Genotyping of ancient *Mycobacterium tuberculosis* strains reveals historic genetic diversity

Romy Müller¹, Charlotte A. Roberts² and Terence A. Brown¹

¹Manchester Institute of Biotechnology, Faculty of Life Sciences, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK
²Department of Archaeology, Durham University, South Road, Durham DH1 3LE, UK

The evolutionary history of the *Mycobacterium tuberculosis* complex (MTBC) has previously been studied by analysis of sequence diversity in extant strains, but not addressed by direct examination of strain genotypes in archaeological remains. Here, we use ancient DNA sequencing to type 11 single nucleotide polymorphisms and two large sequence polymorphisms in the MTBC strains present in 10 archaeological samples from skeletons from Britain and Europe dating to the second–nineteenth centuries AD. The results enable us to assign the strains to groupings and lineages recognized in the extant MTBC. We show that at least during the eighteenth–nineteenth centuries AD, strains of *M. tuberculosis* belonging to different genetic groups were present in Britain at the same time, possibly even at a single location, and we present evidence for a mixed infection in at least one individual. Our study shows that ancient DNA typing applied to multiple samples can provide sufficiently detailed information to contribute to both archaeological and evolutionary knowledge of the history of tuberculosis.

1. Introduction

Tuberculosis (TB) has caused millions of deaths throughout history and is still a major burden in many parts of the world. Especially in the seventeenth–nineteenth centuries AD, TB was highly prevalent throughout Europe, urbanization facilitating spread of the disease in overcrowded environments [1]. The improved living standards in the late nineteenth century AD led to a decline in incidence rates and a further drop was achieved by the use of antibiotics in the mid-twentieth century [1,2]. Since its re-emergence in the 1980s, however, TB has once again become one of the leading infectious diseases, causing morbidity and mortality in all parts of the world, with an estimated 1.4 million deaths in 2011 [3].

TB is caused by the members of the *Mycobacterium tuberculosis* complex (MTBC), with *M. tuberculosis* being the most common infecting species in humans. The MTBC also comprises the human pathogens *M. africanum* and *M. canetti* as well as the primarily animal infecting species *M. bovis*, *M. microti*, *M. pinnipedii* and *M. caprae*, all of which have been identified as causative agents of TB in humans [4–8]. The continuing appearance of antibiotic-resistant MTBC strains has stimulated interest in the evolutionary history of TB, in particular the possible coevolution between MTBC lineages and human populations [9]. Modern genetic data indicate that the MTBC may have coexisted with humans for at least 15 000 years [10–14], and archaeological evidence suggests that it has afflicted humankind since the Neolithic [15–18]. Throughout the past two decades, studies of ancient DNA (aDNA) in archaeological remains have begun to contribute to our understanding of the evolutionary history of the MTBC, several publications reporting the presence of MTBC aDNA in human remains, and some attempting to classify the infecting MTBC strains based on the identities of various genetic markers [17–28].
Several methods are used in clinical research to classify MTBC isolates into groups of related strains, including IS6110 restriction fragment length polymorphism (IS6110 RFLP) [29], mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing [30,31], spacer oligotyping (spoligotyping) [32], targeting of large sequence polymorphisms (LSPs) [33–35] and typing of single nucleotide polymorphisms (SNPs) [13,36–39]. RFLP analysis of IS6110, MIRU-VNTR typing and spoligotyping are less suitable for phylogenetic analyses as convergent evolution may result in homoplasy of the targeted loci [40,41]. Spoligotyping has been applied in studies of ancient TB [17–24] but its use has been questioned [42], not just because of its limited phylogenetic value but also because of its methodological problems when highly degraded aDNA is being analysed. LSPs and SNPs are considered to be the most suitable markers for strain identification and phylogenetic analysis, although they are not exempt from convergent evolution [43,44]. Early sets of SNPs were discovered by analysing selected genes or comparing genome sequences from only few strains, thereby not accessing a large part of the global variation [45]. More recently, de novo sequencing of 89 genes in 108 strains from all parts of the world identified 488 SNPs and resulted in a detailed phylogeny of the MTBC [36]. Whole genome comparison of 22 globally representative, mainly newly sequenced strains subsequently revealed 9037 unique SNPs and further resolved the phylogeny of the MTBC [47], and 34 167 SNPs identified from 259 strains have been used to study the coevolutionary history of M. tuberculosis and prehistoric human populations [14]. Targeting such large sets of loci is not feasible in aDNA studies if conventional PCR procedures are applied because the amount of extract available for analysis is usually very limited. These methodological constraints can be overcome by next generation sequencing (NGS), which is now being adopted in human aDNA studies and has recently been applied to historic strains of plague [48], TB [49] and leprosy [50]. However, NGS methods have their own limitations, requiring relatively large amounts of aDNA, being computationally intensive, and suffering from missing data owing to the absence of sequence reads covering particular SNPs, even ones that can be typed in the same sample by conventional PCR [49].

In this study, we show that an informative comparison of MTBC strains in archaeological human bone and dental samples is possible by conventional PCR of eleven SNPs and two LSPs (table 1). These markers enable MTBC strains to be classified into principal genetic groups (PGGs) 1–3 [36], lineages I–IV and M. bovis [38], SNP cluster groups (SCGs) 1–7 [39], ‘modern’ M. tuberculosis [33] and the Euro-American lineage of modern M. tuberculosis [34,35]. Five additional SNPs [51] allow a more precise classification of strains of the Euro-American lineage. We typed these markers in British and other European archaeological bone and dental samples from skeletons dated to the second–nineteenth centuries AD, revealing historic variations in genotype identities.

