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INVITED REVIEW ARTICLE

Insights from ancient DNA analysis of Egyptian human mummies: clues to disease and kinship

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Abstract

The molecular Egyptology field started in the mid-eighties with the first publication on the ancient DNA (aDNA) analysis of an Egyptian mummy. Egypt has been a major interest for historians, archeologists, laymen as well as scientists. The aDNA research on Egyptian biological remains has been fueled by their abundance and relatively well-preserved states through artificial mummification and by the advanced analytical techniques. Early doubts of aDNA integrity within the Egyptian mummies and data authenticity were later abated with studies proving successfully authenticated aDNA retrieval. The current review tries to recapitulate the published studies presenting paleogenomic evidence of disease diagnosis and kinship establishment for the Egyptian human remains. Regarding disease diagnosis, the prevailing literature was on paleogenomic evidence of infectious diseases in the human remains. A series of reports presented evidence for the presence of tuberculosis and/or malaria. In addition, there were solitary reports of the presence of leprosy, diphtheria, bacteremia, toxoplasmosis, schistosomiasis and leishmaniasis. On the contrary, paleogenomic evidence of the presence of rare diseases was quite scarce and mentioned only in two articles. On the other hand, kinship analysis of Egyptian human remains, including that of Tutankhamen, was done using both mitochondrial DNA sequences and nuclear DNA markers, to establish family relationships in four studies. It is clear that the field of molecular Egyptology is still a largely unexplored territory. Nevertheless, the paleogenomic investigation of Egyptian remains could make significant contributions to biomedical sciences (e.g. elucidation of coevolution of human host–microbe interrelationship) as well as to evidence-based archeology.

Introduction

The term Paleogenetics, introduced by Pauling *et al*., in 1963 (1), entails the study of the past through the examination of preserved genetic material from the remains of ancient organisms (2). In 1981, the field of ancient DNA (aDNA) analysis was started, in the Chinese literature, with the first-ever published article on the analysis of two ancient Chinese mummies (3); however, the first manuscript on aDNA analysis in the English literature was

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in 1984 by Higuchi *et al.* (4) on a museum specimen of a horse ancestor, the extinct quagga. The following year witnessed the launching of the field of molecular Egyptology with the first publication on the aDNA analysis of an Egyptian mummy (5).

Egypt has always drawn the interests of historians, archeologists, laymen and scientists. Egypt has been a major hub of the old world, the site of one of the earliest ancient civilizations and a major exit corridor for human beings out of Africa according to a prevailing theory (6). The aDNA research on Egyptian biological remains has been fueled by their abundance and relatively well-preserved state through artificial mummification, as well as by the advanced next-generation sequencing (NGS) technology (7–9).

The mummification process stood the test of time to various degrees, as evidenced by the preservation status of the Egyptian human remains. This old technology, which is believed to have its roots in predynastic times (~4000 BC), evolved over the ages. Thus, the used resin constituents varied according to the era, the social class of the deceased and the mummy species (i.e. humans or animals) (10,11). Although artificial mummification helped to preserve the bodies against decay, it has an impact on the technical feasibility of aDNA analysis from Egyptian mummies. The inherent challenges of working with the highly degraded and low copy number aDNA are additionally supplemented with the effects of an impressive arsenal of unknown components in the embalming resins that are coextracted with the aDNA and effectively inhibit the polymerase chain reaction (PCR) even at a concentration of one part per 4000 (12).

Due to doubts regarding aDNA integrity within the Egyptian mummies, research on them was hampered by suspicions of the authenticity of the resulting data (3). This situation has stalled the widespread progress of the field and caused scarcity of the related scientific literature. However, the preservation of the aDNA in the Egyptian mummies and biological remains was proven in principle (13,14). Despite its highly degraded state and low copy number, aDNA studies have generated comprehensive genomic data, using the NGS technology, to taxonomically classify Egyptian animal remains (9,15–17).

Reports of aDNA analysis of Egyptian human remains, though scanty, continued to be published by various groups from all over the world, giving impetus to evidence-based archeology. The current review tries to recapitulate the published studies presenting paleogenomic evidence of disease diagnosis and kinship establishment for Egyptian human remains.

Paleogenomic-Based Disease Diagnosis in Ancient Egyptian Human Remains

It has been stated that the impact of human disease on the course of history has been understudied (18). However, biologic evidence is at the core of this dilemma (19). Genomic evidence of a parasitic or microbiological pathological organism in an Egyptian mummy may hint to the presence of acute lethal, chronic or uncleared infection during the life of the subject. The field of paleomicrobiological study of mummies was started in 1910, with Sir Ruffer' histological examination of a 20th dynasty mummy and the finding of *Schistosoma haematobium* ova in the examined tissues (20).

