historical**Perspectives**

# Ancient tuberculosis and leprosy

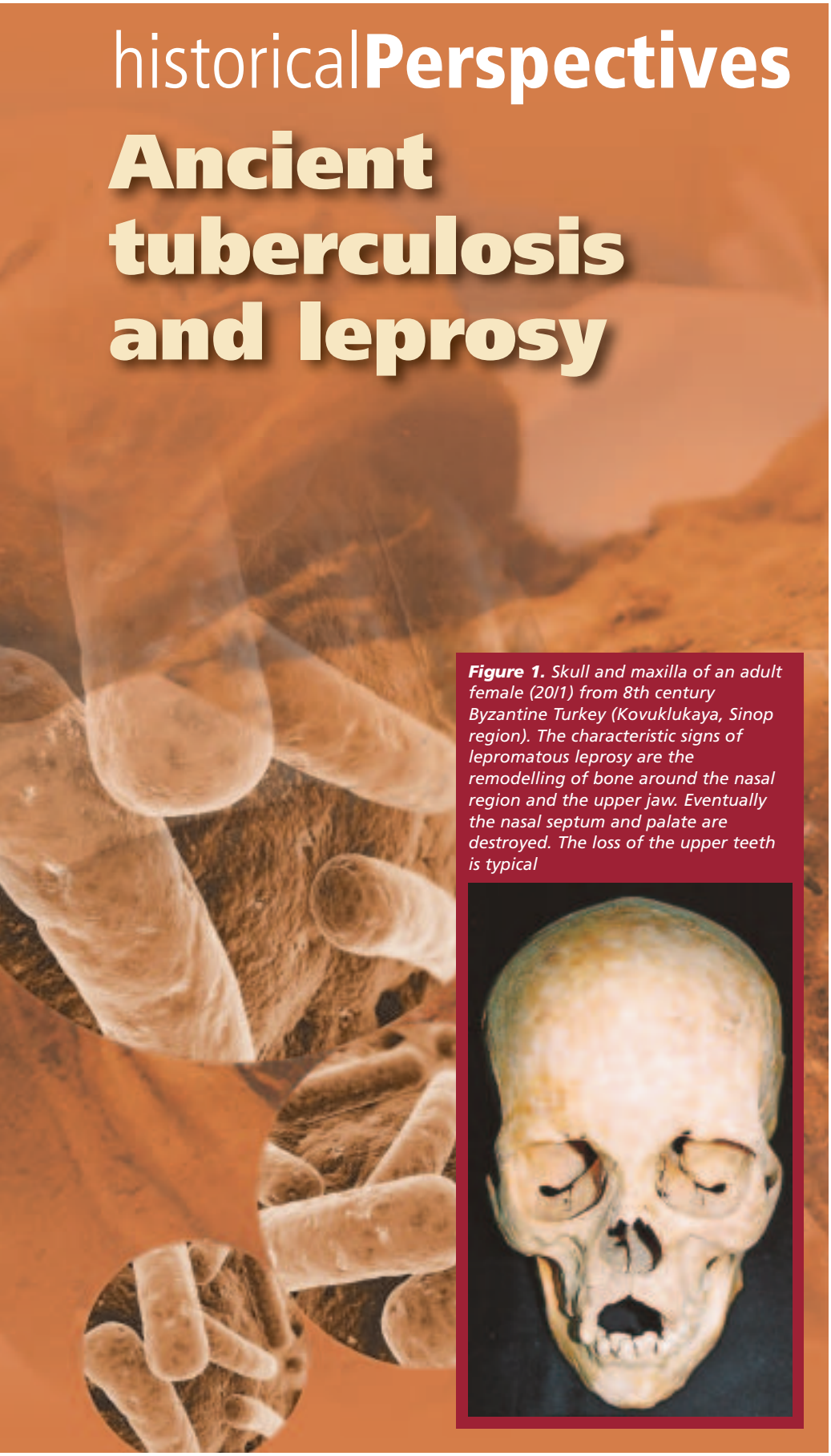


***Figure 1.*** *Skull and maxilla of an adult female (20/1) from 8th century Byzantine Turkey (Kovuklukaya, Sinop region). The characteristic signs of lepromatous leprosy are the remodelling of bone around the nasal region and the upper jaw. Eventually the nasal septum and palate are destroyed. The loss of the upper teeth is typical*

