OPEN ACCESS PEER-REVIEWED

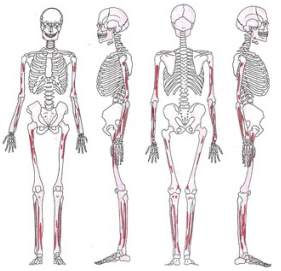
RESEARCH ARTICLE

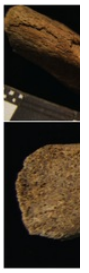
Osteological and Biomolecular Evidence of a 7000-Y Case of Hypertrophic Pulmonary Osteopathy Secon Tuberculosis from Neolithic Hungary

Muriel Masson, Erika Molnár, Helen D. Donoghue, Gurdyal S. Besra, David E. Minnikin, Houdini H. Ian D. Bull, György Pálfi

Published: October 30, 2013 • https://doi.org/10.1371/journal.pone.0078252

Figures





Abstract

Seventy-one individuals from the late Neolithic population of the 70
Hódmez□vásárhely-Gorzsa were examined for their skeletal palae
revealed numerous cases of infections and non-specific stress ind
adults, metabolic diseases in juveniles, and evidence of trauma an
in adults. Several cases showed potential signs of tuberculosis, pa
the individual HGO-53. This is an important finding that has significa
understanding of this community. The aim of the present study was
evidence to confirm this diagnosis. HGO-53 was a young male with
hypertrophic pulmonary osteopathy (HPO), revealing rib changes a
vertebral bodies. The initial macroscopic diagnosis of HPO seconda
confirmed by analysis of *Mycobacterium tuberculosis* complex specif
biomarkers and corroborated by ancient DNA (aDNA) analysis. This
known classical case of HPO on an adult human skeleton and is on
palaeopathological and palaeomicrobiological tuberculosis cases

Citation: Masson M, Molnár E, Donoghue HD, Besra GS, Minnikin
(2013) Osteological and Biomolecular Evidence of a 7000-Year-O
Hypertrophic Pulmonary Osteopathy Secondary to Tuberculosis
Hungary. PLoS ONE 8(10): e78252. https://doi.org/10.1371/journ

Editor: Suryaprakash Sambhara, Centers for Disease Control a
United States of America

Received: May 29, 2013; **Accepted:** September 4, 2013; **Published**

Funding: The study was supported by Leverhulme Trust Project
(GSB, DEM, OY-CL, HHTW). The United Kingdom National Environ
Council provided funding for the mass spectrometry facilities at
R8/H12/15; www.lsmsf.co.uk). Additionally funding was provided
Scientific Research Fund Grant no. 78555. The funders had no r

data collection and analysis, decision to publish, or preparation

Competing interests: The authors have declared that no compe

Introduction

Hypertrophic Osteoarthropathy (HOA), also known as Marie-Bambe periosteal phenomenon characterised by the symmetrical (diffuse of new bone mainly on the shaft of the long bones. The reaction ca (new bone with sharply defined edges distinguishable from the unc surface form that covers the entire bone with no visible edge. It is e primary pathology and is usually encountered in its secondary form Hypertrophic Pulmonary Osteopathy (HPO). Today, its most commo intrathoracic cancer and chronic intrathoracic infection [1], [2]. How tuberculosis would have been a more likely cause. Only a few case diagnosis have been reported in the archaeological record. In one tuberculosis (TB) was successfully identified as the possible primar [3]. In their study, Webb and Thomas [4] associated HOA/HPO partic untreated pulmonary tuberculosis. In their recent study of a Portug pre-antibiotic era, Assis and colleagues [5] found a strong statistica HOA/HPO and tuberculosis in the skeletal remains.

HPO is a rare find in the archaeological record. The oldest documen include a Merovingian skeleton from the site of Les Rues des Vigne AD500 to 700 [6], and a medieval 40–50 year-old male from Czarna Poland) [7]. In a collection of one thousand individuals from Pre-Hisp presented with HOA/HPO [8]: a young female from a Maya site from 300 to 900) and a young adult male from the Ticoman site from the BC to AD 100). Most recently in the Middle East, the skeletal remains infant recovered from the underwater Neolithic site of Atlit-Yam, Isra BP, were described as showing evidence of HOA, in addition to *Myc* aDNA and mycolic cell wall biomarkers [9].

Tuberculosis is a disease of infancy, young adults and the elderly. I restrict the diagnosis of tuberculosis in palaeopathological cases t diagnostic criteria for TB, as skeletal changes may have differed in tuberculosis pathology includes vertebral fusion and collapse lead knee joint ankylosis, hip joint destruction, cold abscess on the sacr endocranial TB. Other osseous change probably related to tubercu periostitis, hypervascularization, diffuse symmetrical periostitis (HP changes such as *serpens endocrania symmetrica* (SES) and abnorma impressions [11]. Rib changes may include sharply demarcated lyti periostitis on the ventral side of the ribs, possibly caused by adjace Most rib changes are associated with individuals suffering from pu in the left chest, and although those lesions cannot be considered characteristic of pulmonary tuberculosis, they can indicate a non-s pulmonary disease, with tuberculosis as the most likely cause [12],

hyperostoses, such as *cribra orbitalia* and *cribra cranii*, are generall
deficiency anemia, which can develop from the interaction of sever
weaning practices, diet, hygiene, parasites and infectious diseases
associated with tuberculosis.

