USE OF THE AMPLIFICATION REFRACTORY MUTATION SYSTEM (ARMS) IN THE STUDY OF HBS IN PREDYNASTIC EGYPTIAN REMAINS

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INTRODUCTION

Paleopathological studies provide important information about the diffusion of diseases and the living conditions in ancient populations. Investigations of hair, dental, skeletal and mummified tissues have contributed to an understanding of the evolutionary processes of these peoples. In particular, extensive research has been carried out on Egyptian mummies. Research on ancient remains was revolutionized by the techniques of molecular biology. The invention of PCR (Polymerase Chain Reaction) made it possible to study ancient DNA, thus opening new areas of research. In fact, molecular studies can provide a wide range of information about familial relationships, genetic diseases and bacterial or fungal infections.

Unfortunately, investigations of DNA in ancient samples encounter some technical problems. First of all, it is possible to obtain only tiny amounts of DNA, which is often physically and chemically modified by fragmentation, transformation of bases into others, or insertion of other chemical compounds (e.g. humic acids) in the DNA molecule. Such alterations can be severe impediments to the analysis: for example, humic acids can inhibit PCR (1).

Another, but no less important, limitation of studies of ancient DNA is the contamination by modern DNA: even a small degree of pollution can have a great influence on the results of the research.

MATERIALS AND METHODS

We investigated the presence of HbS in predynastic Egyptian mummies (about 3200 BC) from the "Marro" collection of the Anthropological and Ethnographic Museum of Turin. We selected 6 individuals on the basis of their good state of preservation, in order to extract a sufficient quantity of non-degraded DNA. In addition, we considered other criteria, such as typical bone markings and malarial infection, indicating a high probability of the presence of the HbS mutation. In fact, these subjects exhibited extensive bone pathologies characteristic of hemoglobinopathies and thalassemias and they were negative to an ELISA immunoenzymatic assay for a protein (PfHRP-2) derived from Plasmodium falciparum infection. It is common knowledge that some mutations, e.g. HbS, represent ecological adaptations that provide protection against various diseases, including malaria.

The DNA was extracted from dental samples (500 mg). To prevent contamination with modern DNA, the external surfaces of the teeth were washed with a hydrochloric acid solution. All the reactions were carried out in sterile conditions and pre- and post-PCR work was performed in separate laboratories. We applied a silica-gel method specific for ancient remains, which purifies the DNA by removing components inhibiting amplification (2).

To amplify the DNA, we employed the amplification refractory mutation system (ARMS) (3), with appropriate modifications to adapt the method to ancient DNA.

ARMS permits the diagnosis of single nucleotide substitutions and it is based on specific priming of the PCR. In this method, amplification can occur only in the presence of the specific mutation being studied. This technique can be very useful in the study of genetic diseases: once it has been verified that the operator's DNA does not contain the mutation, any possible contamination cannot influence the result of the amplification. The amplified products were analyzed by electrophoresis in 2% agarose gel. In the series of samples, we included modern DNA controls for HbS

(negative and positive) and a control to monitor contamination (PCR without extract).

RESULTS

Three of the 6 individuals show amplification products obtained with specific priming for the HbS mutation. Fig. 1 illustrates a band at the level of HbS in these ancient samples, indicating the probable presence of the HbS mutation. At the moment, we are sequencing the amplification products to verify the presence of the beta mutation.

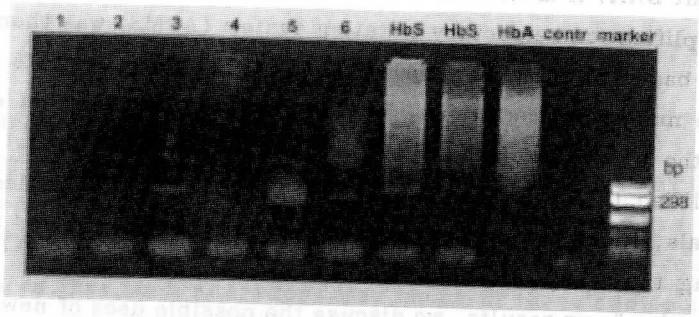

Fig. 1 - Electrophoretic analysis of 6 ancient Egyptian DNA samples (lanes 1-6), 2 positive controls (homozygous and heterozygous) and one negative control (not containing the mutation) from modern DNA, a control amplified without extract and a PM marker. Lanes 3, 5 and 6 show a band at the level of the HbS positive control, indicating the presence of the HbS mutation in these three ancient samples.

DISCUSSION

In three subjects, the extensive bone pathologies characteristic of severe anemia and the associated molecular results may indicate that they were affected by sicklemia.

Therefore, the results of our molecular study represent additional confirmation of the presence of this hereditary disease in Ancient Egypt. Indeed, previous histological studies had revealed sickle cells in a mummy from the same collection (4).

The present results also highlight the great potential for the use of these new molecular investigation systems in paleopathological diagnoses of

genetic diseases and of viral, bacterial, fungal infections and parasitic infestations.

We conducted a molecular investigation of the presence of sicklemia in six predynastic Egyptian mummies (about 3200 BC) from the Anthropological and Ethnographic Museum of Turin. Previous studies of these remains showed the presence of severe anemia, while histological preparations of mummified tissues revealed hemolytic disorders.

DNA was extracted from dental samples with a silica-gel method specific for ancient DNA. A modification of the polymerase chain reaction (PCR), called amplification refractory mutation system (ARMS) was then applied. ARMS is based on specific priming of the PCR and it permits diagnosis of single nucleotide mutations. In this method, amplification can occur only in the presence of the specific mutation being studied.

The amplified DNA was analyzed by electrophoresis. In samples of three individuals, there was a band at the level of the HbS mutated fragment, indicating that they were affected by sicklemia.

On the basis of our results, we discuss the possible uses of new molecular investigation systems in paleopathological diagnoses of genetic diseases and viral, bacterial and fungal infections.

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