How old are these human infectious diseases? There are several strands of evidence we can use to attempt to answer this question. Many historical texts contain recognizable descriptions of tuberculosis, where it is named as consumption, King's Evil, lupus vulgaris, phthisis or scrofula. The Greek physician Hippocrates (460 to 370 BC) clearly described the vertebral changes associated with Pott's disease — the formation of a gibbus due to tuberculosis of the spine, while leprosy is described in ancient Chinese medical texts of a similar date but based on older material (Skinsnes & Chang, 1985). However, written descriptions alone can be misleading. For example, there has been much debate over the actual disease described as leprosy in the Bible.

Skeletal remains from past populations are the most obvious sources of scientific information. Many diseases leave traces on bones and teeth: some are non-specific — such as abscesses, others are so specific that a diagnosis is possible. Both tuberculosis and leprosy (Figure 1) can be recognized in archaeological skeletal remains, based on very characteristic combinations of changes, some of which are visible to the naked eye, others are disclosed by imaging and histology. Palaeopathology has emerged from physical anthropology, biomedical science and forensic science as a distinct discipline. Diagnostic criteria have been agreed, based on skeletal changes verified by archived patient records.

A totally different way to estimate the age of infectious diseases is that of genomics and ancestral sequence inference. Molecular sequences from different strains and species of modern pathogenic microorganisms are compared and bioinformatics lead to a calculation of 'the most recent common ancestor'.

Examination of human remains for molecular traces of past pathogens is a direct way to study ancient infections. Early studies tackled the host response and sought specific proteins or antibodies. The development of PCR led to much over-enthusiastic research on ancient animal and plant remains until the problems of contamination with modern DNA were appreciated. It was after this phase that the first attempt was made, in our department at University College London (UCL), to

detect ancient pathogenic bacterial DNA in human skeletal specimens (Spigelman & Lemma, 1993). Tuberculosis was the targeted disease due to a fortuitous combination of circumstances. There was consensus on the diagnostic criteria based on skeletal changes, and the molecular diagnosis of tuberculosis had been driven by the extremely slow growth of the causative organism and the need for speedier results. Also, there is no environmental reservoir of the pathogen. We published the first report of ancient PCR-confirmed leprosy a year after that of tuberculosis (Rafi *et al.*, 1994).

*Mycobacterium tuberculosis* and *Mycobacterium leprae* have GC-rich DNA enclosed in a resistant hydrophobic lipid-rich cell wall, both traits which are believed to contribute to the persistence of their ancient DNA (aDNA). This enables the molecular typing of both pathogens and characterization of individual members of the closely related *Myco. tuberculosis* complex. The aDNA findings are supported by parallel developments in organic chemistry, enabling the direct detection of protein and lipid biomolecules using techniques such as fluorescence high-performance liquid chromatography and negative ion chemical ionization gas chromatography mass spectrometry (Gernaey *et al.*, 2001; Redman *et al.*, 2009; Donoghue *et al.*, 2010). As a result the new field of palaeo-microbiology has emerged during the past 18 years (Table 1).

Tuberculosis is still a major global cause of death and disease, even though antimicrobial therapy has been available for over half a century. Around two billion people, about one third of the world's total population, are infected with tubercle bacilli (http://www.who.int/tb/publications/factsheets/en/index.html, accessed 7th September 2011) and in 2009 1.7 million people died from the disease. It is primarily a respiratory infection spread by infectious aerosols. Swallowing infected sputum or ingestion of infected animal products can cause gastrointestinal tuberculosis. Skeletal tuberculosis is rare as these are disseminated infections yet the host needs to survive for sufficient time for lesions to develop. Even in the pre-antibiotic era it is estimated that only 3 to 5% of tuberculosis infections were associated with bony changes. The great majority of human infections are caused by *Myco. tuberculosis sensu strictu*. *Mycobacterium bovis* is responsible for about 6% of human deaths from tuberculosis.