### Table 1. Loci targeted in this study in order to classify ancient MTBC strains.

| locus type | classification into | references |
|------------|--------------------|------------|
| SNP        | PGGs 1–3           | [36]       |
| gyrA        |                    |            |
| katG        |                    |            |
| oxyR        |                    |            |
| oxyR        |                    |            |
| rpoB        |                    |            |
| rpoB        |                    |            |
| oxyR        |                    |            |
| oxyR        |                    |            |
| rpoB        |                    |            |
| rpoB        |                    |            |
| leuB        | SNP cluster groups 1–7 | [39]       |
| qcrB        |                    |            |
| recN        |                    |            |
| Rv0083      | (92197)            |            |
| Rv2802c     | (3111473)          |            |
| LSP         | ancient/modern M. tuberculosis | [33] |
| TbD1        |                    |            |
| pks15/1     | Euro-American lineage | [34,35] |

*aNumbers in parentheses denote the nucleotide position in H37Rv, as given in Filliol et al. [39].*

2. Material and methods

Thirty-four bone and dental samples were selected based on the positive outcomes of PCRs directed at the IS6110 and IS1081 insertion sequences [52], which are looked on as specific for the MTBC group of bacteria. The samples come from skeletons dated to the second–nineteenth centuries AD and most but not all displayed lesions specific or non-specific for TB (electronic supplementary material, table S1). We have previously reported NGS and conventional SNP typing results for one of these samples, St George’s Crypt 4006 [49].

Samples were taken under clean conditions by personnel wearing forensic suits, hair nets, face masks and sterile gloves, and stored in sterile plastic bags under dry conditions. Work was performed at the University of Manchester and the Complutense University of Madrid. The aDNA facility at the University of Manchester comprises independent, physically isolated laboratories for extraction and PCR set-up, each with an ultrafiltered air supply maintaining positive displacement pressure. DNA extractions were prepared in a Class II biological safety cabinet, and PCRs were set up in a laminar flow hood. Surfaces were sterilized by UV irradiation and regularly cleaned with 5% bleach and 70% ethanol. All equipment was treated with DNA-Away (Molecular Bioproducts) and tubes, pipettes and aqueous solutions...
were UV irradiated (254 nm, 120,000 μJ cm⁻²) for at least 10 min before use. Personnel wore protective clothing at all times, including forensic suits, face masks, hair nets, goggles and two pairs of sterile gloves. Work in Madrid was also carried out in physically separated laboratories for DNA extraction and PCR set-up, UV irradiated both before and after use. Surfaces and laboratory equipment were regularly cleaned with bleach. Personnel wore disposable forensic suits, face masks, caps, glasses, shoe covers and gloves. All reagents and consumables were DNase and RNase free. All procedures were carried out in a laminar flow cabinet, UV irradiated and cleaned with bleach before use. DNA extractions were accompanied by two blanks (extraction without skeletal material) per five samples (Manchester) or one blank per extraction (Madrid). A set of 5–7 PCRs was always performed in a final volume of 30 μl comprising 1× Taq buffer (New England Biolabs), 200 nM each primer, 200 μM dNTPs and 0.625 units Taq DNA polymerase (New England Biolabs), with cycling at: 95°C for 3 min; 30 cycles of 30 s at 95°C, 1 min at 72°C; followed by a 1 min extension at 72°C. All PCR products were examined by electrophoresis in 2% agarose gels, purified either from the gel or directly using Qiaquick columns (Qiagen) and subsequently cloned into Escherichia coli XL1-Blue competent cells (Agilent) using the CloneJet PCR cloning kit (Fermentas). Colony PCR was performed in 20 μl comprising 1× Tag buffer (New England Biolabs), 200 nM each primer, 200 μM dNTPs and 0.625 units Taq DNA polymerase (New England Biolabs), with cycling at: 95°C for 3 min; 30 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C; 10 min at 72°C. PCR products were then sequenced (GATC Biotech, Cologne) and sequences aligned with the M. tuberculosis H37Rv reference sequence using Geneious v. 6.0.3 (http://www.geneious.com/).

To compare our results with 21 extant MTBC strains, targeted regions available from the six samples that provided unambiguous typing results were concatenated and aligned with the equivalent concatenated regions of the extant MTBC strains. For Auldhame 43, this procedure was repeated for all available sequences, including the four additional SNPs taken from Abadia et al. [51]. Neighbour-joining trees were created using MEGA v. 5.2.1 [56] and visualized with DENDROSCOPE v. 3.2.4 [57].

