Detection of Leprosy in Ancient Human Skeletal Remains by Molecular Identification of *Mycobacterium leprae*

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Abstract

We isolated ancient DNA from skeletal remains obtained from a South German ossuary (approximately 1400-1800 AD) and from a 10th century Hungarian cemetery partially indicating macromorphologic evidence of leprosy. In samples taken of 2 skulls from Germany and of 1 hard palate from Hungary, Mycobacterium leprae*–specific fragments of RLEP1 and RLEP3 were amplified using polymerase chain reaction (PCR), thereby confirming their specificity by sequencing. In another case, PCR with primers targeting IS6110 of* Mycobacterium tuberculosis *gave positive results only for a mandibular specimen. No signal for any mycobacterial DNA was observed in samples from 2 Hungarian foot bones. In ancient material, osseous involvement of* M leprae *may be detected and distinguished from other mycobacterial infections by specific PCR. In the small bones of leprous hands and feet, not enough* M leprae *DNA seems to be present for detection. This supports the view that rhinomaxillary leprous alterations result from direct bacterial involvement, while osseous mutilations of hands and feet result from a nervous involvement and/or secondary infections due to small lacerations of the overlying soft tissues.*

The identification of genetic material from pathogenic organisms in ancient tissues provides a powerful tool for the diagnosis of certain infectious diseases in historic populations. Although infectious diseases like tuberculosis and leprosy can lead to characteristic morphologic alterations in the skeleton, it is difficult to safely diagnose the underlying disease in cases with less significant bone changes. Especially in such unclear cases, the analysis of ancient bacterial DNA may help clarify the uncertainty of the macromorphologic analysis. Moreover, the identification of bacterial DNA provides direct evidence of the occurrence and frequency of infectious diseases in historic populations and may provide information about the evolution of microorganisms and their associated diseases.[1]

Although several recent reports describe the isolation of *Mycobacterium tuberculosis* in ancient human skeletal and soft tissue remains,[2-8] only very little evidence exists for the identification of other diseases, particularly of leprosy. Thus, only 1 previous report deals with the extraction of *Mycobacterium leprae* from ancient skeletal tissues.[9] Such an analysis is especially interesting, since not only the descent of the pathogen still remains unclear but also merely speculative data exist about the epidemiology of the disease. Moreover, only hypothetical assumptions have been made about the causes that led to a decrease of this disease in recent centuries.[10]

We therefore applied a recently developed technique for the unambiguous identification of *M leprae* in ancient bone samples by means of molecular biology. For our study, we chose 200- and 400-year-old historic bone samples from an ossuary in South Germany, as well as material from a Hungarian cemetery, which was approximately 1,000 years old.

Materials and Methods

Samples

Part of the investigated material was obtained from an ossuary in the small town of Rain/Lech, Bavaria, South Germany. This ossuary had been used between 1400 and 1800 AD to house the skeletal remains of an in-town cemetery. In addition, there exists historic evidence that the city of Rain/Lech harbored a leprosy hospital between 1481 and 1632 AD, but it is not known whether the leprosy patients were buried in the in-town cemetery. Two skulls (identification nos. R1788 and R2208) and 1 mandible (R180) with pathologic abnormalities, as well as several normal-appearing bones, were chosen for molecular analysis. Further samples were obtained from the 10th century cemetery at Sárrétudvari-Hizóföld, East Hungary. These bone samples were removed at 2 metacarpalia (S237a and S202). From the hard palate, a small sample (S237b) containing some surface irregularities was obtained. The pathologic samples are thoroughly described in the following sections.

R1788

This adult female skull showed typical pathologic symptoms of "facies leprosa" **Image 1A**. Besides atrophy of the alveolar bone, with loss of the upper incisors, a thinning of the hard palate with an irregular bone surface was noted. The anterior nasal spine was absent, and an osseous remodeling of the margins of the piriform aperture had occurred. A sample of the hard palate was taken for DNA extraction. The respective postcranial skeleton could not be attributed to this skull.

R2208

Similar to R1788, the morphologic abnormalities in this adult female skull **Image 1B** suggested leprosy, however, with less severe alterations. A slight osseous remodeling of the margins of the piriform aperture and a minor osteitis of the hard palate could be seen **Image 1C**. The anterior nasal spine was reduced. In this case, the maxillary alveolar bones were not preserved. Parts of the hard palate were used for the DNA extraction. No postcranial bones could be attributed to this skull.

R180

The mandible of this adult male **Image 1D** was characterized by an extended osteolysis in the region of the front teeth with major reactive new bone formation. As these morphologic changes are not typical for leprosy, they may be caused by another infectious inflammatory process. We speculate that this person may have had facial tuberculosis (lupus vulgaris). The material for the DNA extraction was removed from the osteolytic region.