Microbiological infections

Mycobacteria. Human mycobacterial infections comprise two highly morbid chronic diseases, i.e. tuberculosis (TB) and leprosy that are caused by *Mycobacterium tuberculosis* and *Mycobacterium leprae*, respectively, in addition to a group of atypical mycobacteria with high pathological diversity. It is believed that due to the thick lipid-rich bacterial cell wall and the high guanine–cytosine content of its DNA, mycobacterial aDNA might be more persistent than the surrounding host aDNA within the human remains (21). A series of studies has provided molecular evidence of TB infection in ancient Egyptian subjects (22–31). However, it was concluded that TB was expanded in ancient Egypt with no proof for the *Mycobacterium bovis* presence (29). Another study provided evidence of the presence of *M. leprae*, in a 1500-year-old mummy from Dakhla Oasis (fourth–fifth century AD) (32). Using metagenomic analysis, a recent report could detect and construct a near full genomic sequence of *M. leprae* organism in a ~2200-year-old mummy from Abusir (33). This represents the oldest reported full genomic construct of the *M. leprae* organism.

Other bacteria and viruses. It was suggested that diphtheria, another lethal disease, was present in ancient Egypt. The analysis of the 16S rDNA in the tooth sample of an infant mummy provided an evidence for the presence of the causing organism, *Corynebacterium* spp. (34). Another study presented molecular evidence of bacteremia by gastrointestinal pathogenic bacteria in an infant Egyptian mummy (35).

The recent study of Neukamm *et al.* (33) reported on metagenomic analysis of >100 Egyptian human remains from two anthropological collections. The study could construct hepatitis B viral genome from a 2000-year-old Egyptian mummy as well as detecting a variety of bacteria related to oral pathology among a number of mummies.

Parasitic infections

Malaria. In humans, malaria is caused by five species of plasmodium parasites (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi*) and transmitted through the bites of female *Anopheles* mosquitoes (36). Several molecular studies investigated the presence of malaria parasite in ancient Egyptian remains. Nerlich *et al.* (37), identified *P. falciparum* in two Egyptian mummies who tested positive for the 134-bp fragment of the chloroquine resistance transporter gene. A study on late 18th dynasty provided the earliest evidence of the presence of *P. falciparum* in the remains of four mummies (Tutankhamun, Thuya, Yuya and TT320-CCG61065) through the identification of plasmodial Merozoite surface protein1, Subtelomeric variable open reading frame and Apical membrane antigen 1 (AMA1) gene fragments in their extracted DNA samples (38). A study on embalmed heads from Lower Egypt also showed positive results for partial AMA1 gene fragments (31). The first published report on the metagenomic NGS analysis of Egyptian mummies provided evidence for the presence of *P. falciparum* DNA sequences in some of the embalmed heads (7).

Toxoplasmosis. Toxoplasmosis is a zoonotic disease, caused by the *Toxoplasma gondii*, which is transmitted from animals to humans via the fecal–oral route as well as through eating infected meat and by transfer from mother to fetus through placenta (39). The population of domestic cats in North Africa is believed to be high, especially in urban areas (40). According to studies, the seroprevalence of *T. gondii* in contemporary Egyptian cats reached 97%. Consequently, the soil is suspected to be heavily contaminated with oocytes. This lends support to the hypothesis that contact with cats is a main risk factor for toxoplasmosis transmission in Egypt (41,42). The historical perspective of this rampant infection status within the Egyptian

society needs further investigation, as ancient Egyptian culture was well known for its reverence and mummification of cats (43). In their role as totemic animals of Bastet (daughter of Ra and goddess of domesticity and protection), votive cat mummies were purchased by worshiping pilgrims, offered at temples, and then buried in catacombs. Recent genetic evidence supports the notion that ancient Egyptians used domesticated cats and implies that taming of the cats occurred prior to or during Predynastic and Early Dynastic Periods (44). Toxoplasma DNA was identified in soft tissue biopsies from five embalmed heads, using metagenomic NGS analysis. It could be postulated that contact with cats might explain the presence of this specific pathogen in these Late and Graeco-Roman human mummies (7,16).

Schistosomiasis. Schistosomiasis transmission depends on the active role of the human host in the transmission process, through excretory contamination of snail habitats and direct contact with infective water. This ecological relationship thus makes schistosomiasis a disease closely linked to rural water resource development, population increase, inadequate sanitation and lack of effective medical treatment (45). Egypt, which depends on the waters of the Nile for nearly all of its agricultural output, used to have some of the highest schistosomal infection rates in the world (46).

Schistosomal DNA was extracted from a liver sample found in the canopic jar of an Egyptian mummy. The DNA of the intestinal form, *Schistosoma mansoni*, was detected in the liver sample by two laboratories and that of the urinary form, *S. haematobium*, was also detected by one laboratory. This study concluded to the presence of both types of *Schistosoma* from about 3900 years ago, during the Middle Kingdom in Central Egypt (47).