### 3. Results
We analysed 34 samples from 31 individuals from 22 archaeological sites and one pathological reference collection (electronic supplementary material, table S1) for 11 SNPs and two LSPs (table 1). We obtained data for ten samples from seven archaeological sites (electronic supplementary material, table S3), enabling us to classify the infecting MTBC strains according to their SNP identities, the presence/absence of the TbD1 locus, and/or the presence of deletions within pks15I (table 2). Six of the samples (Auldhame 43, Saint Amé 20, St George’s Crypt 4006, St Peter’s Church 1390, St Peter’s Collegiate Church 62, Whitefriars 10466) provided sufficient information, reproducible with two independent extracts, to assign the infecting strains to both their PGGs [36] and SCGs [39]. Two other samples, St Peter’s Collegiate Church 28 and

---

**Table 2. Summary of SNP and LSP data.** —, no result obtained; n.d., not done; TbD1⁺, deletion of TbD1 has occurred; Δ7 bp, 7 bp deletion in pks15I has occurred.

| sample ID   | sample date       | TbD1   | pks15I   | PGG          | lineage | SCG          |
|-------------|-------------------|--------|----------|--------------|---------|--------------|
| Ashchurch 705a | 129–131 calAD   | TbD1⁻/;/TbD1⁻ | Δ7 bp/Δ7 bp | 2/2         | I or II/– | 3/3 or 4     |
| Auldhame 43a | 1280–1394 calAD | TbD1⁻/n.d. | Δ7 bp/Δ7 bp | 2/2         | II/–    | 5/5          |
| Saint Amé 20 | 16th–18th centuries AD | –/n.d. | –/Δ7 bp | 3/3         | not IV/not III | 6/6       |
| St George’s Crypt 4006b | mid-19th century AD | TbD1⁻/TbD1⁻ | Δ7 bp/Δ7 bp | 3/3         | II/II   | 6/6          |
| St George’s Crypt 5003 | mid-19th century AD | –/TbD1⁻ | Δ7 bp/– | –/–         | not IV/– | 6/–          |
| St Peter’s Church 1390b | 1016–1155 calAD | TbD1⁻/n.d. | Δ7 bp/Δ7bp | 2/2         | I or II/– | 3/3          |
| St Peter’s Collegiate Church 28 | 19th century AD | TbD1⁻/n.d. | Δ7 bp/– | 3/–         | I or II/– | 6/–          |
| St Peter’s Collegiate Church 62b | 19th century AD | TbD1⁻/TbD1⁻ | Δ7 bp/Δ7 bp | 2/2         | I or II/II | 4/4          |
| Whitefriars 657 | 18th–19th centuries AD | –/n.d. | Δ7 bp/– | 2/3         | I or II/– | 6/6          |
| Whitefriars 10466b | 18th–19th centuries AD | TbD1⁻/n.d. | –/Δ7 bp | 2/2         | I or II/– | 4/4          |

aResults listed as first/second extraction.
bSecond extraction performed in Madrid, except for TbD1.

data, material, table S2). For primers with an annealing temperature less than or equal to 60°C, a three-temperature PCR was set up, with each annealing step at x°C followed by a 1 min extension at 72°C. All PCR products were examined by electrophoresis in 2% agarose gels, purified either from the gel or directly using Qiaquick columns (Qiagen) and subsequently cloned into Escherichia coli XL1-Blue competent cells (Agilent) using the CloneJet PCR cloning kit (Fermentas). Colony PCR was performed in 20 μl comprising 1× Tag buffer (New England Biolabs), 200 nM each primer, 200 μM dNTPs and 0.625 units Taq DNA polymerase (New England Biolabs), with cycling at: 95°C for 3 min; 30 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C; 10 min at 72°C. PCR products were then sequenced (GATC Biotech, Cologne) and sequences aligned with the M. tuberculosis H37Rv reference sequence using Geneious v. 6.0.3 (http://www.geneious.com/).

To compare our results with 21 extant MTBC strains, targeted regions available from the six samples that provided unambiguous typing results were concatenated and aligned with the equivalent concatenated regions of the extant MTBC strains. For Auldhame 43, this procedure was repeated for all available sequences, including the four additional SNPs taken from Abadia et al. [51]. Neighbour-joining trees were created using MEGA v. 5.2.1 [56] and visualized with DENDROSCOPE v. 3.2.4 [57].

---

| sample ID   | sample date       | TbD1   | pks15I   | PGG          | lineage | SCG          |
|-------------|-------------------|--------|----------|--------------|---------|--------------|
| Ashchurch 705a | 129–131 calAD   | TbD1⁻/;/TbD1⁻ | Δ7 bp/Δ7 bp | 2/2         | I or II/– | 3/3 or 4     |
| Auldhame 43a | 1280–1394 calAD | TbD1⁻/n.d. | Δ7 bp/Δ7 bp | 2/2         | II/–    | 5/5          |
| Saint Amé 20 | 16th–18th centuries AD | –/n.d. | –/Δ7 bp | 3/3         | not IV/not III | 6/6       |
| St George’s Crypt 4006b | mid-19th century AD | TbD1⁻/TbD1⁻ | Δ7 bp/Δ7 bp | 3/3         | II/II   | 6/6          |
| St George’s Crypt 5003 | mid-19th century AD | –/TbD1⁻ | Δ7 bp/– | –/–         | not IV/– | 6/–          |
| St Peter’s Church 1390b | 1016–1155 calAD | TbD1⁻/n.d. | Δ7 bp/Δ7bp | 2/2         | I or II/– | 3/3          |
| St Peter’s Collegiate Church 28 | 19th century AD | TbD1⁻/n.d. | Δ7 bp/– | 3/–         | I or II/– | 6/–          |
| St Peter’s Collegiate Church 62b | 19th century AD | TbD1⁻/TbD1⁻ | Δ7 bp/Δ7 bp | 2/2         | I or II/II | 4/4          |
| Whitefriars 657 | 18th–19th centuries AD | –/n.d. | Δ7 bp/– | 2/3         | I or II/– | 6/6          |
| Whitefriars 10466b | 18th–19th centuries AD | TbD1⁻/n.d. | –/Δ7 bp | 2/2         | I or II/– | 4/4          |