**Table 1.** How palaeomicrobiology has increased our understanding of ancient human infectious diseases

- Confirmation of visible palaeopathology of specific diseases
- Detection of disease in the absence of specific bony changes, or even of any skeletal or other morphological changes
- Detection of past co-infections, such as tuberculosis with leprosy, leishmaniasis, or intestinal parasites
- Insights into the social context of past human infections
- Evidence of the epidemiology and past geographical range of ancient infections
- Provision of real-time markers of genetic changes, thus enabling better understanding of the evolution of human pathogens
- Potential to increase our understanding of evolution of the host/pathogen relationship

Leprosy continues to be an ongoing problem in many parts of the world. It can be cured with multi-drug therapy but it is often under-reported due to social stigma and is therefore not treated. More than 213,000 people mainly in Asia and Africa are infected (http://www.who.int/wer/2011/wer8636/en/index.html, accessed 7th September 2011), with 228,474 new cases of leprosy detected during 2010. *Myco. leprae* targets Schwann cells and causes nerve damage. The clinical presentation can range from multi-bacillary or lepromatous leprosy, where there is a minimal host response and extremely high numbers of *Myco. leprae* in tissues, to the pauci-bacillary or tuberculoid form, with a good cell-mediated response and very low bacterial load. If left untreated, leprosy causes progressive and permanent damage to the skin, nerves, limbs and eyes in about 10 to 30% of cases, resulting in severe disability.

The very high level of latent tuberculosis, the slow rate of progression and low infectivity of leprosy suggest a long period of co-evolution of the pathogen with its human host. It is believed the long hunter-gatherer stage of human evolution, with small population sizes, selected symbionts or pathogens that could persist until transmitted (Blaser & Kirschner, 2007). In tuberculosis, active disease and transmission generally occurs in those with a less effective immune system such as the very young and old, or who suffer from malnourishment, other diseases, physical or mental stress. The development of agriculture and animal

***Figure 2.*** *Real-time PCR using a 6-carboxyfluorescein (FAM) specific fluorescent probe for an 80 base pair locus in the RLEP region of extinct Myco. leprae strains obtained from European archaeological samples, which span over 1,000 years. The cycle threshold is unrelated to the chronological age of samples but depends upon the local burial conditions and preservation of the aDNA*

