is maternally inherited and evolves quickly, mtDNA has become a powerful tool for unraveling the ancient human migrations. However, although mtDNA variations are indeed a valuable source of relevant data, they are of limited use in studies of human diversity, since gene flow through maternal lineage only is expressed. On the other hand, a large number of nuclear DNA polymorphisms have now been catalogued, and it has been shown that each locus can serve as an independent estimate of the evolutionary history of the population. Thus, data on both nuclear and mitochondrial DNA polymorphisms should be accumulated for a complete molecular genetic analysis of ancient human bones, especially in the case of specimens from human populations considered as "key populations" for human evolution.

It has recently been shown that β-globin haplotypes are significantly diverse when sequenced, making the β-globin gene a good candidate for detailed analysis of allelic sequence variation in human populations (Fuller-

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ton et al. 1994). We began to analyze the conserved intragenic framework of the β-globin gene (Orkin et al. 1982) to prove that analysis of ancient nuclear DNA is actually feasible. Clearly the critical issue is to verify that DNA extracted from fossil material is not chemically altered to such an extent that would prevent further analysis of polymorphisms. Then, as a first approach to analyzing sequence variations in human populations, we focused on the highly polymorphic upstream noncoding region encompassing the microsatellite $(AT)_x(T)_y$ (Moschonas et al. 1982; Semenza et al. 1984; Chebloune et al. 1988).

The populations in Africa today are recognized as being the most diverse, irrespective of the level of genetic analysis (genetic distance, nuclear DNA polymorphism, or diversity of mtDNA). We therefore chose half of our human specimens from archaeological sites in Africa, to increase the likelihood of finding different haplotypes for different individuals.

This article describes experiments in which we successfully extracted, amplified, and sequenced fragments of the β-globin gene from 12,000-, 7,000-, and 1,200-year-old human bone remains. This study demonstrates that ancient human nuclear DNA polymorphisms can be systematically analyzed.

Material and Methods

Specimens of Old Human Bones

The following 10 specimens were studied: two independent specimens (*Taf* 1 and *Taf* 2) from Taforalt, an archaeological site in western Morocco occupied by man 12,000 years before present (BP); one specimen (*Mal*) from Hassi el Abiod in the Malian Sahara dated 7,000 years BP; one specimen (*S'Is*) from S'Isterri d'Olzu, Sassari in Sardinia, dating from the Bronze age, i.e., ~3,000 years BP; one specimen (*Gei*) from Geili, Khartoum in Sudan, selected from the Meroitic period (3d century B.C.), thus dated 2,300 years BP; one specimen (*Cam*) from Il Campo deï Miracoli, Pisa from a 6th century, High Middle Age archaeological site in Italy, i.e., ~1,400 years BP; two specimens (*Vol* 1, *Vol* 2j) from Volonne, an 8th century, High Middle Age archaeological site in France, i.e., 1,200 years BP; one specimen (*Mat*) from Matera, in Lucania, a region in southern Italy, dated as medieval, i.e., 1,100–1,200 years BP; and one specimen (*Tiy*) from Tiya, an Ethiopian site, dated as 620 ± 60 years old.

DNA Extraction and Purification

On arrival in the laboratory, the specimens were cleaned of superficial debris and subsequently handled under sterile conditions. Small samples of bone weighing ~5 g were extensively cleaned by cutting off ≥2 mm of the entire bone surface with scalpel blades. Internal bone fragments (200–300 mg) were placed in a disposable sterile tube containing a sterile stainless steel bead (previously cooled in liquid nitrogen). The bone, in liquid nitrogen, was then pulverized into a fine powder by using a high-speed shaker. Four milliliters of extraction buffer (10 mM Tris-HCl [pH 8.5], 0.5 M EDTA, 65 mM DTT, 0.1% SDS, and 0.5 mg proteinase K/ml) were added, and solubilization continued for 48 h at room temperature with gentle stirring. The aqueous phase was then extracted once with phenol and twice with phenol/chloroform. The buffer was eliminated together with nucleic acids <50 bp by purification over a centricon 30 column. The DNA was finally brought to a volume of 200 μl with filtered sterile TE (10 mM Tris [pH 7.5] and 0.1 mM EDTA) and kept frozen at −20°C. An extraction blank was also run for each extraction. It consisted of a complete mock process without bone fragments, i.e., pulverization, phenol and chloroform extractions, and centricon filtration.