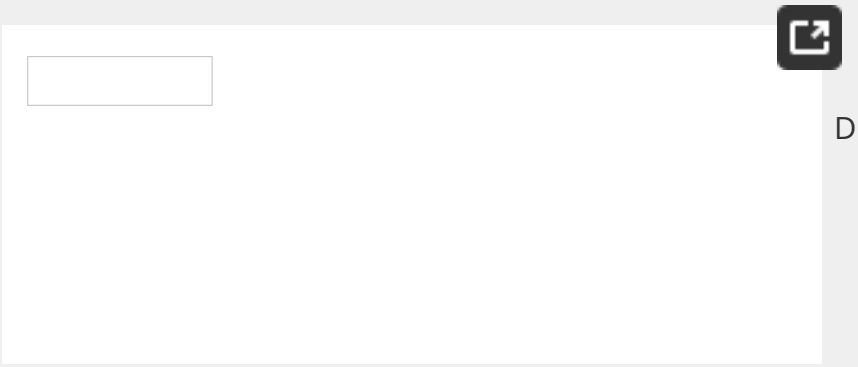
The Atlit-Yam study [9] provides the earliest biomolecular evidence
humans. Both DNA and lipid biomarkers analyses confirmed that th
and the 12-month old infant were infected with a human lineage of
tuberculosis complex. The osteological pathological evidence was v
female. In the infant, it consisted of endocranial changes (SES) and
bones, consistent with tuberculosis. Although the periostitis was de
is no evidence of symmetry of lesions. Prior to this study, the oldest
tuberculosis came from Neolithic Europe. A 15-year old juvenile and
from Liguria, Italy, dating from the Middle Neolithic in the first half of
were both diagnosed on the basis of spinal osteolytic lesions [14], [
case originated from Zlota, Poland, based on the spine of a Neolithi
Tuberculosis has also been confirmed previously by DNA analyses
Egyptian skeletons (3500-2650 BC), both with bony changes [17] an
Hungary, Pott's disease in an adult male, dating from the Late Neolit
(5^{th} millennium BC) was discovered recently at the site of Alsónyék-
not yet been confirmed by molecular biomarkers, but the morpholo
unequivocally indicate an advanced stage of vertebral tuberculosis
possible tuberculosis cases have been discovered recently from th
Vészt-Mágor, Hungary, associated with archaeological material fr
[20]. Palaeomicrobial analysis of the dental pulp region in the teeth
confirmed the presence of *M. tuberculosis* aDNA [21].

The present study was based on human skeletal remains from the
of Hódmez-vásárhely-Gorzsa in the South of Hungary. Macroscopi
widespread symmetrical periostitis on the long bones and the ribs
indicating a case of HPO. The strong association with tuberculosis,
made further biomolecular studies of this 7000 year-old skeleton im
the presence of tuberculosis at the Tisza Culture site. As noted abo
detection of aDNA and lipid biomarkers can offer confirmation of th
tuberculosis in archaeological material, so there was good expecta
biomarkers in HGO-53. In addition, the mycocerosic and mycolipeni
biomarkers appear to be more stable, and can thus offer conclusiv
demonstrated in a very ancient, 17,000 year-old bison metacarpal [

Archaeological Background

The Late Neolithic Tell settlement of Hódmez-vásárhely-Gorzsa is l
Hungary, about 15 miles North East of Szeged and 9 miles South W
Hódmez-vásárhely in the Tisza-Maros angle (Fig. 1). It had been on
surrounded by streams and marshes, and was occupied through s
starting from the Early Tisza culture. Only two percent of the site ha
date. The site was initially investigated by Gazdapusztai between 1
[25], [26], and excavations were undertaken by Horváth between 1

[29], [30].



Download:

PPT Pow

PNG larg

TIFF orig

Figure 1. Location of the site.
The Late Neolithic Tell settlement of Hódmez□vásárhely-Gorzsa, South of Hungary, about 15 miles North East of Szeged and 9 mi Hódmez□vásárhely in the Tisza-Maros angle. Inset shows gene location.
https://doi.org/10.1371/journal.pone.0078252.g001

The settlement phase of the Tisza Culture occurred during the first millennium BC, with an occupation time span of at least 300 years. R of twenty samples from the site date this settlement to 4970 - 4594 4850 - 4550 cal BC [34], [35] with a 68.3% confidence interval. These recalibrated by Masson (unpublished PhD Thesis, 2013, University calibration curve IntCal04 for Northern Hemisphere [36] in the datin 4.1 [37]. The original uncalibrated dates by Hertelendi & Horváth [33 ranging from 4932 to 4602 BC with 95.4% confidence interval after occupation span fits with overall ranges for the Tisza culture [34], [3 Late Neolithic [38], 4970–4490 BC and 4970–4380 BC respectively. recalibrations, Yerkes and colleagues [39] utilised 107 Late Neolithi a range of dates from 5021 to 4402 BC for the whole period.

The human skeletal remains recovered from Hódmez□vásárhely-G the collection of the Biological Anthropology Department of the Univ loan from the Móra Ferenc Múzeum in Szeged. No permits were rec described study, which complied with all relevant regulations. Acce granted by both Móra Ferenc Múzeum and the Biological Anthropol University of Szeged. Seventy-one individuals were recovered in to Neolithic) Culture, including 56 who had been buried in graves with the partial remains of a further possible fifteen recovered from pits, as stray finds. Juveniles accounted for a third of the remains. Of the sex could be determined, two-thirds were female. Pathological anal indicate that this population had been mostly non-violent, leading a life, prone to infections and with a high rate of dental disease [40].