aResults listed as first/second extraction.
bSecond extraction performed in Madrid, except for TbD1.
St George’s Crypt 5003, gave incomplete results that enabled a PGG and/or SCG to be assigned from one extract but not confirmed with the second extract. Ambiguous results were obtained with two additional samples. Whilst Whitefriars 657 was identified as containing a SCG 6 strain, the \textit{gyrA} \textsuperscript{284} SNP was typed as a C in one extract and G in the other, suggesting either PGG 2 or 3. With Ashchurch 705, clone sequences from the first extract gave a mixture of C and G at this position, and the \textit{qcrB} SNP, which distinguishes SCG 3 from SCG 4, could be typed with only one of the two extracts (electronic supplementary material, table S3).

The \textit{oxyR} and \textit{rpoB} PCRs used to assign strains to lineages I–IV [38] were less successful owing to lack of reproducibility and amplification of non-specific \textit{rpoB} \textsuperscript{3243} targets. Nine of the 10 samples could be assigned to lineages I or II, but distinction between these lineages, which requires \textit{rpoB} \textsuperscript{3243}, was only possible for Auldhame 43, St Peter’s Collegiate Church 62 and St George’s Crypt 4006, each of which was identified as lineage II based on results with one or both extracts. St George’s Crypt 5003 could only be identified as not lineage IV. The \textit{pks15/1} PCR revealed a 7 bp deletion for each of the 10 samples, suggesting that the strains belong to the Euro-American lineage, and amplification of the region flanking \textit{TbD1} was achieved for eight samples, again indicating that these are modern \textit{M. tuberculosis} strains.

All but one of the 10 samples for which we obtained strain data came from British excavation sites spanning the second–nineteenth centuries AD, the one exception coming from sixteenth–eighteenth centuries AD Douai in northern France (figure 1). The relationships between these strains and modern ones are depicted as a neighbour-joining tree in figure 2a. Additional targeting of five of the SNPs reported by Abadia \textit{et al}. [51] was attempted with one extract of Auldhame 43, a member of SCG 5. Four SNPs were typed, enabling more accurate resolution of the position of Auldhame 43 compared to the extant MTBC strains (figure 2b).

4. Discussion
We obtained sufficient SNP and/or LSP data to classify the \textit{M. tuberculosis} strains present in 10 of the 34 bone and dental samples that we studied. Our results clearly show that, at least during the eighteenth–nineteenth centuries AD, strains of \textit{M. tuberculosis} belonging to different genetic groups were present in Britain at the same time, possibly even at a single location. Within this time period, we discovered PGG 2/SCG 4 strains in individuals at sites in Norwich, eastern England (Whitefriars 10466), and Wolverhampton, central England (St Peter’s Collegiate Church 62), and a PGG 3/SCG 6 strain at Leeds, northern England (St George’s Crypt 4006). Strains of both types might even have coexisted in the same local areas, as a second Wolverhampton individual (St Peter’s Collegiate Church 28) had a PGG 3/SCG 6 strain, and a second individual from Norwich (Whitefriars 657) had a PGG \textsuperscript{2/3} SCG 6 strain.

One explanation of the ambiguous Whitefriars 657 result is that this individual was co-infected with two strains of \textit{M. tuberculosis}, one strain belonging to PGG 2 and the other to PGG 3. Based on the presence of a T at Rv0083, in both extracts, the infecting strains could further be assigned to SCG 6. However, SNP typing success with the second extract

![Figure 1](http://rspb.royalsocietypublishing.org/Downloaded from http://rspb.royalsocietypublishing.org/)
Figure 2. (a) Neighbour-joining tree comparing the concatenated sequences of eight regions (478 bp — gyrA, katG, leuB, oxyR, pks15/1, qcrB, rpoB and Rv0083) obtained from a total of six samples (Auldhame 43, Saint Amé 20, St Peter’s Church 1390, St Peter’s Collegiate Church 62, St George’s Crypt 4006 and Whitefriars 10466) with the equivalent regions of 21 extant MTBC strains. These extant MTBC strains are M. tuberculosis strains H37Rv (National Center for Biotechnology Information reference sequence NC_000962.2), H37Ra (NC_009525.1), ATCC35801 str. Erdman (AP012340.1), CDC5079 (NC_017523.1), CDC5180 (NC_017522.1), CDC1551 (NC_002755.2), CTRI-2 (NC_017524.1), F11 (NC_009565.1), KZN605 (NC_018078.1), KZN1435 (NC_012943.1), KZN4207 (NC_012943.1), RGTB327 (NC_017026.1), RGTB423 (NC_017528.1), UT205 (NC_016934.1) and HN878 (CM001043.1) as well as M. bovis bacillus Calmette–Güerin str. Mexico, (NC_016804.1), M. canettii GM041182 (NC_015758.1) and M. africanum CIPT140010059 (NC_015848.1). Further strain data from whole genome shotgun sequencing projects was available from the Broad Institute (M. tuberculosis comparative sequencing project, Broad Institute of Harvard and MIT (http://www.broadinstitute.org/)) for: M. tuberculosis C (GenBank accession number AACR00000000), M. tuberculosis Haarlem (AA5N00000000) and M. tuberculosis W-148 (AC5X00000000). (b) Neighbour-joining tree comparing the concatenated sequences of 16 regions (1054 bp) obtained from Auldhame 43 with the equivalent regions of the 21 extant MTBC strains listed in (a). Bootstrap values were weak for both trees, as expected due to the small character set.