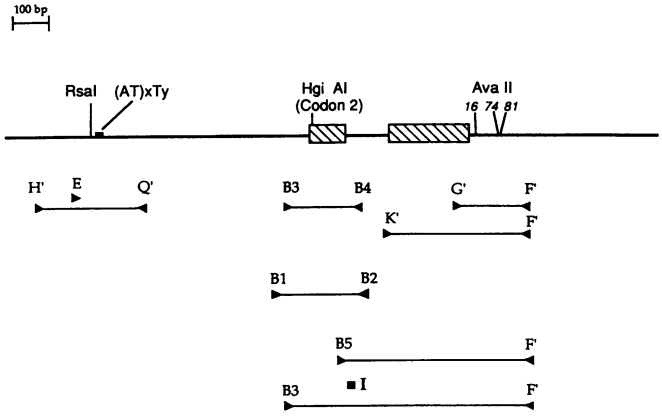


Figure 1 Map of the β-globin gene showing the upstream polymorphic $(AT)_x(T)_y$ microsatellite region, exons 1 and 2 (striped blocks), and the polymorphisms comprising the β-globin gene framework (exon 1, codon 2, and intragenic positions 16, 74, and 81). The position of the primers used for PCR, sequencing, and Southern hybridization are shown. Their nucleotide sequences are as follows: (B1) 5'CAGGAGCAGGGAGGGCAGGA3'; (B2) 5'GTCTCCACATGCCCAGTTTC3'; (B3) 5'GGAAGCTTGGGCTGGGCATAAAAGTCAGG3'; (B4) 5'AATCTAGAGGTCTCCTTAAACCTGTCTTGTAAC3'; (B5)5'GATGAAGTTGGTGGTGAGGCC3'; (E) 5'GCATGAGCAAATTAAG3' (primer for sequencing); (F') 5'CGATCCTGAGACTTCCACACTGATGC3'; (G') 5'GCCACACTGAGTGAGCTGCACTG3'; (H') 5'CTCTTGTTTCCCAAAACCTAATAAG3'; (I) 5'CTGGGCAGGTTGGTATCAA3' (internal probe); (K') 5'CCCACCCTTAGGCTGCTGGTGGT3'; (Q') and 5'GGATCTCTTCCTGCGTCTCCAG3'.

PCR Experiments

Primers.—The sequence of the primers used to study the β-globin gene is given in figure 1, and the combinations are reported in table 1. The primers used for DNA sex determination were those designed from the pub-

Table 1

Summary Table Showing Results of all the PCR Analyses Performed

Specimens	Age or Years BP	Extraction	β-Globin Gene PCR Products (no. of bp) G'F' (200)	B3 B4 (237)	B1 B2 (272)	H'Q' (302)	K'F' (377)	B5F' (515)	53F' (678)
Taf 2	12,000	e	+++ (1)	+++ (2)		+++ (2)	+++ (2)	− (1)	0 (1)
		g	+++ (2)			++ (2)		0 (1)	
Taf 1	12,000	f	+++ (2)	0 (1)			− (1)		
Mal	7,000	a			+ (2)	− (3)			
		c	+++ (1)	++ (1)	+ (3)	− (1)			
						+++ (1)			
						++ (1)	− (1)		
		e	+++ (2)	+++ (2)	+ (1)	− (1)			
				++ (1)		++ (1)			
S' Is	Bronze age	d	− (1)	0 (2)	0 (1)	− (1)			
		e	− (1)	0 (2)					
		f	− (1)	0 (1)		− (1)			
Gei	2,300	a	− (1)		0 (2)				
		b	− (1)		0 (2)				
Mat	Medieval	d	+++ (1)	+++ (2)	+ (1)	+++ (1)			
		f	+++ (1)			+++ (1)	+++ (1)	+++ (1)	0 (2)
Vol 1	Medieval	b		0 (1)	0 (1)			0 (1)	
Vol 2j	Medieval	b	+++ (1)	+++ (2)	+ (2)	− (1)			
		c	+++ (1)		0 (2)				
			+ (1)						
Cam	Medieval	d		− (1)	0 (2)	− (1)	− (2)		
		e		− (1)	0 (1)				
Tiy	620	a	− (1)	0 (1)	0 (1)				
		e	− (1)	0 (1)					