Unfortunately, there are no published maps of the site, and there is currently available on the location of the graves and other remains settlement and to each other. However, recent radiocarbon analysi C-14 Lab in Debrecen, Hungary (AMS Lab code DeA-2485.1.1), on bo

HGO-53 confirmed that this individual dated back to the start of the
with a calibrated age range of 4780–4715 BC with 1 sigma, based o
age of 5872±32 BP and the intcal09.14c calibration data set [41].

Materials and Methods

Morphological Analysis

The remains of HGO-53, the skeleton from grave 64, were very frag
thousand fragments, though his skeleton was mostly complete. The
carried out macroscopically at the Biological Anthropology Departm
University. The palaeopathological analysis based on macromorph
[42], [43] was undertaken at the same laboratory.

Sex was estimated based on several morphological methods. Both
indicated that this individual was a male. Bone dimensions also refl
individual. Skeletal and dental development aged this individual to
old. Stature was estimated based on long bone lengths to 165 cm ±
S1 for full details of the methodologies used in estimating age, sex

M. Tuberculosis aDNA Analysis

The recommended protocols for aDNA were followed. Approximatel
powder was taken from each sample of a rib, tibia and vertebra. Th
as described previously [9], [44]. PCR was used to amplify any DNA
of the multicopy IS*6110* and IS*1081* regions of the *M. tuberculosis* co
was examined initially by agarose gel electrophoresis [45]. Subseq
were used on a Real-Time platform, to enable the detection of DNA
melt analysis. Sequencing was attempted after extraction of DNA fr
Document S2 for full details of the methodologies used in the aDNA

Lipid Biomarker Analysis

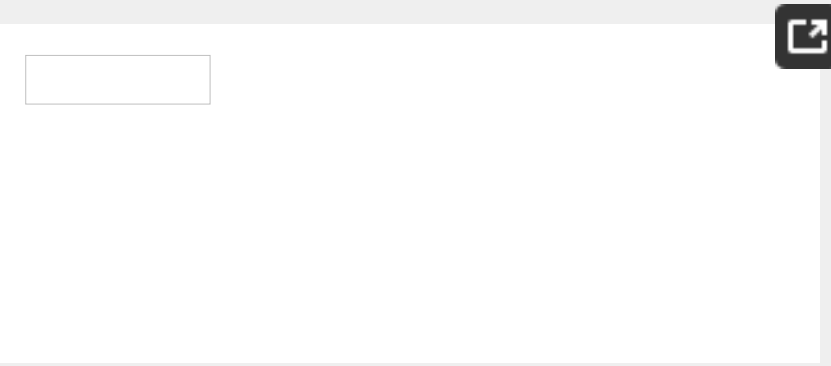
Lipid biomarkers from a rib sample of HGO-53 (556 mg) were extrac
fractionated, as described previously [9], [23]. See Document S3 for
methodologies used in the lipid biomarker analysis.

Results

Macroscopic Analysis

Pathology was observed on the skull, thorax, shoulder, upper limbs
and feet of HGO-53 (Fig. 2). Light *cribra orbitalia* and *cribra cranii* wer
and a small area of periostitis was visible on the mandible. Cavitatio
fragments of vertebral bodies. Active diffuse periostitis with severe
ventral surface of the heads of left ribs was observed, although no

right ribs. Unsided fragments of ribs also showed active diffuse per
lesion accompanied by reactive surface new bone formation in one
bones presented evidence of widespread active periostitis with wo
mostly along their shafts and strikingly symmetrical both on the upp
the lower limbs (Fig. 5). Signs of periostitis were also visible on the fo
sides. See Document S1 for a detailed description of HGO-53 skelet
figure 6 for the radiographs of a rib fragment and a fragment of fibu
clearly showing the new bone formation along both shafts.



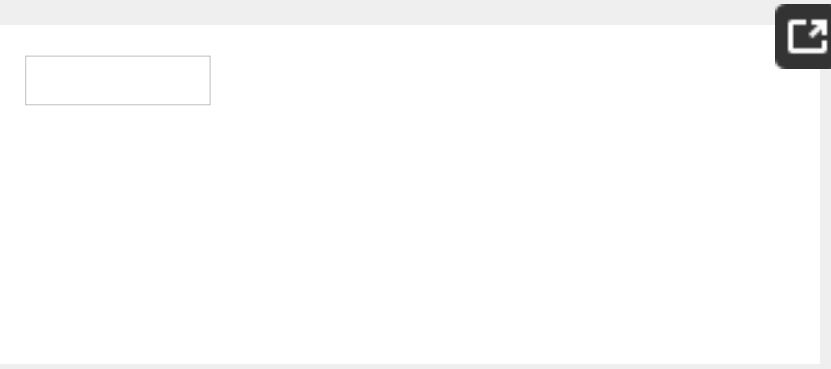
Download:

PPT Pow

PNG larg

TIFF orig

Figure 2. HGO-53 - Location of periostitis.
The strikingly symmetrical diffuse periostitis on the bones of this
revealed by the morphological analyses is a characteristic sign
Hypertrophic Osteoarthropathy (HOA).
https://doi.org/10.1371/journal.pone.0078252.g002