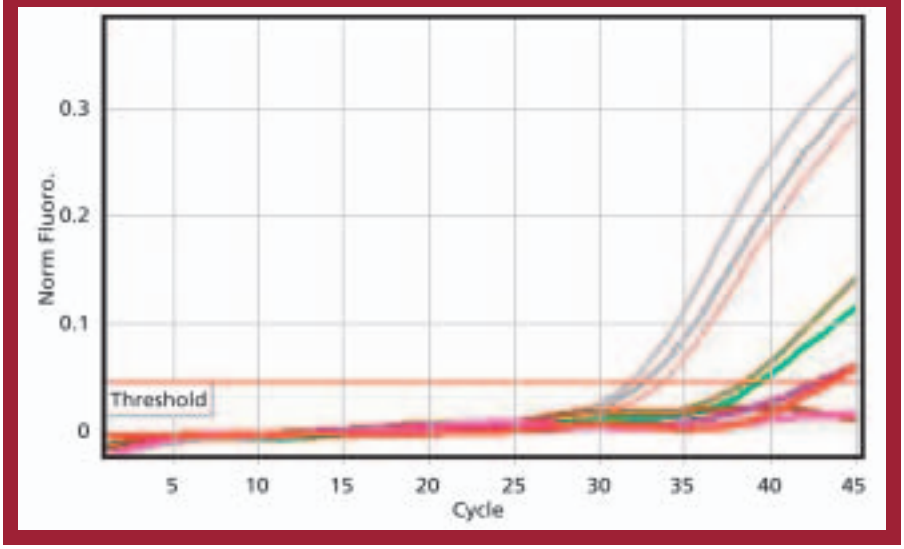
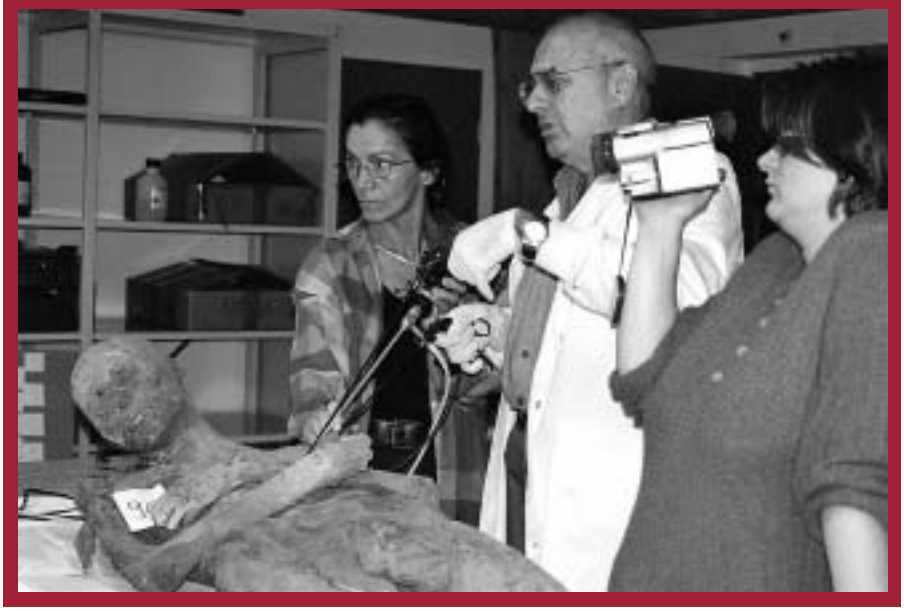


***Figure 3.*** *A naturally mummified body of a 33 year-old man from 18th century Vác, Hungary, one of 263 found in a church crypt during renovation. Chest X-rays suggested that several individuals had tuberculosis, which led to aDNA studies*

domestication in the Neolithic period had a profound impact on human society. There was a switch from foraging and hunting to settled communities, a sedentary lifestyle, less varied diet and pronounced differences in social status. According to the archaeological record tuberculosis is one of several diseases that appeared at this time. This led to the suggestion that humans acquired the infection from domesticated animals and that *Myco. bovis* was the ancestor of *Myco. tuberculosis*. This has now been disproved (see below) but it is certainly true that infectious diseases such as tuberculosis are associated with larger, denser populations.

***Figure 4.*** *Sampling one of the naturally mummified bodies from 18th century Vác, Hungary, using a surgical endoscope. This shows the team working in the Anthropology Department of the Natural History Museum, Budapest, in 1999. Left to right, Ildikó Pap — Head of Department, Mark Spigelman — the pioneer surgeon turned archaeologist who started work in this field, and Ildikó Szikossy — anthropologist in the Budapest department*



The first primers used to detect ancient tuberculosis were based on a specific locus found in all members of the *Myco. tuberculosis* complex, located in the insertion sequence IS6110, usually present in multiple copies in *Myco. tuberculosis*. Early studies confirmed tuberculosis in ancient Egypt, pre-European contact Borneo, China and pre-Columbian South America (Donoghue, 2011). *Mycobacterium africanum* was reported from ancient Egypt (Zink *et al.*, 2003), but the only reported case of ancient *Myco. bovis* infection is of a group of Iron Age pastoralists in Siberia (Taylor *et al.*, 2007). Single nucleotide polymorphisms (SNPs), deletion analysis and other specific loci have confirmed an extant lineage of *Myco. tuberculosis* in a Pre-Pottery Neolithic village in the Eastern Mediterranean from 9,000 years ago (Hershkovitz *et al.*, 2008).