NOTE.—Multiple DNA extractions were made on different days referred to as a, b, c, d, e, f, g, on each of the specimens, and in each extraction the specimens were grouped differently. As depicted in figure 2, the result of the amplifications was marked "+++" when the specific product could be visualized on an ethidium bromide gel and was subsequently sequenced; "++" when the PCR product was revealed by ethidium bromide staining; "+" when the specific product could only be revealed after a Southern blot; zero "0" when no product could be seen on a gel or could be detected by Southern blot; as negative "−" when no band could be seen on the EtBr stained gel and the Southern blot was not performed. The number in parentheses indicates the number of PCRs completed.

lished amelogenin gene sequence (Nakahori et al. 1991): 5'CCCTGGGCTCTGTAAAGAATGTG3' (Amel-A) and 5'ATCAGAGCTTAAACTGGGAAGCTG3' (Amel-B). These flank a 6-bp deletion within intron 1 of the X homologue, resulting in 106-bp and 112-bp PCR products from the X and Y chromosomes, respectively. Amplification of one hypervariable mtDNA region of the D-Loop was performed using primers: 5'GACTCACCCATCAACAACCGCTATG3' (L 16089) and 5'GGTATCCTAGTGGGTGAGGGGTGG3' (H 16256).

Amplifications.—Amplifications were performed in 50 μl of PCR buffer containing 10 mM KCl, 10 mM $(NH4)_2SO_4$, 2 mM $MgCl_2$, 0.1% Triton X-100, 0.1 mg BSA/ml, 20 mM Tris-HCl (pH 8.75), 200 μM dNTPs, 20 pmol each primer, 2 μl or 5 μl of the extracted DNA, and 5–10 U Pfu DNA polymerase (Stratagene). The same PCR profile was used for all primer combinations: an initial 94°C denaturation step for 5 min, followed by 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, for a total of 40 cycles, finishing with a final elongation step at 72°C for 6 min. A large excess of Pfu DNA polymerase was found to be necessary, presumably to overcome the effects of PCR inhibitors in the extracted samples.

Southern Blot

The gel analysis of PCR products (which were not sequenced) were transferred to hybond N membrane by

alkaline transfer and probed with the appropriate γ^{32}P-labeled β-globin internal oligo.

Direct Sequence Analysis

An aliquot of the PCR product was run on a 2% agarose gel, and the relevant specific band was excised and purified using a MerMaid DNA purification kit (Bio 101). The PCR product was then directly sequenced using the dideoxy chain termination method and modified T7 polymerase (Sequenase II sequencing kit, USB).

Sequence Analysis after Cloning

The PCR product was run on a 2% agarose gel. The specific band was excised and cloned after spin elution onto glass wool, using a pCR-Script SK(+) cloning kit (Stratagene). The sequences were determined by cycle sequencing with an automatic DNA sequencer (ALF Pharmacia).

Laboratory Precautions

All experiments, except those of DNA sexing were conducted by the same experimenter (E.B.-C.), in a laboratory where the study and amplification of human DNA were not performed (Unité de Pathogénie des infections à Lentivirus). All buffers and water were autoclaved and purified before use by filtration through disposable centricon 30 microconcentrators (Amicon). A large amount of PCR premix containing all the necessary components, except DNA and Pfu polymerase, was prepared and frozen in small sealed aliquots at −20°C. A "quality control" PCR, containing 10 U Pfu without added DNA was then performed on random aliquots. The products were run on a 2% agarose gel, blotted onto hybond N membrane by alkaline transfer and probed with the appropriate γ^{32}P-labeled β-globin internal oligo to control for laboratory contamination. The preparation of bone samples and the DNA extractions were performed in one room and stored in a freezer in a separate room. Preparation of buffers and PCR set-up were performed in another separate room in a dedicated sterile hood under constant UV illumination (254 nm germicidal lamp) between use. Dedicated pipettes with aerosol resistant plugged tips were used throughout. Subsequent PCR experiments were performed in a dedicated thermocycler in a third room of the laboratory. Gel analysis was also performed in this room. For each set of PCR experiments reaction blanks and mock extraction controls were conducted.

Results

A map of the β-globin gene, showing the position of the polymorphisms studied, the location of the PCR primers and product sizes, is shown in figure 1. Primer sequences were chosen to optimize the yield of product in each case, because we found that many primer combinations commonly employed to amplify modern DNA were not as efficient on ancient DNA in our system (B1,B2, for example). Most often the primers had to be extended for good amplification of ancient DNA.