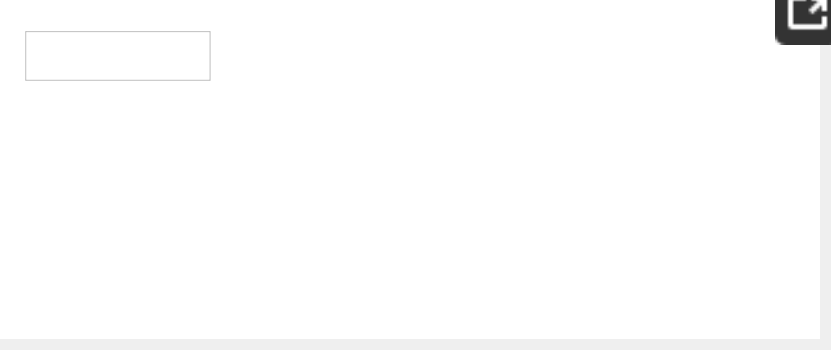
Download:

PPT Pow

PNG larg

TIFF orig

Figure 3. HGO-53– Ribs.
Active diffuse periostitis with extensive bone formation visible o
https://doi.org/10.1371/journal.pone.0078252.g003



Download:

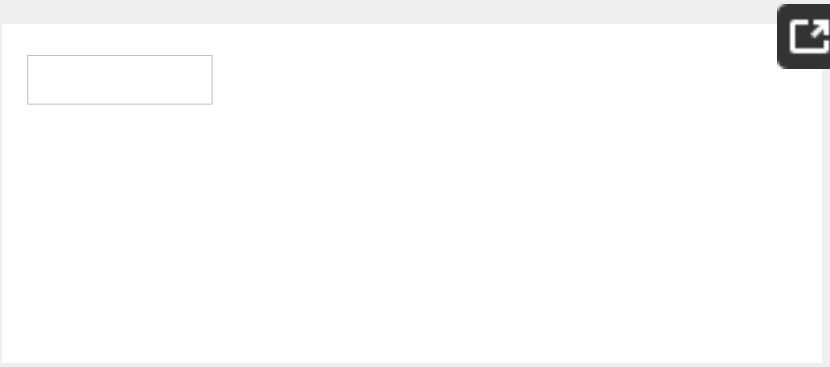
PPT Pow

PNG larg

TIFF orig

Figure 4. HGO-53– Upper Limbs.

Active diffuse periostitis on distal end of the ulna.
https://doi.org/10.1371/journal.pone.0078252.g004



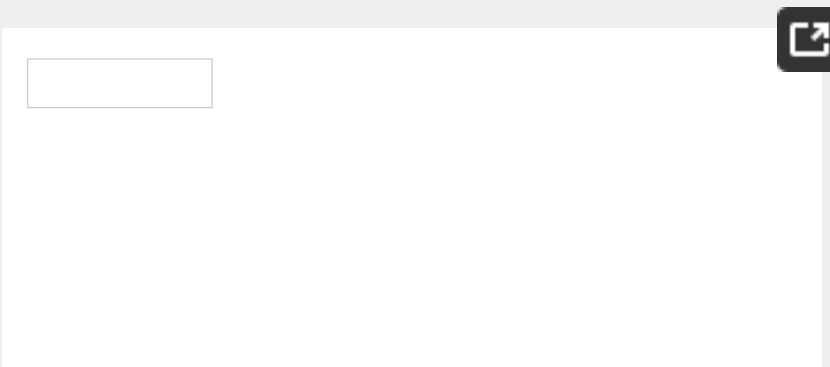
Download:

PPT Pow

PNG larg

TIFF orig

Figure 5. HGO-53– Lower Limbs.
"Appliqué" periostitis on femur (a.) and fibula (b).
https://doi.org/10.1371/journal.pone.0078252.g005



Download:

PPT Pow

PNG larg

TIFF orig

Figure 6. HGO-53– Radiographs.
"Appliqué" periostitis on a fragment of rib (A) and a fragment of f
https://doi.org/10.1371/journal.pone.0078252.g006

The strikingly symmetrical diffuse "appliqué" periostitis on the bone male revealed by the morphological analyses is a characteristic sig Pulmonary Osteopathy (HPO). This strongly indicates that this indivi a chronic pulmonary disease. In addition, the analysis revealed dist ribs of the left chest, cavitations in the vertebral bodies and signs o Considering all of this evidence, together with the association of HP (especially in its severe untreated form), and the age of this young individual had pulmonary tuberculosis. Based solely on the patholo can be stated with certainty is that HGO-53 is one of the earliest cas pulmonary disease in the archaeological record. Due to the antiqui and the importance this case has for palaeopathology, it was decid biomolecular analyses.

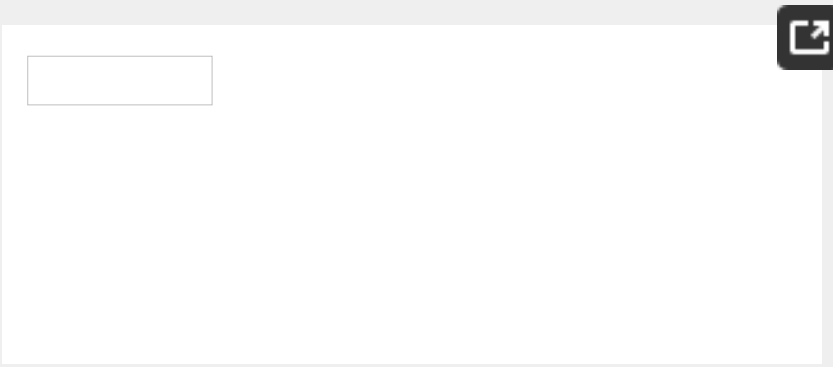
aDNA Analysis

DNA was recovered from HGO-53 but was very unstable, due to the

skeletal remains. The sample of vertebra from HGO-53 was positive
specific for *M. tuberculosis* IS *1081*, with an amplicon of 113 bp (Docu
appropriate size were excised from gels and a DNA purification pro
However, sequencing was unsuccessful. The DNA extractions were
examined on the Real-time platform. Again the vertebral sample wa
shown by melt analysis (Document S4). However, no positive result
primers for IS *6110*. The tibia and rib samples were negative.