was limited (table 2; electronic supplementary material, table S3), and for this reason we look on the individual from Whitefriars (657) as having possible but not definite mixed infection, i.e. TB caused by two different strains. A second, more convincing example of mixed infection was presented by the Roman sample Ashchurch 705. One of the extracts of this sample gave both possible nucleotides for gyrA (284 and 281), indicating an infection with both PGG 2 and PGG 3 strains. Owing to the type of polymorphism, we exclude the disparity as resulting from a miscoding lesion [58–60], and there was no evidence of contamination (electronic supplementary material). Mixed infection has been reported with patients today, either as concurrent infections with multiple MTBC strains or as exogenous re-infections [61]. The markers we typed do not distinguish the a and b subgroups of SCG 6. However, we have previously shown by NGS genotyping that skeleton 4006 from St George’s Crypt had a SCG 6b strain [49]. This is the same SCG as the MTBC reference strain H37Rv [39], which was first isolated at the beginning of the twentieth century from a North American patient [62]. Our results therefore indicate that strains similar to H37Rv might have been present in continental Europe in the sixteenth–eighteenth centuries AD and geographically dispersed in eighteenth–nineteenth centuries AD Britain.

The markers we typed do not distinguish the a and b subgroups of SCG 6. However, we have previously shown by NGS genotyping that skeleton 4006 from St George’s Crypt had a SCG 6b strain [49]. This is the same SCG as the MTBC reference strain H37Rv [39], which was first isolated at the beginning of the twentieth century from a North American patient [62]. Our results therefore indicate that strains similar to H37Rv might have been present in continental Europe in the sixteenth–eighteenth centuries AD and geographically dispersed in eighteenth–nineteenth centuries AD Britain.

Strains belonging to SCG 3b, 3c, 4 and 5 fall into PGG 2, as they harbour a polymorphism at katG but not gyrA [39]. By contrast, SCG 6 strains display polymorphisms at both katG and gyrA and are therefore classified as PGG 3 [39]. Further analyses will be necessary in order to identify whether skeletons from Ashchurch (705) and St Peter’s Church 1390 (1016–1155 calAD), both of whom had strains belonging to PGG 2/SCG 5, and skeleton 43 from Auldhame (1260–1349 calAD), which was identified as PGG 2/SCG 5. The remaining six British samples, dated to the eighteenth–nineteenth centuries AD, were from individuals with either PGG 2/SCG 4 or PGG 3/SCG 6, with skeleton 657 from Whitefriars possibly also harbouring a PGG 2 strain. Additionally, a sixteenth–eighteenth century AD individual from Douai, northern France (Saint Amé 20), was shown to contain a PGG 3/SCG 6 strain.
individuals (39) indeed had strains belonging to SCG 3b or 3c as suggested by the PGG 2 determination or if they had strains belonging to subgroup SCG 3a which is primarily found within PGG 1 (39), with possible exceptions (63). PGGs 2 and 3 (and likewise their associated SCGs) are also known to comprise lineage II strains, as identified by a polymorphism at rpoB (32,33) and, to lack the M. tuberculosis-specific deletion Tbd1 (33) as well as a 7 bp region within pks15/1 (34). Deletion of the 7 bp region was revealed for all 10 of our samples, with eight of them also shown to lack Tbd1, classifying them as strains belonging to the Euro-American clade of modern M. tuberculosis. While deletion of Tbd1 has previously been reported in an individual from Britain dating to approximately 2,200 years BP (25), our results for Ashchurch 705 now additionally disclose that the deletion of the 7 bp region within pks15/1 had already taken place by the second–fourth centuries AD in Britain.

By typing four of the SNPs reported by Abadia et al. (51), we showed that the infecting SCG 5 strain found in the skeleton of Auldhame 43 is a member of the group that is ancestral to extant strains of the Latin-American Mediterranean clade within the Euro-American lineage. The detection of a SCG 5 strain at Auldhame (a coastal settlement east of Edinburgh, Scotland) but a SCG 3 strain at Leicester only about 100–200 years earlier raises the possibility that the Auldhame strain was not introduced into the human population in Scotland from southern parts of Britain but from Scandinavia instead. The first archaeological evidence of TB in Scotland (64) pre-dates Viking invasions in the late eighth century AD and Scandinavian contact in subsequent centuries (65), and stable isotope analysis of samples from individuals from the Auldhame site has indicated that the skeletal population itself is most likely composed of local individuals (66). Nevertheless, introduction of (new) TB strains via this route is a possible scenario as the osteological evidence suggests the presence of TB in Scandinavia during the Iron Age (fifth–first centuries BC) as well as the medieval period (1050–1536 AD) (1), 67–69.