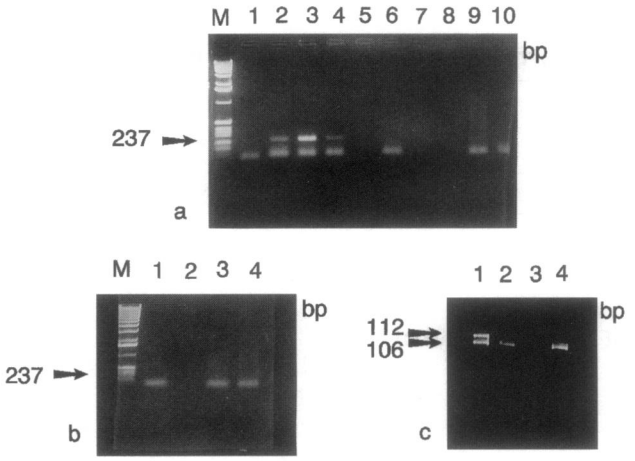


Figure 2 Amplification of a 237-bp fragment from the β-globin gene by using primer pair B3/B4 and DNA extracted from 10 ancient bones (*a* and *b*). Forty cycles of PCR were used for each sample. *a*, DNA size marker (M), PCR from *Mal* (1), *Vol 2j* (2), *Taf 2* (3), *Mat* (4), *Vol 1* (5), *Taf 1* (6), *S'Is* (7), *Cam* (8), *Gei* (9), and blank PCR (10). *b*, DNA size marker (M), PCR from 2 μl (1) and 5 μl (2) DNA extract from *Tiy*, mock extraction performed at the same time as this specimen (3), and blank PCR (4). *c*, PAGE (10%) of PCR product from male DNA template (1), female DNA template (2), *Taf 1* (4), and PCR product from *Taf 1* digested by *Ava*II (3).

Figure 2 (*a* and *b*) shows ethidium bromide–stained gel (representative of 16 experiments) of the PCR products from all 10 specimens and the mock extractions and PCR blanks, after 40 cycles of amplification using B3 and B4 as primers. The expected 237-bp band could be clearly seen in three specimens: *Vol 2j, Mat, Taf 2* (*a*, lanes 2, 3 and 4) and barely visible in one specimen: *Mal* (*a*, lane 1). In all 16 experiments the mock extractions and PCR blanks were negative (see *a*, lane 10; and *b*, lanes 3 and 4) as were all other specimens. Some extracts (*a*, lanes 5, 7, and 8; and *b*, lane 2) contained more PCR inhibitors than others and consequently showed no specific band and no primer dimer. Thus, in these cases we used less extract (two to five times less) in order to bypass the inhibitory effect. In the absence of inhibitors, ancient DNA was either revealed (*a*, lanes 1, 2, 3, and 4) or not (*b*, lane 1).

The crucial problem in interpreting the results from ancient human DNA is the high risk of contamination by modern sequences. This can come from handling the material itself during or subsequent to excavation, from PCR product carryover in the laboratory, or from laboratory contamination. To control for laboratory contamination, stringent precautions were taken (similar to those used by other workers when examining mtDNA),

and to eliminate any modern DNA liable to contaminate the bones, ≥2 mm of the entire surface were cut off with scalpels and only internal bone fragments used. To be sure the PCR products obtained were not due to sporadic contaminations, multiple extractions of the bone samples were performed in different combinations. A summary of the results from all the different PCRs performed is presented in table 1. It can be seen that using a single round of PCR amplification (40 cycles) we were able to amplify multiple fragments of the β-globin gene in 5 of the 10 specimens studied (*Taf* 2, *Taf* 1, *Mal, Mat,* and *Vol 2j).* These results could be reproduced using batches of DNA extracted at different times and in several different PCR experiments. For example, among bone samples yielding positive results three independent extractions were performed on the *Mal* specimen, and each time we obtained amplifiable DNA. Extraction experiment e, table 1, included five specimens; only *Mal* and *Taf*2 were positive, which suggests that contamination of one bone sample by another during extraction and PCR can be eliminated. In contrast, PCR performed on extracts from *S'Is, Gei, Vol* 1, *Cam,* and *Tiy* were consistently negative, irrespective of the primers, extraction batches, or PCR experiment.