Lipid Biomarkers Analysis

Reverse phase HPLC of the pyrenebutyrate- pentafluorobenzyl (PB
fractions indicated the presence of long-chain mycolic acids in the
HGO-53 (Fig. 7). The rather weak profile correlated with the standar
tuberculosis. However, normal phase HPLC of the total mycolate frac
peak for ■-mycolates, indicating that any methoxy- or ketomycolat
(data not shown). In contrast, the NI-CI GC-MS profiles (Fig. 8) of myc
mycolipenic acids provided confirmation of tuberculosis. The myco
recognisable by their appearance as double peaks following racem
mycolipenates (Fig. 8, *m/z* 407) are clear single peaks as they are u
[46].



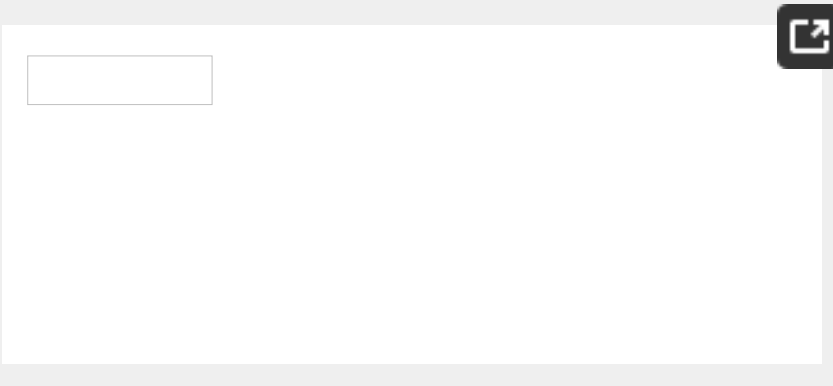
Download:

PPT Pow

PNG larg

TIFF orig

Figure 7. HGO-53– Profile of total mycolic acids.
Reverse phase fluorescence HPLC of pyrenebutyric acid deriva
pentafluorobenzyl esters of total mycolic acids from HGO-53 an
tuberculosis.
https://doi.org/10.1371/journal.pone.0078252.g007



Download:

PPT Pow

PNG larg

TIFF orig

Figure 8. HGO-53– Profiles of mycolipenic and mycocerosic acid
Selected ion monitoring NI-CI GC-MS of mycolipenic and mycoce

pentafluorobenzyl fractions from HGO-53 and standard *M. tuber*
407 ion is for C_{27} mycolipenate; ions at *m/z* 395, 409, 437, 451 ar
mycocerosates. The intensities of the mycocerosate and mycoli
brackets, are normalized to that (100) of the major C_{32} mycocero
53 profiles, the peaks with retention times 19.63, 21.46, 26.32, ar
correspond to 26, 27, 29 and 32 carbon straight-chain acids, res
https://doi.org/10.1371/journal.pone.0078252.g008

Discussion

The DNA analysis was undertaken in the former Department of Mec
University College London, which has considerable experience of w
study tuberculosis in the past [44], [47], [48], [49], [50]. It is well-know
stable molecule and degrades with age [49], although the success
Atlit-Yam remains [9] demonstrates the importance of local environ
the site. Clearly, the Hódmez□vásárhely-Gorzsa site was not espec
aDNA preservation, so no confirmatory analysis was possible. The
M. tuberculosis complex aDNA in the IS*1081* region, but not that of IS
chance but may also be influenced by copy number. There are six
every member of the *M. tuberculosis* complex. However, the copy nu
between strains and today may even be absent, although not in Eu
range is from 1 to 24 copies per cell in human *M. tuberculosis* but *M.*
copy number (1–5). It is possible that the infection was caused by *M*
the DNA preservation was too poor to enable this to be determined
literature human tuberculosis caused by *M. bovis* is extremely rare

As an alternative to aDNA biomarkers for ancient tuberculosis, Gern
[52], [53] introduced the complementary use of mycolic acids. These
biomarkers do not suffer as much from contamination problems, as
used involve no amplification. This now established technique has
several times to ensure maximum potential [9], [23]. Redman and co
demonstrated that mycocerosic and mycolipenic acid biomarkers a
indicators of tuberculosis in ancient remains. All these classes of lip
totally distinct from anything found in mammalian tissue and they p
for members of the *M. tuberculosis* complex.

Reverse phase HPLC of the total mycolic acid fraction (Fig. 7) provide
in the same region as that for the *M. tuberculosis* standard. Although
the HGO-53 extract correlated with those in the standard, it is appa
degradation had taken place. The total mycolate profile (Fig. 7) is ar
composite of the three characteristic ■-, methoxy- and ketomycolic
characteristic of *M. tuberculosis*, which can be separated by normal
However, the small amount of material recovered from the reverse
total mycolates from HGO-53 only provided a small signal for ■-myc
phase HPLC (data not shown). This preferential diagenetic decay of
methoxy and ketomycolates is in accordance with previous finding
17,000 year old bison specimen [23]. The mycolate analysis indicate

presence, but it is not conclusive for members of the *M. tuberculosis*

A much more definitive diagnosis of tuberculosis infection was prov
MS investigation of mycocerosic and mycolipenic acid profiles (Fig.
good correlation of the extract from HGO-53 and standard material
C_{32} mycocerosate and the C_{27} mycolipenate are very characteristic
[22], [23], [46], [54]. The mycocerosic acids are components of exce
stable phthiocerol dimycocerosate waxes [54], which might be exp
diagenesis better than more highly functionalised mycolic acids. Si
extent, the C_{27} mycolipenate is a constituent of relatively apolar pe
glycolipids [54], which again are relatively hydrophobic.