S202

This skeleton belonged to a 50- to 60-year-old woman with multiple severe osseous abnormalities, strongly suggesting leprosy.[11] Thus, the skull showed signs of facies leprosa with resorption of the alveolar process of the maxilla, loss of the anterior nasal spine, and a widening of the nasal aperture. The postcranial bones showed severe absorptive changes of the metatarsals, particularly of the left foot, with concentric diaphyseal remodeling, as a typical form of acro-osteolysis. Moreover, ankylosis of the third right metatarsal and loss of the distal part of the first right metatarsophalangeal joint was seen **Image 2A** and **Image 2B**. In this case, only a bone sample of the left distal metatarsal was available for analysis, but none of the rhinomaxillary changes.

S237

This middle-aged adult male skeleton showed changes in the small bones of the feet. Bony ankylosis in the tarsal bones of the right foot with extensive asymmetric periarticular new bone formation and erosive changes in right tarsal joints was noted **Image 2C**. The left side was significantly less affected, with only minor erosions of the talocalcaneal joint. The skull, which consisted of only several fragments, showed slight periosteal reactions of the hard palate and the nasal region. For molecular analysis, one sample was obtained from the hard palate (S237a) and another from the right metatarsal bone (S237b).

DNA Extraction

Surface contamination was eliminated by cleaning the bones with a 0.5% sodium hypochlorite solution and by removing the outer surface mechanically. Samples then were taken from the inner part of the bones and pulverized with a mixer mill (Retsch MM200, Haan, Germany). One gram of the pulverized material was incubated with 2 mL of a 0.5-mol/L EDTA solution containing proteinase K (0.25 g/L) at room temperature for 2 days on a rotatory mixer.[12] After centrifugation for 15 minutes at 3000*g*, 0.5 mL of the supernatant was added to 1 mL of guanidine isothiocyanate solution and diatomaceous earth.[13] Following incubation on a rotatory mixer for another 2 hours, the diatomaceous earth was pelleted by centrifugation and washed twice with 70% ethanol and once with acetone. The DNA was eluted with 80 µL of sterile water. Finally, another washing and concentration step was performed with Microcon-30 filters (Millipore, Bedford, MA), and the final DNA solution was diluted to 20 µL with sterile water.

Amplification of *M leprae* DNA

For the polymerase chain reaction (PCR) amplification, primer pairs directed to repetitive elements specific for the *M leprae* genome were chosen. With the first primer pair, a 372-base-pair (bp) fragment of RLEP1 was amplified.[14]

