RECOVERY AND IDENTIFICATION OF MITOCHONDRIAL AND NUCLEAR DNA SEQUENCES OF A 500-YEAR-OLD INCA MUMMY

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Background

Advances in molecular genetic techniques in the last decade have enabled the recovery of ancient DNA from a different range of samples, including bone and dry tissue. This paper describes the successful extraction, amplification, STR and mitochondrial sequence analysis of anthropological informative DNA from an ancient Inca human hone discovered in 1999 by anthropologist Dr. Johan Reinhard atop the snowcapped mountain called *Nevado Quewar*, in Argentina. The molecular analysis of this particular human remain proved informative and useful in part because the sanctuary where the mummy was buried 500 years ago was dynamited by looters in the attempt to steal precious ancient handmade crafts and jewelry.

Goals and Objectives

The purpose of the research was to answer questions regarding individual characterization, gender, and population affiliation for the Quewar mummy. This study had three main objectives:

(1) To extract and amplify the nuclear and mitochondrial DNA from a mummy bone sample, in order to elucidate the extent to which it could yield endogenous, ancient DNS.
(2) To characterize the mitochondrial DNA sequence variations at three specific anthropologically informative regions (the HV1 and HV2 regions, and the 9-bp intergenic detection at region V) and to compare those variations with reference samples, revealing the occurrence of a particular mtDNA haplotype.
(3) To determine the gender of the Quewar mummy by means of STR profiling.

Results

Mitochondrial DNA products were observed with good yield in the size range of around 350 base pairs, suggesting the extracted ancient mtDNA was of excellent quality. The sequence of both mtDNA strands was determined from several PCR amplification fragments, with multiple overlapping primers using fluorescent automated DNA sequencing technology. The Quewar mummy's mitochondrial DNA showed 11 base differences from the Anderson reference sequence. This information was used to search databases and published data for similar sequences. The array of polymorphisms found in the mummy's HV1 and HV2 sequences were unique when compared to more than 3,8000 HV1/HV2 sequences in current databases. No contamination from the mummy's manipulators was detected. Furthermore, a novel polymorphism never reported before in the literature was found at position 16178 (T→C). As expected, the Quewar mummy proved to be related to other Native Americans; the closest related sequences differed only at four nucleotides (with the Quiche people of Guatemala) and five nucleotides (with the Argentinean Mapuche group) at the HV1 region. The Quewar mummy sequence can be assigned to the Native American haplogroup B, since it showed both the 9bp deletion in the mtDNA region V and the substitutions 16189C/16217C characteristic of this group. But the mummy sequence also shared genetic patterns found in people from China, bolstering the hypothesis that the earliest Americans came from Asia. Concerning the gender identification of the Quewar mummy, amelogenin gene amplification indicated that the individual studied here was a female.

Conclusions

The present study shows that the Quewar mummy's mtDNA HV1 and region V sequences place her precisely in the Native American Indian haplogroup B. Her mtDNA sequence represents a new variant not found in the current mitochondrial DNA sequence databases and is most closely related to the Quiche or Mapuche people of Central/South America. Analysis of the Quewar mummy's DNA offered an important opportunity for understanding her genetic origin and this molecular information offered elements that complement archaeological evidence. This research will have a significant impact on the field of ancient DNA analysis, and will assist specifically in the procedures of ancient DNA extraction, amplification, and characterization.