As a proof of ancient DNA authenticity we compared the osteological sexing of one specimen, *Taf* 1, with that obtained by DNA sexing. The *Taf* 1 specimen comes from a tomb containing three adult female skeletons. By use of a *Taf* 1 DNA extract and the Amel A and B primer pair, a PCR fragment of 106 bp was generated, the size expected for DNA from a female individual (fig. 2*c*). Identical results were obtained when two workers of different sex performed independent extractions and amplifications. As another proof of ancient DNA authenticity, the hypervariable D-Loop region in mtDNA was amplified and both DNA strands sequenced after cloning. A 166-nt fragment (nt 16090–16255) was amplified from *Taf1* and *Mat* DNA extracts. Duplicate PCR were cloned for each specimen. The same sequence was obtained three and five times for the two PCRs performed with the *Taf1* specimen. On *Mat* it was possible to analyze eight clones from each of the two independent cloning procedures that exhibited similar sequences. *Taf1* and *Mat* sequences were compared with the sequence of the main experimenter (E.B.-C.). *Mat* and the experimenter exhibited similar sequences. Indeed, this sequence is shared by 70% of Europeans. The *Taf1* sequence was found to be different from those collected in the different databases (table 2). It differs from the reference sequence by five known polymorphic sites, never yet described in association (Horai and Hayasaka 1990; Torroni et al. 1993).

The stringent precautions taken and the number of controls carried out are strong evidence that the PCR products obtained were generated from ancient DNA and were not due to contamination with modern DNA. It was thus possible to analyze the β-globin gene. Direct sequencing of the amplified products is shown in figure 3. We analyzed the three β-globin gene frameworks (gene polymorphisms) as defined by Orkin et al. (1982). Type 1 is characterized by the absence of polymorphism; type 2 genes have a single polymorphism, the G-T substitution at IVS-2 position 74; and type 3 genes display five polymorphisms, one in codon 2 (C-T) and four in IVS-2 at positions 16 (C-G), 74 (G-T), 81 (C-T) (see fig. 1), and 666 (T-C). We were able to analyze these polymorphisms (with the exception of the one at position 666) by sequencing the B3/B4 and G′/F′ PCR products. The 237-bp B3/B4 PCR product was sequenced entirely, but the other amplified fragments were only sequenced around the targeted regions of polymorphism. In all cases an identical sequence was obtained from two independent extractions of the same specimen. Concerning the framework polymorphism, two specimens, *Mal* and *Vol 2j,* were homozygous type2/type2; *Taf* 2 and *Mat* were heterozygous type1/type2; and *Taf*1 was heterozygous type2/type3.

In addition, we analyzed the highly variable repeated purine/pyrimidine sequence $(AT)_x(T)_y$, which lies 500 bp upstream of the β-globin gene, by sequencing the 302-bp H′/Q′ PCR product with primer E. The nucleotide sequence around this microsatellite could be established for four individuals, *Taf* 2, *Mal, Vol 2j,* and *Mat. Mal* was homozygous $(AT)_9(T)_5$; and two specimens, *Vol 2j* and *Mat,* were homozygous $(AT)_7(T)_7$, which is the allele most commonly found in nonthalassemic contemporary humans. *Taf* 2 was found to be heterozygous $(AT)_9(T)_5$/$(AT)_7(T)_7$ and also heterozygous for the often polymorphic *Rsa*I restriction site at position −551 (relative to the cap site of the β-globin gene). Thus, these two alleles were present in Morocco 12,000 years ago.

Discussion

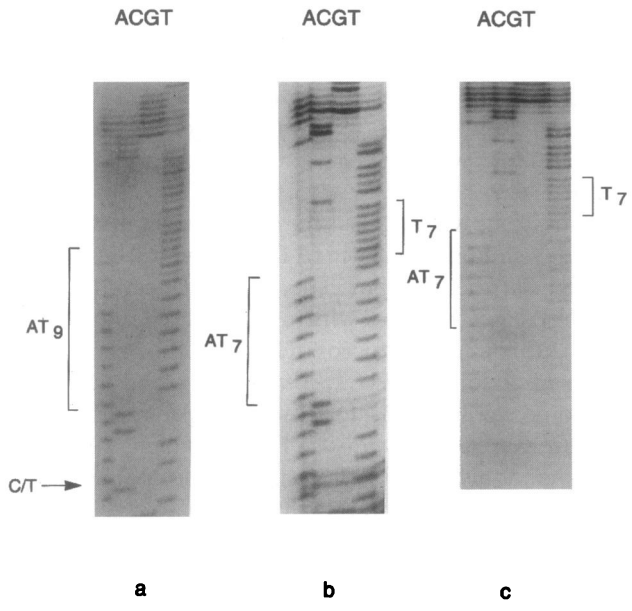
The macroscopic study of ancient human skeletons in association with the analysis of cultural and religious environments has led to the identification of populations that may have been vital links in human evolution. These ancient human bones represent an important source of material that has greatly aided our understanding of the chronology of events because they can be dated in several ways. Analyzing the DNA from these human specimens would be an essential step to the understanding of genetic diversity in current populations. For this analysis to be informative, two conditions have to be met. We must be able to study both mitochondrial and nuclear genes, since extensive sequencing of only one locus or the mitochondrial control region alone will not solve the puzzle of human population origins. We must be able to study human DNA ≥10,000–15,000 years old,