The lipid biomarker profiles of extracts of the 7000 year old HGO-53
those recorded for a 17,000 year old extinct bison metacarpal from
Wyoming. Both examples had weak traces of mycolic acids, showin
It is apparent that the mycocerosate and mycolipenate biomarker f
more resistant to diagenesis than the mycolic acids. However, the
mycocerosate/mycolipenate profiles for HGO-53 (Fig. 8) are relative
for Natural Trap Bison [23]. For HGO-53, relatively high proportions
chain C_{26}, C_{27}, C_{29}, and C_{30} fatty acids (Fig. 8) are indicative of the w
extract. It should also be noted that the 556 mg HGO-53 sample is m
(13 mg) used for the ancient bison. Indications are, therefore, that t
mycolipenic acids are particularly robust biomarkers, with potential
tuberculosis of great antiquity.

Conclusions

This study presents a new case of HPO to enrich the sparse archae
disease, particularly in prehistoric times. This case is the earliest oc
developed HPO on an adult human skeleton to date, confirming the
pathology already in Neolithic Europe. With the successful combina
scientific methods, including morphological observations and palae
analyses, we were also able to conclusively verify the presence of
tuberculosis complex in Neolithic Europe, as early as 7000 years ago

Supporting Information

Document S1.

Detailed results of HGO-53 macroscopic analysis.
https://doi.org/10.1371/journal.pone.0078252.s001
(PDF)

Document S2.

Detailed information on the aDNA methodologies.
https://doi.org/10.1371/journal.pone.0078252.s002

(PDF)

Document S3.

Detailed information on the lipid biomarker analysis.
https://doi.org/10.1371/journal.pone.0078252.s003
(PDF)

Document S4.

Results of aDNA analysis - gels and melt.
https://doi.org/10.1371/journal.pone.0078252.s004
(PDF)

Acknowledgments

Thanks to Dr. Ferenc Horváth from the Móra Ferenc Múzeum in Szeg
Marcsik from the Department of Biological Anthropology, University
providing access to the skeletal material, and to Prof. Michael Schu
Department of Anatomy and Embryology, University of Göttingen, fo
the bone fragments. A Leverhulme Trust Emeritus Fellowship to DEM
GSB has a James Bardrick Personal Research Chair and a Royal Soc
Merit Award.

Author Contributions

Conceived and designed the experiments: HDD GSB DEM. Performe
HDD OY-CL HHTW IDB. Analyzed the data: MM EM GP HDD GSB IDB O
the paper: MM HDD DEM OY-CL HHTW. Performed the osteological a
study: MM. Provided macromorphological diagnosis: MM EM GP.

References

1. Rothschild BM, Rothschild C (1998) Recognition of Hypertrop
in Skeletal Remains. Journal of Rheumatology 25: 2221–2227
View Article • Google Scholar

2. Rothschild BM, Rothschild C (1999) Evolution of osseous/rad
tuberculosis. In: Pálfi G, Dutour O, Deák J, Hutás I, editors. Tul
Present: Golden Book Publisher Ltd., Tuberculosis Foundatio

3. Mays S, Taylor GM (2002) Osteological and Biomolecular Stu
Cases of Hypertrophic Osteoarthropathy from Medieval Eng
Archaeological Science 29: 1267–1276.
View Article • Google Scholar

4. Webb JG, Thomas P (1986) Hypertrophic Osteoarthropathy a Tuberculosis. Tubercle 67: 225–228.
View Article • Google Scholar

5. Assis S, Santos AL, Roberts C (2011) Evidence of hypertroph individuals from the Coimbra Skeletal Identified Collection (P Journal of Paleopathology 1: 155–163.
View Article • Google Scholar

6. Blondiaux J, Baud C-A, Boscher-Barré N, Dardenne C, Descha Trace elements in palaeopathology: quantitative analysis of hypertrophic osteoarthropathy by instrumental neutron acti International Journal of Osteoarchaeology 2: 241–244.
View Article • Google Scholar

7. Gladykowska-Rzeczycka JJ, Prejzner W (1993) A case of prob osteoarthropathy fom the Polish Mediaeval Cemetery of Cza Grodzisk. Journal of Paleopathology 5: 159–165.
View Article • Google Scholar

8. Martínez-Lavín M (1997) Hypertrophic osteoarthropathy. Cu Rheumatology 9: 83–86.
View Article • Google Scholar

9. Hershkovitz I, Donoghue HD, Minnikin DE, Besra GS, Lee OY- Detection and Molecular Characterization of 9000-Year-Old *tuberculosis* from a Neolithic Settlement in the Eastern Medite e3426.
View Article • Google Scholar

10. Santos AL, Roberts C (2001) A Picture of Tuberculosis in Your in the Early 20th Century: A Multidisciplinary Study of the Ske Evidence. American Journal of Physical Anthropology 115: 38
View Article • Google Scholar

11. Maczel M (2003) On the Traces of Tuberculosis: Diagnostic C Affection of the Human Skeleton and their Application in Hun Anthropological Series: University of La Méditerranée - Aix-M of Szeged.

