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Palaeopathology and genes: Investigating the genetics of infectious diseases in excavated human skeletal remains and mummies from past populations

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ABSTRACT

The aim of this paper is to review the use of genetics in palaeomicrobiology, and to highlight the importance of understanding past diseases. Palaeomicrobiology is the study of disease pathogens in skeletal and mummified remains from archaeological contexts. It has revolutionarised our understanding of health in the past by enabling a deeper knowledge of the origins and evolution of many diseases that have shaped us as a species. Bacterial diseases explored include tuberculosis, leprosy, bubonic plague, typhoid, syphilis, endemic and epidemic typhus, trench fever, and *Helicobacter pylori*. Viral diseases discussed include influenza, hepatitis B, human papilloma virus (HPV), human T-cell lymphotrophic virus (HTLV-1) and human immunodeficiency virus (HIV). Parasitic diseases investigated include malaria, leishmaniasis, Chagas' disease, roundworm, whipworm, pinworm, Chinese liver fluke, fleas and lice. Through a better understanding of disease origins and their evolution, we can place into context how many infectious diseases are changing over time, and so help us estimate how they may change in the future.

1. Introduction

Study of disease in past populations (palaeopathology) has traditionally focused on the analysis of the appearance and prevalence of pathological lesions present in excavated human skeletal remains. This approach has certain limitations (see Wood et al., 1992), as it is not easy to differentiate an individual who died quickly from an infectious disease before they developed lesions on their bones, from someone who never contracted the disease in the first place. Furthermore, a number of different diseases may produce similar lesions on the bones and many diseases cause only soft tissue damage and no bony lesions. Therefore, in the absence of mummified remains, using traditional methods palaeopathology is limited to the identification of a small proportion of individuals who suffered for a long time with those diseases that cause pathognomonic lesions on the skeleton. However, with the development of molecular tools that allow the extraction and identification of pathogen DNA from human remains, a greater number of disease pathogens can now be identified, improving our understanding of health and disease in the past.

Since the first study to extract pathogen DNA (*Mycobacterium tuberculosis*) from skeletal remains in 1993 (Spigelman and Lemma, 1993), the extraction and identification of pathogen DNA and other biomolecules from ancient remains has revolutionised the field of palaeopathology, providing new, exciting tools for reconstructing past health and disease. The identification of pathogen biomolecules from ancient skeletal and mummified specimens, from coprolites (faeces), teeth and paraffin embedded tissues in pathology museums provides new insights into the co-evolutionary relationship between a pathogen and its human host. Furthermore, the field provides new means with which to address palaeoepidemiological questions about the prevalence and distribution of infectious diseases in past populations (Drancourt and Raoult, 2005; O'Rourke et al., 2000; Tsangaras and Greenwood, 2012; Zink et al., 2002).

Palaeomicrobiological studies also provide information about the molecular evolution and the phylogenetic relationship of infectious diseases through the identification of extinct pathogens or pathogen strains and through the comparative analysis of modern and ancient microbial sequences (O'Rourke et al., 2000). Although the analysis of modern pathogen DNA can provide information about the evolution and phylogeny of different disease agents, some pathogens evolve rapidly and thus their origin, phylogeny and genetic evolution are traced more accurately through the analysis of ancient pathogen DNA. In understanding the evolution of infectious diseases, the sequencing of the whole genome of pathogens extracted from ancient specimens has an

Abbreviations: aDNA, Ancient DNA; HTLV-1, Human T-cell lymphotrophic virus type I; HIV, Human immunodeficiency virus; HPV, Human papilloma virus; MNI, Minimum number of individuals; PCR, Polymerase chain reaction; RLEP, Repetitive elements; SNPs, Single nucleotide polymorphisms; TB, Tuberculosis.

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important role to play, since whole genome analysis provides information about the mechanisms of pathogen evolution and adaptation, which is crucial for understanding emerging and re-emerging infections (Bos et al., 2011).

Before discussing the palaeomicrobiological studies so far undertaken, it must be highlighted that the extraction and identification of the genetic material of pathogens from archaeological remains is subject to the same problems and limitations as the analysis of human aDNA from archaeological remains. These problems have been presented in our paper on human evolution and genetics in this volume (Anastasiou and Mitchell, 2013). Contamination of archaeological remains with pathogen DNA can occur both with environmental microorganisms in the soil and with modern pathogen DNA, during excavation and later analysis in the laboratory. A thorough set of controls is required in order to ensure that any positive results genuinely reflect the presence of an infectious disease in the ancient context under study (Cooper and Poinar, 2000; Cipollaro et al., 2005).

2. Bacterial diseases

2.1. Mycobacterium tuberculosis (TB)

In palaeomicrobiology, the organism that has most frequently undergone DNA extraction is *M. tuberculosis*. Mycobacteria are considered to be particularly suitable targets for aDNA research because of their waxy, hydrophobic and lipid-rich cell wall that helps to protect against DNA degradation and environmental destruction (Donoghue et al., 2004; Zink et al., 2002).

One of the first studies to extract and analyse pathogen DNA from archaeological specimens was carried out by Salo et al. (1994), who demonstrated the presence of *M. tuberculosis* DNA in a 1000 year old Peruvian mummy and proved the presence of tuberculosis in pre-Columbian South America. In their study, the authors followed the example of Spigelman and Lemma (1993) and employed a primer pair designed to bind to a 123 bp product of the repetitive insertion element IS6110 associated with *M. tuberculosis*. IS6110 remains even today one of the basic methods for the detection of the *M. tuberculosis* DNA in ancient samples (Donoghue et al., 2004; Stone et al., 2009). It is worth noting here that Spigelman and Lemma's original results were subsequently confirmed when more modern methods became available, indicating that high quality research in palaeomicrobiology was carried out already from the earliest days of the field (Spigelman et al., 2003).