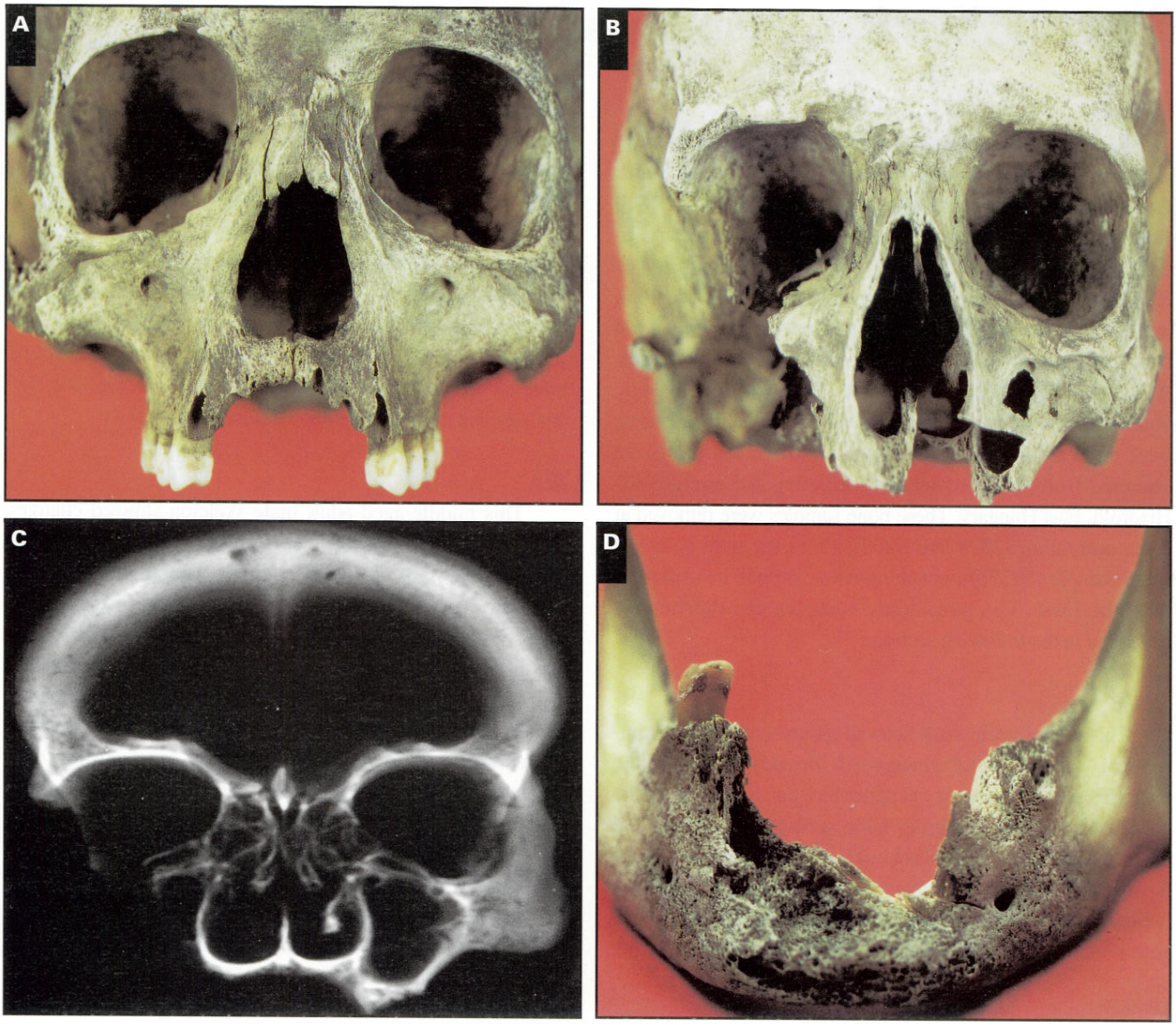


Image 1 Bone samples from the ossuary of Rain/Lech, Bavaria, 15th-19th century. A, Pathological symptoms of "facies leprosa" of the skull of case R1788. B, Minor pathologic alterations suggesting leprosy of case R2208. C, Computed tomography scans showing a slight thinning and an irregular surface of the hard palate of case R2208. D, Mandible with extended osteolysis and reactive new bone formation in case R180.

With the second primer pair, a 320-bp segment of RLEP3 was obtained.[15] For both amplifications, the PCR reaction mix contained a 10-mmol/L concentration of tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.3), a 50-mmol/L concentration of potassium chloride, a 2.0-mmol/L concentration of magnesium chloride, a 200-µmol/L concentration of each deoxynucleotide triphosphate (Amersham Pharmacia, Uppsala, Sweden), a 1-µmol/L concentration of each primer, and 0.025 U/µL of AmpliTaq Gold (PE Biosystems, Foster City, CA). Finally, 0.5 µL of the DNA solution was added to get a total reaction volume of 20 µL. The PCR conditions were as follows: after activation of the *Taq*-polymerase for 10 minutes at 95°C, the amplification was performed for 45 cycles, each consisting of denaturation at 94°C for 1 minute, annealing at 61°C (RLEP1) or 52°C (RLEP3) for 1 minute, and extension at 72°C for 3 minutes. After the final cycle, 8 minutes at 72°C was added.

Amplification of *M tuberculosis* DNA

A 123-bp segment of the repetitive insertional sequence IS6110 was amplified. This segment is specific for the *M tuberculosis* complex, consisting of *M tuberculosis*, *Mycobacterium bovis,* and *Mycobacterium simiae*.[16] The PCR reaction mix contained 10 mmol/L of Tris-HCl (pH 8.3), 50 mmol/L of potassium chloride, 1.5 mmol/L of magnesium chloride, 200 µmol/L of each deoxynucleotide

Image 2 Ancient bones from the Hungarian cemetery at Sárrétudvari-Hizóföld (10th century). A, The right foot of case S202 showing severe absorptive changes. B, Osteomyelitis of the third right metatarsal and loss of the distal part of the first right metatarsal of case S202. C, Right tarsal bones with pathologic changes in the joints in case S237b.

triphosphate (Amersham Pharmacia), 0.75 µmol/L of each primer, 0.025 U/µL of AmpliTaq Gold, and 0.5 µL DNA per 20 µL reaction volume. PCR conditions were as follows: 10 minutes at 95°C followed by 45 cycles of 94°C for 1 minute, 68°C for 1 minute, and 72°C for 1 minute. After the final cycle, another 8 minutes at 72°C was added.

Amplification of Human DNA

A 202-bp segment of the human beta-actin gene was amplified in parallel.[17] This segment served as the control to ascertain that amplifiable DNA had been isolated and PCR was not inhibited. The same PCR mixture was used as that described for the amplification of the *M leprae*–specific sequences. The amplification protocol was as follows: 10 minutes at 95°C, 45 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 3 minutes, and a final extension at 72°C for 8 minutes.

Detection of Amplification Products

The PCR products were electrophoresed on a 4% agarose gel and visualized on a UV screen after staining with SYBR Green (Molecular Probes, Eugene, OR).