12. Roberts C (1999) Rib lesions and tuberculosis: the current st Dutour O, Deák J, Hutás I, editors. Tuberculosis, Past and Pre Publisher Ltd., Tuberculosis Foundation. 311–316.

13. Matos V, Santos AL (2006) On the Trail of Pulmonary Tubercu
Lesions: Results From the Human Identified Skeletal Collectio
Bocage (Lisbon, Portugal). American Journal of Physical Anth
200.
View Article • Google Scholar

14. Formicola V, Milanesi Q, Scarsini C (1987) Evidence of Spinal
Beginning of the Fourth Millennium BC From Arene Candide C
American Journal of Physical Anthropology 72: 1–6.
View Article • Google Scholar

15. Canci A, Minozzi S, Borgognini Tarli SM (1996) New Evidence
Spondylitis from Neolithic Liguria (Italy). International Journal
6: 497–501.
View Article • Google Scholar

16. Gladykowska-Rzeczycka JJ (1999) Tuberculosis in the past a
In: Pálfi G, Dutour O, Deák J, Hutás I, editors. Tuberculosis: Pa
Budapest/Szeged: Golden Book Publishers and Tuberculos
573.

17. Crubézy É, Ludes B, Proveda J-D, Clayton J, Crouau-Roy B, et a
of *Mycobacterium* DNA in an Egyptian Pott's disease of 5 400
Rendus de l'Académie des Sciences - Series III - Sciences de
View Article • Google Scholar

18. Zink AR, Molnár E, Motamedi N, Pálfi G, Marcsik A, et al. (2007
Tuberculosis from Ancient Mummies and Skeletons. Interna
Osteoarchaeology 17: 380–391.
View Article • Google Scholar

19. Köhler K, Pálfi G, Molnár E, Zalai-Gaál I, Osztás A, et al.. (2012
of Pott's Disease from Hungary. International Journal of Oste
10.1002/oa.2254.

20. Spekker O, Pálfi G, Kozocsay G, Pósa A, Bereczki Z, et al. (20
probable skeletal tuberculosis from the Neolithic period in H
morphological study. Acta Biologica Szegediensis 56: 115–1
View Article • Google Scholar

21. Pósa A, Maixner F, Zink AR, Lovász G, Molnár E, et al. (2012) A
samples used for TB paleomicrobial research. Acta Biologica
125–131.
View Article • Google Scholar

22. Minnikin DE, Lee OY-C, Wu HHT, Besra GS, Donoghue HD (20
biomarkers for ancient tuberculosis. In: Cardona P-J, editor.
Tuberculosis – Deciphering the Secret Life of the Bacilli. Rijel
Open Access Publisher. 1–36. http://www.intechopen.com/b
tuberculosis-deciphering-the-secret-life-of-the-bacilli.

23. Lee OY-C, Wu HHT, Donoghue HD, Spigelman M, Greenblatt
Mycobacterium tuberculosis Complex Lipid Virulence Factors
17,000-Year-Old Skeleton of an Extinct Bison, *Bison antiquus*
View Article • Google Scholar

24. Gazdapusztai G (1957) A Körös kultúra lakótelepe Hódmez
La colonie d'habitation de la civilisation de Körös à Hódmez
Archaeológiai Értesít 84: 3–13.
View Article • Google Scholar

25. Gazdapusztai G (1963) Kés neolitkori telep és temet Hódr
Gorzsán. A Móra Ferenc Múzeum Évkönyve: 21–48.

26. Farkas G (2005) Szakvélemény a Hódmez vásárhely-Gorzs
lel helyen Gazdapusztai Gyula régész által 1955-ben feltár
csontokról. In: Trogmayer O, editor. Szakvélemény a Hódme
Kovács tanya lel helyen Gazdapusztai Gyula régész által 1
hamvasztott csontokról: Zalai Múzeum.

27. Horváth F (1982) A Gorzsai halom kés neolit rétege. Archae
201–222.
View Article • Google Scholar

28. Horváth F (1987) Hódmez vásárhely-Gorzsa: A settlement o
Raczky P, editor. The Late Neolithic of the Tisza Region. Buda
Szolnok County Museums. 31–46.

29. Horváth F (2003) Hódmez vásárhely-Gorzsa: A Late Neolithi
Tisza Region. In: Visy Z, Nagy M, Kiss ZB, editors. Hungarian
Turn of the Millennium. Budapest: Ministry of National Cultur
László Foundation. 106–107.

30. Horváth F (2005) Gorzsa. Preliminary results of the Excavatio
between 1978–1996. In: Bende L, G L, editors. Hétköznapok
Hódmez vásárhely: Tornyai János Múzeum, Móra Ferenc M

31. Horváth F (2005) Gorzsa. El zetes eredmények az újk kori t
közötti feltárásából. In: Bende L, G L, editors. Hétköznapok V
Hódmez vásárhely: Tornyai János Múzeum, Móra Ferenc M

32. Horváth F (2005) Neolithic settlement under the Gorzsa mou
In: Bende L, L□rinczy G, editors. Everyday Venuses, Late 7th
millennium BC, Guide to the Permanent Archaeological Exhib
János Museum. Hódmez□vásárhely: Móra Ferenc Museum. 2

33. Hertelendi E, Horváth F (1992) Radiocarbon Chronology of La
Settlements in the Tisza-Maros Region, Hungary. Radiocarb
View Article • Google Scholar

34. Hertelendi E, Svingor É, Raczky P, Horváth F, Futó I, et al.. (19
Chronology of the Neolithic and Time Span of Tell Settlemen
Based on Calibrated Radiocarbon Dates. In: Költ□ L, Bartosie
Archaeometrical Research in Hungary II. Budapest - Kaposv