Leishmanaisis. The presence of visceral leishmaniasis was reported by Zink *et al.* (48), where evidence of *Leishmania donovani* DNA [a 120-bp fragment of a conserved region of the minicircle molecule of kinetoplastid mitochondrial DNA (mt DNA) of the parasite] was detected in four Egyptian and nine Nubian samples from the Middle Kingdom. The relative high prevalence of the disease among the Nubian samples supports the notion that Nubia (or modern Sudan) could be a focus of visceral leishmaniasis in the past.

Noncommunicable diseases

Several lines of evidence pointed to the presence of rare and common noncommunicable diseases in ancient Egyptians. This could be exemplified by studies on skeletal dysplasias (49,50) and atherosclerosis (51,52). However, reports of genetic testing-based diagnosis for these disease types are quite scarce. Marin *et al.* (53), using amplification refractory mutation system, detected a band at the level of the HbS mutated fragment in DNA samples that were extracted from the teeth of three predynastic Egyptian mummies (~3200 BC), suggesting the diagnosis of sickle cell anemia. Another study by Pusch *et al.* (54), reported a G > A transition in the FGFR3 gene at cDNA position 1138, diagnostic of achondroplasia, in cloned PCR products obtained from a first dynasty mummy (~3000 BC).

Paleogenomic-Based Kinship Analysis of Ancient Egyptian Human Remains

The genetic relatedness of individuals from archeological sites has been utilized to elucidate family relationships. A number of studies on Egyptian human remains assessed the maternal and paternal lineages using both mtDNA sequences and nuclear DNA markers, including autosomal and Y-chromosome short tandem repeats (STRs) (38,55–57). To the best of our knowledge, no full NGS autosomal study has been published yet in this regard, only uniparental markers were utilized.

An investigative study was carried out on the familial relationships of a number of late 18th dynasty mummies (ca. 1550–1295 B.C.), including that of Tutankhamen. The study was based on the analysis of the autosomal and Y-chromosome STR markers in addition to mitochondrial hypervariable region 1 sequences. A 4-generation pedigree of Tutankhamun's immediate lineage and the identity of his ancestors were established. The Royal male lineage was the Y-chromosome haplogroup R1b that was passed from the grandparent (Amenhotep III) to the father (KV55, Akhenaten) to the grandchild (Tutankhamen). The maternal lineage, the mitochondrial haplogroup K, extended from the great-grandmother (Thuya) to the grandmother (KV35 Elder lady, Queen Tiye) to the yet historically unidentified mother (KV35 Younger lady) to Tutankhamen (38,55).

Another genetic examination was done as a part of a multidisciplinary study on the mummies of Ramesses III and the Unknown Man E (20th dynasty, circa 1190–1070 BC). Ramesses III was subjected to an assassination attempt by members of his harem as part of a palace coup, historically known as the Harem conspiracy and recorded in the Judicial Papyrus of Turin. The Unknown Man E was suggested to be Pentaware, the son sharing in the coup. The study concluded, on the basis of radiographic evidence, that the pharaoh was murdered during the attempt. The genetic results confirmed that both mummies had the same Y-chromosome molecular signature, E1b1a haplotype and one set of identical autosomal alleles, suggesting a father–son relationship (56).

Genomic analysis could resolve a longstanding question regarding the kinship of two high-status Egyptians from the 12th dynasty (1985–1773 BC), known as two brother mummies, Nakht-Ankh and Khnum-Nakht. The two mummies shared the same tomb and inscriptions on their coffins stated that they had the same mother name, Khnum-aa. However, when unwrapped, they were different morphologically from each other (47). Using hybridization capture of the mitochondrial and Y chromosome fractions, followed by NGS, the results revealed that both mummies were assigned to the same mtDNA haplogroup, M1a1, indicating a maternal relationship. On the other hand, incomplete profiles of Y-STRs were obtained but showed variations between the two mummies, suggesting that they belonged to different fathers and may likely be half-brothers (57).

Concluding Remarks

The field of molecular Egyptology is still a largely unexplored territory. Exploitation of the advanced genomic analytical techniques can make significant contributions to the progress and understanding of many scientific disciplines. Improvements in the NGS technical capabilities using for example single-stranded DNA library protocols can augment the number of retrieved DNA sequence messages despite the highly degraded DNA state (58).

It is anticipated that the research line of molecular Egyptology will be a cornerstone in the coming age of evidence-based archeology. The paleogenomic investigation will definitely help in filling defined gaps of knowledge within the fields of anthropology, archeology, evolution and history, as well as

providing paramount information about the coevolution of human host–microbe interrelationship throughout history.

Conflict of Interest statement. None declared.

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