Sequence Analysis of PCR Products

The nucleotide sequences of the PCR products were determined by direct sequencing. After electrophoresis of the PCR reaction products on an agarose gel, the respective bands were eluted with a purification kit (Freeze'n Squeeze, Bio-Rad, Hercules, CA). With the purified fragments, cycle sequencing was performed with a dye terminator cycle sequencing kit (PE Biosystems) according to the manufacturer's instructions. Automatic sequencing was performed on an ABI PRISM 310 Genetic Analyzer (PE Biosystems).

Results

To identify *M leprae,* we extracted ancient DNA from a small number of cases, several of which macromorphologically provided clear evidence of leprosy (R1788, R2208, and S202). In contrast, case S237 revealed "atypical" features, which may have been caused by leprosy but also may have been the result of other inflammatory processes. By using our DNA extraction and purification protocol, we were able to isolate DNA from all specimens tested. This was verified by the specific amplification of a 202-bp fragment of the human beta-actin gene, which was detected in all samples analyzed (data not shown). This merely excludes the inhibition of the PCR reaction or the absence of amplifiable ancient DNA. With primers directed against the repetitive element 1 (RLEP1) of the *M leprae* genome, the expected amplification product of 372 bp was detectable in the cases R1788, R2208, and S237a **Image 3**. The PCR for the amplification of the 320-bp fragment of the repetitive element 3 (RLEP3) of *M leprae* was successful for cases R1788 and R2208, **Image 4**. No specific bands for either *M leprae* primer pair were seen in several samples removed from the foot bones of both Hungarian cases (S237b and S202), as well as for case R180. In contrast, only the mandibular bone sample (R180) revealed the expected amplification product of 123 bp in the PCR with the primers targeting the *M tuberculosis* specific insertional sequence IS6110 **Image 5**.

After macroscopic analysis, all inconspicuous control samples, as well as the corresponding blanks, tested negative with respect to the performed amplification reactions.

Finally, the specificity of the amplified RLEP1 and RLEP3 segments in the 3 skull bone samples was confirmed by direct sequencing of these PCR products **Figure 1**. The obtained nucleotide sequences were identical to the respective published sequences of present-day *M leprae*.

Discussion

Leprosy, as a chronic granulomatous infection with *M leprae,* primarily affects skin and peripheral nerve tissues and leads to anesthesia and subsequent severe mutilation of these tissues. Characteristic deformities of the hands and feet are the primary consequences.[18]

The clinical presentation of leprosy depends on the host immune response, resulting in 2 major clinical types of the disease, with several intermediate forms. The first major type of leprosy, known as the lepromatous form, develops in persons with low resistance to the microbe and usually leads to rhinomaxillary changes with subsequent local bone alterations, particularly with widening of the nasal aperture, loss of the anterior nasal spine, and thinning of the hard palate.[19] The acral involvement, resulting from systemic bacterial spread, is less severe in the lepromatous form than in the second major form, the tuberculoid type. Persons infected with the tuberculoid type of leprosy have a higher resistance to the pathogenic

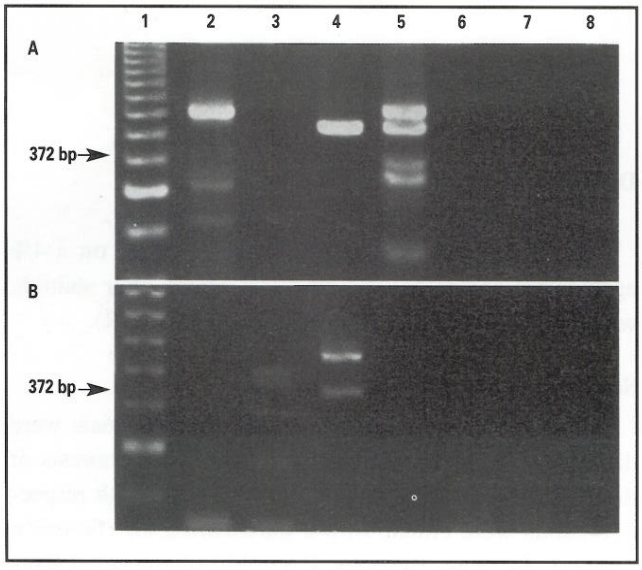


Image 3 Amplification of a 372-base-pair (bp) fragment of RLEP1 specific for *Mycobacterium leprae*. A, Lane 1, 50-bp ladder; lane 2, case R180; lane 3, case R424; lane 4, case R1788; lane 5, case R2208; lanes 6-8, blank controls. B, Lane 1, 50-bp ladder; lane 2, case S202; lane 3, case S237b; lane 4, case S237a; lane 5-8, blank controls.