Table 2

Differences between mtDNA Sequences of Specimens in the Present Article and that Provided in Anderson et al. (1981)

	Polymorphic Sites within Hypervariable Region of D-Loop					
	16189	16193			16223	16248
Anderson et al.	T	C			C	C
E.B.-C. (experimenter)	T	C			C	C
Mat	T	C			C	C
Taf 1	C	C	C[a]	C[a]	T	T

[a] Nucleotides insert at position 16193.



FOSSIL	ORIGIN	AGE	β-GLOBIN GENE FRAMEWORK	(AT)x Ty
Taf 1	Morocco	12 000 yrs	2/3	ND
Taf 2	Morocco	12 000 yrs	1/2	(AT)9 T5/ (AT)7 T7
Mal	Mali	7 000 yrs	2/2	(AT)9 T5/(AT)9 T5
Mat	Italy	Medieval	1/2	*(AT)7 T7/(AT)7 T7
Vol 2 j	France	Medieval	2/2	*(AT)7 T7/(AT)7 T7

Figure 3 DNA polymorphisms of the studied regions of the β-globin gene, as determined by direct sequencing. *Top,* Autoradiogram of a DNA sequencing gel of the double-stranded PCR product generated by the primers H′ and Q′. Sequencing was performed with the primer E. *a,* Taf 2 extraction e. *b,* Mat extraction f. *c,* Mat extraction d. Arrow points to C/T polymorphism at the position −551 corresponding to a polymorphic *RsaI* site. Brackets indicate $(AT)_x$ and $(T)_y$ parts of $(AT)_x(T)_y$ microsatellite. *Bottom,* Summary of sequencing data on the five studied specimens for the β-globin gene framework and $(AT)_x(T)_y$ microsatellite An asterisk (*) indicates results obtained for two independent extractions.

old enough to observe a difference in human evolution. For Taforalt, this corresponds to the Epipaleolithic period, which formed a key transition between two civilizations: the hunters-gatherers from the Paleolithic and the shepherds-farmers from the Neolithic. During this period the climate and biotope evolved progressively toward the conditions prevailing today, making this period of unique interest in the understanding of mankind's evolution.

We show in this report that it is possible to analyze ancient nuclear DNA polymorphisms in bone specimens dated between 1,200 and 12,000 years BP. Indeed, direct sequencing of PCR products demonstrated that the only variations detected match those of the known intragenic polymorphic sites. All the cases were free of nucleotide substitution, which could be caused by chemical modification of the ancient DNA in the fossilization process. It appears that only relatively intact molecules can be used as templates, as already noted by other authors (Rogan and Salvo 1990). Thus, we were able to analyze a highly polymorphic $(AT)_x(T)_y$ microsatellite upstream of the β-globin gene in five specimens and to assign β-globin gene frameworks to the same five specimens. The haplotypes have previously been described and some must therefore have existed in Morocco ≥12,000 years ago.

From table 1 it can be seen that the success of PCR amplification was not directly related to the age of the specimen. We were able to consistently obtain PCR fragments from *Taf* 1 and *Taf* 2 that date from 12,000 years BP, whereas no product could be obtained with much more recent samples. That suggests that specimen age is not necessarily the limiting factor in the study of ancient organic material, a finding already noted by us and others in studies of fossil proteins (Ball et al. 1987; Ulrich et al. 1987; Béraud-Colomb 1993). The prime factor determining fossil preservation is the soil surrounding the bone remains in the archaeological site. In the present study we chose the oldest specimens among the bones collected from Taforalt. This archaeological site is known for its particularly well-preserved skeletons,

suggesting that the directed choice of specimens is of great importance in the successful isolation of DNA molecules from very ancient bones.

Although the data reported here are in themselves uninformative because of limited sample size and location, they suggest that a systematic study is feasible, since we have isolated and analyzed DNA from bones of two individuals belonging to a population of Cro-Magnon-like Africans who had settled on the site at Taforalt during the Epipaleolithic period.

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