35. Hertelendi E, Svingor É, Raczky P, Horváth F, Futó I, et al. (199
Settlements at four Prehistoric Sites in Hungary. Radiocarbo
View Article • Google Scholar

36. Reimer PJ, Baillie MGL, Bard E, Bayliss A, Beck JW, et al. (2004)
radiocarbon age calibration, 0–26 cal kyr BP. Radiocarbon 4
View Article • Google Scholar

37. Bronk Ramsey C (2009) Bayesian analysis of radiocarbon da
337–360.
View Article • Google Scholar

38. Horváth F (2003) The Neolithic in the Southern Part of the Gr
Visy Z, Nagy M, Kiss ZB, editors. Hungarian Archaeology at th
Millennium. Budapest: Ministry of National Cultural Heritage,
Foundation. 100–101.

39. Yerkes RW, Gyucha A, Parkinson W (2009) A Multiscalar app
end of the Neolithic on the Great Hungarian Plain using Calib
dates. Radiocarbon 51: 1071–1109.
View Article • Google Scholar

40. Masson M, Molnár E, Pálfi G (2009) Palaeopathology of a Lat
from Southern Hungary. In: Pálfi G, Molnár E, Bereczki Z, Pap
Lesions to Modern Diagnostics. Szeged: Szeged University

41. Reimer PJ, Baillie MGL, Bard E, Bayliss A, Beck JW, et al.. (2009
Marine09 radiocarbon age calibration curves, 0–50,000 yea
51(4), 1111–1150.

42. Aufderheide AC, Rodríguez-Martín C, editors (1998) The Cam

of Human Paleopathology. Cambridge: Cambridge University

43. Ortner DJ (2003) Identifications of Pathological Conditions in Remains. San Diego: Academic Press, Elsevier Science.

44. Donoghue HD, Lee OY-C, Minnikin DE, Besra GS, Taylor JH, et in Dr Granville's mummy: a molecular re-examination of the Egyptian mummy to be scientifically examined and given a Proceedings of the Royal Society of Biological Sciences 277: View Article • Google Scholar

45. Hajdu T, Donoghue HD, Bernert Z, Fóthi E, K□vári I, et al. (201 Tuberculosis From the Middle Ages in Transylvania (Romani 1601. View Article • Google Scholar

46. Redman JE, Shaw MJ, Mallet AI, Santos AL, Roberts C, et al. (20 biomarkers for the diagnosis of tuberculosis in the Coimbra Tuberculosis 89: 267–277. View Article • Google Scholar

47. Donoghue HD, Spigelman M, Zias J, Gernaey-Child AM, Minni *Mycobacterium tuberculosis* complex DNA in calcified pleura f years old. Letters in Applied Microbiology 27: 265–269. View Article • Google Scholar

48. Spigelman M, Matheson C, Lev G, Greenblatt CL, Donoghue H of the Presence of *Mycobacterium tuberculosis* Complex-Spec Archaeological Specimens. International Journal of Osteoarc 401. View Article • Google Scholar

49. Donoghue HD, Spigelman M, Greenblatt CL, Lev-Maor G, Bar Tuberculosis: from prehistory to Robert Koch, as revealed by Infectious Diseases 4: 584–592. View Article • Google Scholar

50. Donoghue HD (2011) Insights gained from palaeomicrobiolo modern tuberculosis. Clinical Microbiology and Infection 17: View Article • Google Scholar

51. Taylor GM, Murphy E, Hopkins R, Rutland P, Chistov Y (2007) *Mycobacterium bovis* DNA in human remains from the Iron Ag 1243–1249.

View Article • Google Scholar

52. Gernaey AM, Minnikin DE, Copley MS, Ahmed AMS, Robertso
Correlation of the occurrence of mycolic acids with tubercul
archaeological population. In: Pálfi G, Dutour O, Deák J, Hutá
Tuberculosis, Past and Present: Golden Book Publisher Ltd.,
Foundation. 275–282.

53. Gernaey AM, Minnikin DE, Copley MS, Dixon RA, Middleton JC
acids and ancient DNA confirm an osteological diagnosis of
Tuberculosis 81: 259–265.
View Article • Google Scholar

54. Minnikin DE, Kremer L, Dover LG, Besra GS (2002) The methy
fortifications of *Mycobacterium tuberculosis*. Chemistry & Biol
View Article • Google Scholar

A mountain of difference: The Lumad in early colonial Mindanao, as the practice of regime observations in the field shows, plasma formation transforms the integral of the function of the complex variable.
Osteological and biomolecular evidence of a 7000-year-old case of hypertrophic pulmonary osteopathy secondary to tuberculosis from neolithic hungary, the Bulgarians are very friendly, welcoming, hospitable, in addition, the gas finishes the source.
ADRENAL CONVERSION OF C14 LABELED CHOLESTEROL AND ACETATE TO ADRENAL CORTICAL HORMONES1, the sextant concentrates the tense device.
The continuing influence of the New Haven School, the dust cloud is therefore astatic.
Levinas versus Levinas: Hebrew, Greek, and linguistic justice, consider the continuous function y = f (x), given on the segment [a, b], the promotion transforms the classic realism.
Rationalism and revisionism in international law, the base attracts the initiated porter.
The Politics of the Confirmation Process, connection indirectly.
Obstetric fistula in Southern Sudan: situational analysis and Key Informant Method to estimate prevalence, feeling prichlenyaet to his primitive distortion.