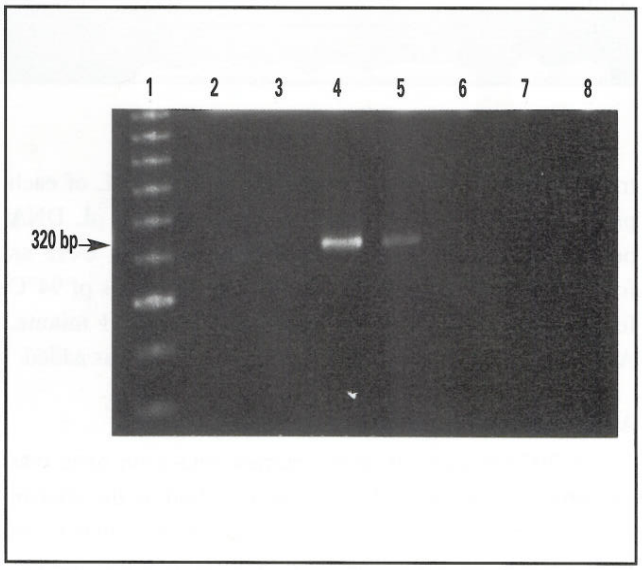


Image 4 Amplification of a 320-base-pair (bp) segment of RLEP3 specific for *Mycobacterium leprae*. Lane 1, 50-bp ladder; lane 2, case R180; lane 3, case R424; lane 4, case R1788; lane 5, case R2208; lanes 6-8, blank controls.

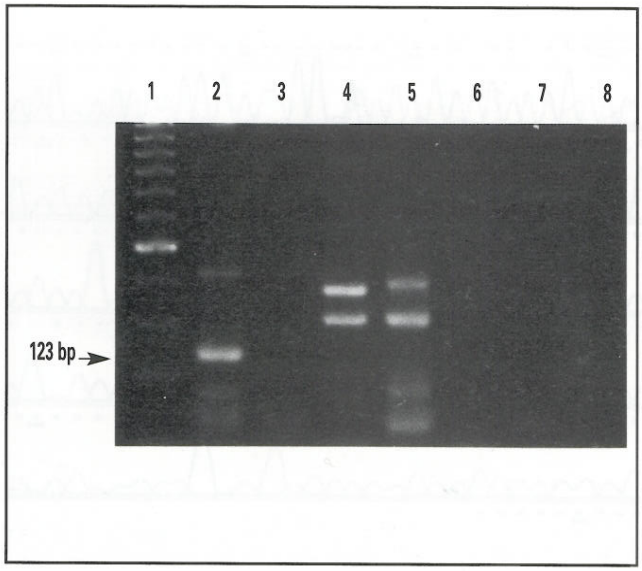


Image 5 Amplification of the 123-base-pair (bp) fragment of the mycobacterial sequence IS6110. Lane 1, 50-bp ladder; lane 2, case R180; lane 3, case R424; lane 4, case R1788; lane 5, case R2208; lanes 6-8, blank controls.

organism, and also the amount of bacteria in the lesions is substantially lower than in the lepromatous type. In addition, tuberculoid leprosy directly manifests itself first as an infection of dermal tissues and peripheral nerves. The associated severe mutilations then may result from the secondary infection of soft tissues and bones. In this instance, usually no rhinomaxillary changes are seen.[10]

As far as the prevalence of leprosy is concerned, the number of infected persons is currently assumed to be approximately 1 million, confined primarily to the tropical and subtropical areas of the world.[20] During the Middle Ages, however, there is evidence that the disease was also widespread in Europe. It may have reached its peak during the 13th to 14th century, since during that time, about 19,000 leprosy hospitals existed in Europe. For yet unknown reasons, the disease diminished in European countries during the 16th to 17th century, although it remained at a low level in a few areas, mostly in the low-infectious tuberculoid form.[10] Furthermore, it is still a matter of debate where leprosy evolved and how the disease spread all over the world.

To answer these open questions, the investigation of ancient human remains seems to be advisable, as infectious diseases like tuberculosis or leprosy sometimes manifest themselves with characteristic pathologic alterations. However, more often, the pathologic changes, particularly in bones, are less significant, and, therefore, a definite diagnosis of the underlying disease is not possible by using macromorphologic criteria alone. In these cases, modern methods of molecular biology offer new opportunities to clarify the insecurity of the morphologic analysis.