Acknowledgements. We thank Darlene Weston (University of British Columbia) for collection of samples and Abigail Bouwman (University of Zürich) for sampling and extraction of a subset of samples at Manchester. We further thank Eva Fernández-Dominguez (John Moores University of Liverpool) for extraction of samples and helping R.M. in Madrid, Cristina Gamba (University College Dublin) for supporting R.M. and Eduardo Arroyo-Pardo (Complutense University of Madrid) for the invitation to analyse our samples in Madrid. We also thank the following people and/or institutions for providing skeletal samples: Bedford Museum (Kempston); Wessex Archaeology (West Thurrock, Purfleet); Gloucester County Archaeology Service (Ashchurch Bridge, Ashchurch); Division of Archaeological and Environmental Sciences, University of Bradford (Kingsholm; St Peter’s Collegiate Church, Wolverhampton); Cotswold Archaeology (Wheatpieces, Tewkesbury); Museum of London Archaeology Services (St Benet Sherehog, London); Department of Archaeology, Durham University (Manchester Hanging Ditch); North Hertfordshire Museums Resource Centre (Baldock); University of Leicester Archaeological Services (St Peter’s Church, Leicester); English Heritage (Ancaster); Lindsey Archaeological Services (Horncastle); Cambridgeshire Archaeology and Norfolk Museums and Archaeology Service (Whitfariers, Norwich); Peterborough Archaeology (Ashton); Northamptonshire Archaeology (Water Lane, Towcester); Oxfordshire Museum Service (Quornsford MiB); MAP Archaeological Consultancy Ltd (St George’s, Leeds); Prof. D. Rimantas Jankauskas, Department of Anatomy, Histology, and Anthropology, University of Vilnius, Lithuania (Obelia); Dr Inna Potekhina and Dr Aleksandra Kozak, Institute of Archaeology, Ukrainian Academy of Sciences, Kiev, Ukraine (Shechekasvita; Naberezhno-Kreschatistskaya, Kiev); Dr William Devriendt, Direction de l’Archéologie Préventive, Communauté d’Agglomération du Douaisis (Saint Arnaud, Douai); Prof. Bernd Herrmann and Dr Birgit Großkopf, Historical Anthropology and Human Ecology, Georg-August University Göttingen (Göttingen, Anthropology Pathology Collections); Jaime Jennings (Durham University), who sampled the Auldhame skeleton that was excavated by AOC Archaeology on behalf of Historic Scotland.

funding statement. This work was supported by NERC grants NE/E015697/1 and NE/E018564/1 awarded to C.A.R. and T.A.B. R.M. acknowledges support of a Natural Environment Research Council studentship.

References

1. Roberts CA, Buikstra JE. 2003 The bioarchaeology of tuberculosis. A global view on a reemerging disease. Gainesville, FL: University Press of Florida.

2. Länneroth K, Jaramillo E, Williams BG, Dye C, Raviglione M. 2009 Drivers of tuberculosis. A global view on a reemerging disease. Emerg. Infect. Dis. 8, 1342–1346. (doi:10.1371/journal.ppat.0010005)

3. Lo¨nnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M. 2009 Drivers of tuberculosis. A global view on a reemerging disease. Emerg. Infect. Dis. 8, 1342–1346. (doi:10.1371/journal.ppat.0010005)

4. Aranaz A, Cousins D, Mateos A, Domı´nguez L. 2003 A novel pathogenic taxon of the Mycobacterium tuberculosis complex, canetti: characterization of an exceptional isolate of Africa. Int. J. Syst. Bacteriol. 47, 1236–1245. (doi:10.1099/00207713-47-4-1236)

5. Van Sooelingen D et al. 1998 Diagnosis of Mycobacterium microti infections among humans by using novel genetic markers. J. Clin. Microbiol. 36, 1840–1845.

6. Van Sooelingen D et al. 1998 Diagnosis of Mycobacterium microti infections among humans by using novel genetic markers. J. Clin. Microbiol. 36, 1840–1845.

7. Kiers A, Klarenbeek A, Mendelts B, Van Soolingen D, Koeter G. 2008 Transmission of Mycobacterium pinnipedii. J. Clin. Microbiol. 46, 3243–3245. (doi:10.1128/jcm.01255-08)

8. Aranaz A, Cousins D, Mateos A, Domı´nguez L. 2003 A novel pathogenic taxon of the Mycobacterium tuberculosis complex, canetti: characterization of an exceptional isolate of Africa. Int. J. Syst. Bacteriol. 47, 1236–1245. (doi:10.1099/00207713-47-4-1236)

9. Kiers A, Klarenbeek A, Mendelts B, Van Sooeling D, Koeter G. 2008 Transmission of Mycobacterium pinnipedii to humans in a zoo with marine mammals. Int. J. Tuberc. Lung Dis. 12, 1469–1473.

10. Gagneux S, Komfa M. 2008 Pathogen coevolution in human tuberculosis. Phil. Trans. R. Soc. B 367, 850–859. (doi:10.1098/rstb.2011.0136)

11. Kapers V, Whittam TS, Musser JM. 1994 Is Mycobacterium tuberculosis 15,000 years old? J. Infect. Dis. 170, 1348–1349. (doi:10.1093/jid/170.5.1348)

12. Kapers V, Whittam TS, Musser JM. 1994 Is Mycobacterium tuberculosis 15,000 years old? J. Infect. Dis. 170, 1348–1349. (doi:10.1093/jid/170.5.1348)
Italy), Am. J. Phys. Anthropol. 72, 1–6. (doi:10.1002/aja.1330720102)

16. Cani A, Minozzi S, Borgognini Tarsitani SM. 1996 New evidence of tuberculosis spondylitis from Neolithic Liguaria (Italy). Int. J. Osteoarchaeol. 6, 497–501. (doi:10.1002/(SICI)1099-1212(199612)6:5<497::AID-OA291>3.0.CO;2-O)