The *Myco. leprae* genome shows evidence of widespread deletions and the organism is uncultivable. Ancient DNA studies have focused on repetitive elements such as RLEP, which has 37 copies per cell, thereby increasing the sensitivity of detection. Leprosy has been confirmed by aDNA in 1st century Palestine, Roman Egypt and the early Byzantine Empire (Donoghue *et al.*, 2005; Monot *et al.*, 2009). Some *Myco. leprae* aDNA is remarkably well preserved (Figure 2) enabling both SNP analysis, and identification of individual strains by microsatellite analysis (Taylor & Donoghue, 2011). In several cases of lepromatous leprosy, the individuals were shown by aDNA analysis to be co-infected with tuberculosis (Donoghue *et al.*, 2005). Reports of this phenomenon were found subsequently in historical accounts of the disease from the pre-antibiotic era.

In both *Myco. tuberculosis* and *Myco. leprae* there is a virtual lack of horizontal gene exchange, reducing the likelihood of SNPs. Therefore SNPs are phylogenetically informative and have enabled the identification of lineages in both species. It also appears that genomic regions lost by deletions are not re-acquired. Therefore the global population structure of both the *Myco. tuberculosis* complex and of *Myco. leprae* can be defined by these lineages. Because the sequence of deletion events is one-way, the evolutionary pathway and possible timescale can be inferred. This has clearly demonstrated that the

*Myco. tuberculosis* lineage is more ancestral than that of *Myco. bovis* (Gordon *et al.*, 2009). The discrimination between SNPs or principal genetic groups 1 to 3 in the *Myco. tuberculosis* complex is based on functionally neutral base changes in the catalase-peroxidase encoding gene *katG* and a subunit of the DNA gyrase gene *gyrA*. Little work has been done on the timescale of the emergence of these SNPs, but a study on naturally mummified bodies from 18th century Hungary, with exceptionally good preservation (Figures 3 and 4), showed that the population was infected with *Myco. tuberculosis* SNP types 2 and 3, thus demonstrating that these had emerged before the antibiotic era (Fletcher *et al.*, 2003).

It appears that both *Myco. tuberculosis* and *Myco. leprae* have undergone an evolutionary bottleneck and have a clonal relationship with their human host. Different human lineages have an association with a particular lineage of these pathogens, even in the modern world and in second-generation or third-generation inhabitants of ethnically diverse cities. In the case of the *Myco. tuberculosis* complex, one estimate based on bioinformatics is that the major lineages emerged 10,000 to 20,000 years ago (Wirth *et al.*, 2008). Therefore, direct detection of *Myco. tuberculosis* lineages from ancient human remains of known date, such as the detection of a lineage from 9,000 years ago which lacks the TbD1 deletion (Hershkovitz *et al.*, 2008) provides a marker in real-time.

Palaeomicrobiology has been especially helpful in elucidating the phylogeography of leprosy as it has enabled the genetic analysis of European indigenous strains of *Myco. leprae* from around 1,500 years ago that are now extinct (Monot *et al.*, 2009). Sub-genotyping of both extant and extinct strains of *Myco. leprae* from around the world showed a strong geographical association, suggesting migration patterns of early humans and trade routes. One conclusion of this study was that leprosy originated in Europe or the Middle East and then spread to the Far East.

Improvements in PCR procedures have increased the sensitivity and detection rate of aDNA from microbial pathogens. One example is that of specific DNA probes, which incorporate a fluorescent reporter that is quenched unless the probe has bound to the specific target sequence. This enables the design of primers with a far smaller target sequence, which is needed as strand separation during PCR leads to fragmentation of aDNA. The norm now is a target fragment size of around 70 base pairs. Next generation technologies that incorporate direct sequence capture to surface-bound primers or aptomers should facilitate the enrichment and detection of aDNA in this challenging field.

Until now most work has been centred on the microorganism. However, the characteristics of the host are of equal importance. In this regard, the exceptionally well-preserved human remains from 18th century Hungary, with a contemporary archive giving details of family members, suggest a possible strategy for the future, as it appears possible to discriminate between individuals with active and latent tuberculosis infections. A study based on material such as this may enable us to address the question of the changing nature of the interactions between host and pathogen over time.

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