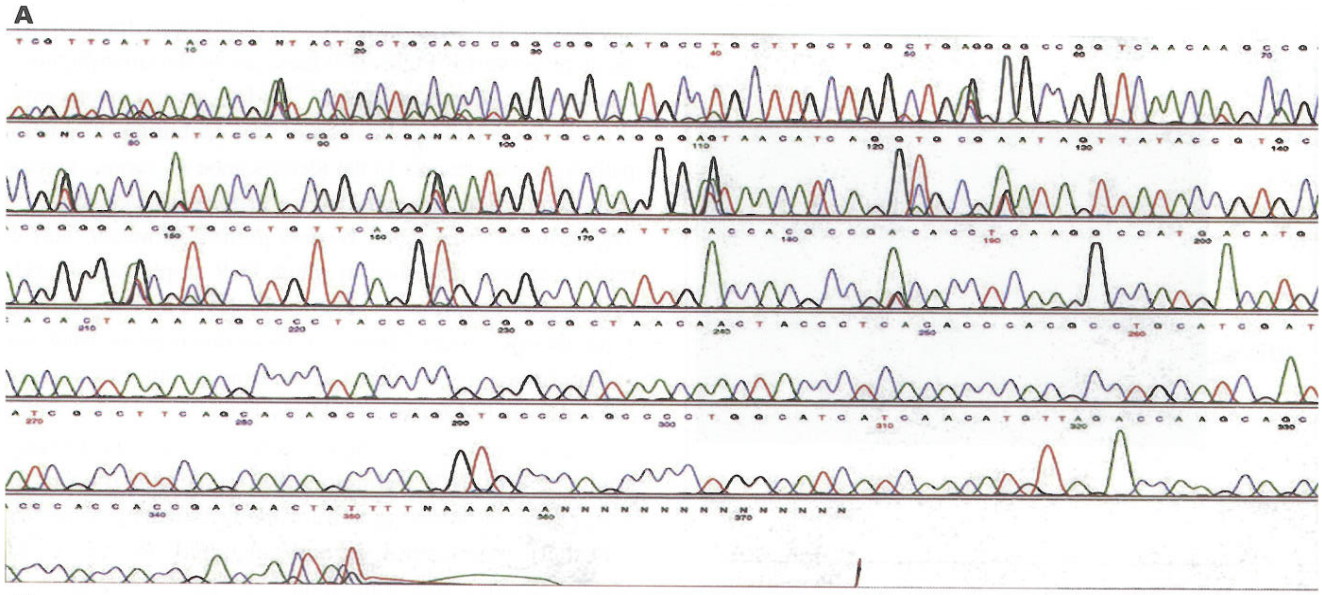
Recent technical improvements, particularly the development of powerful PCR strategies, allow the unambiguous identification of ancient DNA.[2,21] To date, several reports exist on the successful application of this technique to paleopathology with respect to the identification of various human pathogens, eg, *M tuberculosis*,[3,7] *Plasmodium falciparum*,[22] *Trypanosoma cruzi*,[23] and *Yersinia pestis*.[24] However, only 1 report has been published in which PCR amplification products suggest the presence of *M leprae* DNA in bone material from ancient sources.[9] However, these observations were not yet confirmed, eg, by direct sequencing or other techniques such as restriction fragment analysis.

In addition to the diagnostic possibilities, the broader application of PCR technology to the identification of *M leprae* offers a promising tool for the investigation of further aspects of paleopathology and paleoepidemiology of *M leprae*. Besides questions about the historic spread of *M leprae*, ie, time and place of the early occurrence of leprosy in general, the definite diagnosis of leprosy in individual cases of historic relevance could be addressed. Moreover, this approach may help estimate the frequency of *M leprae* infections in historic populations more precisely. However, it must be considered that negative results may not be conclusive, since a loss of *M leprae* DNA may lead to false-negative results. Nevertheless, the identification of possible coinfections with other infectious diseases, especially *M tuberculosis*, may be of substantial interest with respect to presumed immunologic reactions to those diseases. Finally, along with sequencing the amplified DNA from *M leprae,* information about the evolution of the mycobacteria and possible changes in their genetic make-up may be obtained.

We therefore have established a protocol to successfully amplify DNA of *M leprae* from ancient bone samples. With this method, we analyzed several historic bone samples to identify an infection with *M leprae*. In addition, we tested these specimens for an infection with *M tuberculosis*. The material was taken from an ossuary in Southern Germany, dated approximately between 1400 and 1800 AD, and from a cemetery in Eastern Hungary from the 10th century. The 2 skulls from the ossuary (R1788 and R2208), as well as the foot bones of 1 case (S202), highly indicated an *M leprae* infection, while the pathologic alterations in case S237 were unspecific. Case R180 was probably infected with facial tuberculosis.

In all samples, a 202-bp fragment of the human beta-actin gene was amplified, serving as a control for the PCR reaction, thus excluding inhibition of the PCR and/or absence of amplifiable DNA in our samples.