By amplifying a portion of the IS6110 element, Faerman et al. (1997) were able to detect *M. tuberculosis* DNA in skeletal remains from Lithuania, dated to the 15th–17th century AD and to demonstrate the high prevalence of tuberculosis exposure in the population. A year later, Braun et al. (1998) recovered IS6110 from 11th to 13th century AD Mississippian samples demonstrating the presence of tuberculosis in pre-contact North America. More studies followed, confirming the presence of *M. tuberculosis* in mummified remains (Arriaza et al., 1995; Crubézy et al., 1998; Donoghue et al., 2010; Nerlich et al., 1997), calcified pleura (Donoghue et al., 1998) and paraffin embedded tissues (Zink et al., 2005), as well as in skeletal remains (Bachmann et al., 2008; Gernaey et al., 2001; Jaeger et al., 2012; Klaus et al., 2010; Mays et al., 2001; Raff et al., 2006; Zink et al., 2005).

Spoligotyping has also been employed for the detection of *M. tuberculosis* DNA in multiple studies. This technique was developed by Kamerbeek et al. (1997) and is based on the variation of the DR region in *M. tuberculosis* complex members that allows the identification of different strains to the subspecies level. Spoligotyping has been used among others by Zink et al. (2003), Fletcher et al. (2003b), Taylor et al. (1999), Rothschild et al. (2001) and Mays et al. (2001).

In 2008, Hershkovitz et al. using both PCR and high performance liquid chromatography reported the extraction of *M. tuberculosis* DNA from a female and an infant excavated from the site of Atlit-Yam, Israel and dated to 9250–8160 BP. The aDNA result was further supported by the finding of mycolic acids unique to the cell wall of tuberculosis. This identification of tuberculosis in the Atlit-Yam specimens provides the oldest currently known cases of *M. tuberculosis* in humans confirmed with molecular tools (Hershkovitz et al., 2008).

Although the majority of early palaeomicrobiological studies focus on pathogen DNA extracted from one or a few specimens, more recently studies on larger series of individuals have also been carried out to assess the disease at a population level (Roberts and Ingham, 2008). In the case of *M. tuberculosis* DNA these studies provide an important insight into the distribution and prevalence of the disease in different ancient populations. Examples include the analysis of 37 skeletal tissue specimens from Egypt, dated between 3000 BC and 500 BC (Zink et al., 2001), the analysis of 85 Egyptian mummies dated between 2050 and 500 BC (Zink et al., 2003), as well as the extraction of *M. tuberculosis* DNA from seven bones from English cemeteries dated between the 9th and 19th century AD (Bouwman and Brown, 2005). Fletcher et al. examined 168 individuals from an 18th to 19th century AD burial context in Hungary for the presence of *M. tuberculosis* and found that the pathogen had a prevalence of 55% in the population (Fletcher et al., 2003a), whilst the prevalence in rib samples from 93 naturally partially mummified 18th century AD Hungarians was 78% (Donoghue et al., 2011). Of course it must be stressed that such studies do not necessarily demonstrate the proportion of individuals that died from TB, but rather the proportion that had contracted TB at some stage of their lives, so that the organisms were present in their skeletal tissues in either an active or dormant state at the time of death. However, this research has shown that many more people in the past were infected by infectious diseases such as TB than could ever be detected from the analysis of their bones or mummified bodies for lesions visible to the eye (Donoghue, 2011).

Study of the strains of tuberculosis in 160 mummies and skeletons from ancient Egypt using spoligotyping has shown evolution in the forms of the disease present in the region over a 3000-year period. Samples from the pre/early dynastic period dating from 3500 to 2650 BC were found to have TB due to a mixture of strains of *M. tuberculosis*, one of which presents a hybridization pattern suggestive of an ancestral *M. tuberculosis* strain. *Mycobacterium africanum* was found in a Middle Kingdom tomb dating from 2050 to 1650 BC. However, as time passed it seems the situation changed, since in the samples from the New Kingdom to Late Period dating from 1500 to 500 BC modern strains of *M. tuberculosis* became dominant, whilst ancient strains and also *M. africanum* were not found (Zink et al., 2003, 2007).

Perhaps the most important contribution of the analysis of *M. tuberculosis* DNA from ancient remains has been to disprove the traditional hypothesis as to the origin of the disease (Rothschild et al., 2001; Zink et al., 2002, 2003). According to the original hypothesis, *M. bovis* was the probable ancestor of tuberculosis, which was transmitted from cattle to humans for the first time during the domestication of animals in the Neolithic period (Cockburn, 1967). However, whole genome sequencing and comparative genomic studies illustrated that *Mycobacterium bovis* represents a later lineage of the *M. tuberculosis* complex than *M. tuberculosis* (Brosch et al., 2002; Hershberg et al., 2008; Mostowy et al., 2002; Wirth et al., 2008). This is supported by the finding of *M. tuberculosis* (and not *M. bovis*) aDNA and lipid biomarkers in a Pleistocene bison from the USA dating to 17,500 BP (Lee et al., 2011; Rothschild et al., 2001). Indeed, the earliest archaeological example of *M. bovis* aDNA so far identified seems to be in Iron Age (c.2000 BP) Siberian pastoralists (Taylor et al., 2007). Based on genetic evidence it is possible to suggest that an early progenitor of the pathogen, which was similar to *Mycobacterium canettii*, was present in East Africa as early as 3 Ma ago. Thus, based on molecular data it is possible to argue that *M. tuberculosis* has a long co-evolutionary history with its human hosts (Gutierrez et al.,