17. Nickisch N, Maixner F, Ganslmeier R, Friederich S, Desely V, Meller H, Zink A, Alt KW. 2012 Rib lesions in skeletons from Early Neolithic sites in Central Germany: on the trail of tuberculosis at the onset of agriculture. Am. J. Phys. Anthropol. 149, 391–404. (doi:10.1002/aja.22137)

18. Hershkovitz I et al. 2008 Detection and molecular characterization of 9000-year-old Mycobacterium tuberculosis from a Neolithic settlement in the Eastern Mediterranean. PLoS ONE 3, e3426. (doi:10.1371/journal.pone.0003426)

19. Fletcher HA, Donoghues HD, Taylor GM, van der Zanden AGM, Spigelman M. 2003 Molecular analysis of Mycobacterium tuberculosis DNA from a family of 18th century Hungarians. Microbiology 149, 143–151. (doi:10.1099/mic.0.25961-0)

20. Taylor GM, Goyal M, Legge AJ, Shaw RJ, Young D. 1999 Genotypic analysis of Mycobacterium tuberculosis from medieval human remains. Microbiology 145, 899–904. (doi:10.1099/13500872-145-4-899)

21. Zink AR, Sola C, Reichsl C, Grabner W, Rastogi N, Wolf H, Nerlich AG. 2004 Molecular identification and characterization of Mycobacterium tuberculosis complex in ancient Egyptian mummies. Int. J. Osteoarchaeol. 14, 404–413. (doi:10.1002/oa.724)

22. Zink AR, Sola C, Reichsl C, Grabner W, Rastogi N, Wolf H, Nerlich AG. 2003 Characterization of Mycobacterium tuberculosis complex DNAs from Mycobacterium tuberculosis from medieval human remains. J. Clin. Microbiol. 41, 359–367. (doi:10.1128/JCM.41.1.359-367.2003)

23. Zink AR, Molnár E, Motamedi N, Palfy G, Marcsik A, Nerlich AG. 2007 Molecular history of tuberculosis in 14th–15th century Hungarians. Am. J. Phys. Anthropol. 137, 150 – 158. (doi:10.1002/ajpa.002154-0)

24. Marmiesse M, Brodin P, Buchrieser C, Gutierrez C, Simoes N, Vincent V, Glaser P, Cole ST, Brosch R. 2004 Macro-array and bioinformatic analyses reveal mycobacterial ‘core’ genes, variation in the ESAT-6 gene family and new phylogenetic markers for the Mycobacterium tuberculosis complex. Microbiology 150, 483–496. (doi:10.1099/mic.0.26662-0)

25. Gagneux S et al. 2006 Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc. Natl. Acad. Sci. USA 103, 2689–2693. (doi:10.1073/pnas.0511240103)

26. Greub G et al. 2003 Spoligotyping of Mycobacterium tuberculosis complex. Emerg. Infect. Dis. 9, 1142–1149. (doi:10.3201/eid9408.020873)

27. Taylor GM, Murphy E, Hopkins R, Rutland P, Chistov Y. 2007 First report of Mycobacterium bovis DNA in human remains from the Iron Age. Microbiology 153, 1243–1249. (doi:10.1099/mic.0.2006/002154-0)

28. Fletcher HA, Donoghue HD, Holton H, Pap I, Spigelman M. 2003 Widespread occurrence of Mycobacterium tuberculosis DNA from 18th–19th century Hungarians. Am. J. Phys. Anthropol. 120, 144 – 152. (doi:10.1002/aja.10114)

29. Van Embden JDA et al. 1993 Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol. 31, 406–409.

30. Supply P, Mazars E, Lesjean S, Vincent V, Gicquel B, Locht C. 2000 Variable human minisatellite-like regions in the Mycobacterium tuberculosis genome. Mol. Microbiol. 36, 762–771. (doi:10.1046/j.1365-2958.2000.01905.x)

31. Supply P et al. 2006 Proposal for standardization of mycobacterial interspersed repetitive unit—variable-number tandem repeat typing of Mycobacterium tuberculosis. J. Clin. Microbiol. 44, 4496–4510. (doi:10.1128/JCM.001392-06)

32. Kamerbeek J et al. 1997 Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J. Clin. Microbiol. 35, 907–914.

33. Brosch R et al. 2002 A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc. Natl. Acad. Sci. USA 99, 3684–3689. (doi:10.1073/pnas.052549299)

34. Marmiesse M, Brotin P, Buchrieser C, Gutierrez C, Simoes N, Vincent V, Glaser P, Cole ST, Brosch R. 2004 Mac-array and bioinformatic analyses reveal mycobacterial ‘core’ genes, variation in the ESAT-6 gene family and new phylogenetic markers for the Mycobacterium tuberculosis complex. Microbiology 150, 483–496. (doi:10.1099/mic.0.26662-0)

35. Gagneux S et al. 2006 Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc. Natl. Acad. Sci. USA 103, 2689–2693. (doi:10.1073/pnas.0511240103)

36. Greub G et al. 2003 Spoligotyping of Mycobacterium tuberculosis complex. Emerg. Infect. Dis. 9, 1142–1149. (doi:10.3201/eid9408.020873)

37. Gutacker MM et al. 2002 Genome-wide analysis of synonymous single nucleotide polymorphisms in Mycobacterium tuberculosis complex organisms: resolution of genetic relationships among closely related microbial strains. Genetics 162, 1533 – 1543.