By using the sequences RLEP1 and RLEP3, specific amplification products of the expected sizes of 372 bp and 320 bp, respectively, were obtained from all 3 skull bone samples. The identity of these PCR products was further



C

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                    10        20        30        40        50        60        70        80
R1788 RLEP1         TGTTCACTAA CACGATACTG CTGCACCCGG CGGCATGCCT GCTTGCTGGC TGAGGGCCGG TCAACAAGCC GCCGACACCG
                    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Ref. RLEP1          tgttcactaa cacgatactg ctgcacccgg cggcatgcct gcttgctggc tgagggccgg tcaacaagcc gccgacaccg
                    <==
R2208 RLEP3         CGATGCAGGC GTGAGTGTGA GGATAGTTGT TAGCGCCGCG GGGTAGGGGC GTTTTAGTGT GCATGTCATG GCCTTGAGGT
                    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Ref. RLEP3          cgatgcaggc gtgagtgtga ggatagttgt tagcgccgcg gggtaggggc gttttagtgt gcatgtcatg gccttgaggt
                              90        100       110       120       130       140       150       160
R1788 RLEP1         ATACCAGCGG CAGAAATGGT GCAAGGGATA ACATCAGGTG CGAATAGTTA TACCGTGCAC GGGGACGTGC CTGTTCAGGT
                    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Ref. RLEP1          ataccagcgg cagaaatggt gcaagggata acatcaggtg cgaatagtta taccgtgcac ggggacgtgc ctgttcaggt
                    <==
R2208 RLEP3         GTCGGCGTGG TCAATGTGGC CGCACCTGAA CAGGCACGTC CCCGTGCACG GTATAACTAT TCGCACCTGA TGTTATCCCT
                    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Ref. RLEP3          gtcggcgtgg tcaatgtggc cgcacctgaa caggcacgtc cccgtgcacg gtataactat tcgcacctga tgttatccct
                              170       180       190       200       210       220       230       240
R1788 RLEP1         GCGGCCACAT TGACCACGCC GACACCTCAA GGCCATGACA TGCACACTAA AACGCCCCTA CCCCGCGGCG CTAACAACTA
                    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Ref. RLEP1          gcggccacat tgaccacgcc gacacctcaa ggccatgaca tgcacactaa aacgccccta ccccgcggcg ctaacaacta
                    <==
R2208 RLEP3         TGCACCATTT CTGCCGCTGG TATCGGTGTC GGCGGCTTGT TGACCGGCCC TCAGCCAGCA AGCAGGCATG CCGCCGGGTG
                    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Ref. RLEP3          tgcaccattt ctgccgctgg tatcggtgtc ggcggcttgt tgaccggccc tcagccagca agcaggcatg ccgccgggtg
                              250       260       270       280       290       300       310       320
R1788 RLEP1         CCCTCACACC CACGCCTGCA TCGATATCGC CTTCAGCACA GCCCAGGTGC CCAGCCCCTG GCATCATCAA CATGTAGACC
                    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Ref. RLEP1          ccctcacacc cacgcctgca tcgatatcgc cttcagcaca gcccaggtgc ccagccccTG gcatcatcaa catgtagacc
                    <==
R2208 RLEP3         CAGCAGTATC GTGTTAGTGA ACAGTGCATC GATGA-CCGG CCGTCGGCGG
                    ---------- ---------- ---------- ------*----- ----------
Ref. RLEP3          cagcagtatc gtgttagtga acagtgcatc gatgatccgg ccgtcggcgg
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Figure 1 Nucleotide sequences of the *Mycobacterium leprae*–specific polymerase chain reaction products. A, Electropherogram of the 372-base-pair (bp) amplicon of RLEP1 (case R1788). B, Electropherogram of the 320-bp amplicon of RLEP3 (case R2208). C, Comparison of the obtained sequences with the published respective sequences of recent *M leprae*.

confirmed by direct sequencing, thereby confirming the macromorphologic diagnosis. Thus, the presence of *M leprae* in the hard palate sample of S237 clearly attributes this case to leprosy. However, despite macromorphologic evidence, no *M leprae* DNA was detected in the 2 foot bone samples, not even in the specimen in which the hard palate sample was positive for *M leprae*. This observation suggest that peripheral bone alterations during leprosy may contain either substantially fewer bacteria or they may be caused by secondary processes, such as secondary osteomyelitis due to lacerations of the surrounding soft tissues and infection with nonspecific bacteria. While the very typical morphologic features of leprous bone alterations in S202 suggest that the amount of bacterial DNA may have been too small, acro-osteolysis was probably due to nervous involvement, and the more atypical lesions in the foot bones of S237 are more in favor of secondary osteomyelitic changes. The coexistence of nervous involvement in leprosy and that of secondary infection may be suggested in the previous case (S202), as the osteomyelitis and ankylosis of the third right metatarsophalangeal joint reveal the traces of a probable pyogenic infection.