38. Baker L, Brown T, Maiden MC, Drobniewski F. 2004 Hypertrophic osteoarthropathy from Mediaeval skeletons from Early Neolithic sites in Central Europe. Int. J. Osteoarchaeol. 14, 2236 – 2240. (doi:10.1111/j.10.1038/nature10549)

39. Bouwman AS, Kennedy SL, Müller R, Stephens RH, Holst M, Caffelli A, Behr MA, Victor TA. 2012 Genotype of a historic strain of Mycobacterium tuberculosis. Proc. Natl. Acad. Sci. USA 109, 18511–18516. (doi:10.1073/pnas.120944109)

40. Schuenemann VJ et al. 2013 Genome-wide comparison of medieval and modern Mycobacterium leprae. Science 341, 179 – 183. (doi:10.1126/science.1238286)

41. Abadía E et al. 2010 Resolving lineage assignment on Mycobacterium tuberculosis clinical isolates classified by spoligotyping with a new high-throughput 3R SNPs based method. Infect. Genet. Evol. 10, 1066–1074. (doi:10.1016/j.meegid.2010.07.006)

42. Müller R, Roberts CA, Brown TA. 2013 Biomolecular identification of ancient Mycobacterium tuberculosis complex DNA in human remains from Britain and Continental Europe. Am. J. Phys. Anthropol. 153, 178–189. (doi:10.1002/aja.22417)
53. Bouwman AS, Brown TA. 2005 The limits of biomolecular palaeopathology: ancient DNA cannot be used to study venereal syphilis. J. Archaeol. Sci. 32, 703 – 713. (doi:10.1016/j.jas.2004.11.014)

54. Rohland N, Hofreiter M. 2007 Ancient DNA extraction from bones and teeth. Nat. Prot. 2, 1756 – 1762. (doi:10.1038/nprot.2007.247)

55. Rohland N, Siedel H, Hofreiter M. 2010 A rapid column-based ancient DNA extraction method for increased sample throughput. Mol. Ecol. Resour. 10, 677 – 683. (doi:10.1111/j.1755-0996.2009.02824.x)

56. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731 – 2739. (doi:10.1093/molbev/msr121)

57. Huson DH, Scornavacca C. 2012 Dendroscope 3: an interactive viewer for rooted phylogenetic trees and networks. Syst. Biol. 61, 1061 – 1067. (doi:10.1093/sysbio/sys062)

58. Gilbert MT, Hansen AJ, Willerslev E, Rudbeck L, Barnes I, Lynnerup N, Cooper A. 2003 Characterization of genetic miscoding lesions caused by postmortem damage. Am. J. Hum. Genet. 72, 48 – 61. (doi:10.1086/345379)

59. Gilbert MTP, Binladen J, Miller W, Wuf C, Willerslev E, Poinar H, Carlson JE, Lembergs-Mack JH, Schuster SC. 2007 Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. Nucleic Acids Res. 35, 1 – 10. (doi:10.1093/nar/gkl483)

60. Brotherton P, Endicott P, Sanchez JJ, Beaumont M, Barnett R, Austin J, Cooper A. 2007 Novel high-resolution characterization of ancient DNA reveals C->U-type base modification events as the sole cause of post mortem miscoding lesions. Nucleic Acids Res. 35, 5717 – 5728. (doi:10.1093/nar/gkm588)

61. Chiang C-Y, Riley LW. 2005 Exogenous reinfection in tuberculosis. Lancet Infect. Dis. 5, 629 – 636. (doi:10.1016/S1473-3099(05)70240-1)

62. Steenken Jr W, Gardner LU. 1946 History of H37 strain of tubercle bacillus. Am. Rev. Tuberc. 54, 62 – 66.

63. Ilina EN et al. 2013 Comparative genomic analysis of Mycobacterium tuberculosis drug resistant strains from Russia. PLoS ONE 8, e56577. (doi:10.1371/journal.pone.0056577)

64. Cardy A. 1997 The environmental material: the human bones. In Whithorn and St Ninian. The excavation of a monastic town (ed. P Hill), pp. 519 – 562. Stroud, UK: Sutton Publishing.

65. Barrell ADM. 2000 Medieval Scotland, pp. 5 – 10. Cambridge, UK: Cambridge University Press.

66. Lamb AL, Melikian M, Ives R, Evans J. 2012 Multi-isotope analysis of the population of the lost medieval village of Auldhame, East Lothian, Scotland. J. Anal. At. Spectrom. 27, 765 – 777. (doi:10.1039/C2JA10363J)

67. Bennike P. 1985 Palaeopathology of Danish skeletons: a comparative study of demography, disease and injury, pp. 183 – 194. Copenhagen, Denmark: Akademisk Forlag.

68. Bennike P. 1999 Facts or myths? A re-evaluation of cases of diagnosed tuberculosis in the past in Denmark. In Tuberculosis past and present (eds G Pálfi, O Dutour, J Deák, I Hutas), pp. 561 – 573. Budapest, Hungary: Golden Book Publisher.

69. Kjellström A. 2012 Possible cases of leprosy and tuberculosis in medieval Sigtuna, Sweden. Int. J. Osteoarchaeol. 22, 261 – 283. (doi:10.1002/oa.1204)