We were able to isolate high quality DNA from the *M leprae*–containing sample. The quality of the DNA could be determined by the length of the amplified segments and the quality of the electropherograms of the sequencing reactions. This result is not surprising, since a recent study reported that in general, the amplification of DNA fragments of more than 300 bp may be possible, even from ancient samples,[25] provided the extraction and amplification techniques have been optimized for the specific material under investigation. Furthermore, it has been shown that ancient DNA is more stable in bones than in soft tissue.[26] And finally, the excellent preservation of *M leprae* DNA may be due to the extraordinary resistance of the cell walls of these bacteria to degradation.[9]

Along with the DNA amplification specific for *M leprae,* we also tested the presence of DNA from *M tuberculosis* with primers specific for the insertional sequence IS6110.[16] By using this approach, we identified *M tuberculosis* only in the mandibular sample (R180), but not in any of the other samples. This coincides with the morphologic diagnosis that osteolysis and reactive bone formation around the mandibular front teeth are usually not induced by leprosy but are much more suggestive of facial tuberculosis (lupus vulgaris). In addition, we could rule out any double infection by leprosy and tuberculosis in our small series.

Our study provides clear evidence that the application of PCR following careful DNA extraction and purification may prove a mycobacterial infection in ancient bone material with a "suspicious" morphologic appearance. Moreover, in our series, the specificity of the primers allowed us to distinguish between *M leprae* and *M tuberculosis* in the historic samples. Thus, this approach may be suitable to identify affected samples in order to establish the origin and spread of the disease. Thereby, various aspects of the paleopathology of *M leprae* can be addressed. Finally, the high quality of the amplification and sequencing results in ancient samples suggests that different mycobacterial genes can be analyzed, including those of ribosomal RNA, and, thus, it will be possible to reach conclusions about the evolution of mycobacteria.

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References

1. Buikstra JE, Baker BJ, Cook DC. What diseases plagued ancient Egyptians? a century of controversy considered. In: Davies WV, Walker R, eds. *Biological Anthropology and the Study of Ancient Egypt*. London, England: British Museum Press; 1993:24-53.
2. Spigelman M, Lemma E. The use of polymerase chain reaction to detect *Mycobacterium tuberculosis* in ancient skeletons. *Int J Osteoarchaeol*. 1993;3:137-143.
3. Salo WL, Aufderheide AC, Buikstra J, et al. Identification of *Mycobacterium tuberculosis* DNA in a pre-Columbian Peruvian mummy. *Proc Natl Acad Sci U S A*. 1994;91: 2091-2094.
4. Taylor GM, Crossey M, Saldanha J, et al. DNA from *Mycobacterium tuberculosis* identified in mediaeval human skeletal remains using polymerase chain reaction. *J Archaeol Sci*. 1996;23:789-798.
5. Baron H, Hummel S, Herrmann B. *Mycobacterium tuberculosis* complex DNA in ancient human bones. *J Archaeol Sci*. 1996;23:667-671.
6. Faerman M, Jankauskas R, Gorski A, et al. Prevalence of human tuberculosis in a medieval population of Lithuania studied by ancient DNA analysis. *Ancient Biomolecules*. 1997;1:205-214.
7. Nerlich, AG, Haas CJ, Zink A, et al. Molecular evidence for tuberculosis in an ancient Egyptian mummy. *Lancet*. 1997; 350:1404.
8. Haas CJ, Zink A, Molnar E, et al. Molecular evidence for tuberculosis in Hungarian tissue samples. In: Pálfi G, Dutour O, Deák J, et al, eds. *Tuberculosis: Past and Present*. Budapest-Szeged, Hungary: Golden Book-TB Foundation; 1999: 385-391.
9. Rafi A, Spigelman M, Stanford J, et al. DNA of *Mycobacterium leprae* detected in ancient bone. *Int J Osteoarchaeol*. 1994;4:287-290.

10. Aufderheide AC, Rodriguez-Martin C. *The Cambridge Encyclopedia of Human Paleopathology*. Cambridge, England: Cambridge University Press; 1998:141-154.
11. Pálfi G. The first osteoarchaeological evidence of leprosy in Hungary. *Int J Osteoarchaeol*. 1991;1:99-102.
12. Evison MP, Smillie DM, Chamberlain AT. Extraction of single-copy nuclear DNA from forensic specimens with a variety of postmortem histories. *J Forensic Sci*. 1997;42: 1032-1038.
13. Boom R, Sol CJA, Salimans MMM, et al. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol*. 1990;28:495-503.
14. Yoon K-H, Cho S-N, Lee M-K, et al. Evaluation of polymerase chain reaction amplification of *Mycobacterium leprae*–specific repetitive sequence in biopsy specimens from leprosy patients. *J Clin Microbiol*. 1993;31:895-899.
15. Jamil S, Wilson SM, Hacket M, et al. A colorimetric PCR method for the detection of M *leprae* in skin biopsies from leprosy patients. *Int J Lepr*. 1994;62:512-520.
16. Eisenach KD, Cave MD, Bates JH, et al. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. *J Infect Dis*. 1990;161: 977-981.
17. Ghossein RA, Ross DG, Salomon RN, et al. A search for mycobacterial DNA in sarcoidosis using the polymerase chain reaction. *Am J Clin Pathol*. 1994;101:733-737.
18. Møller-Christensen V. *Leprosy Changes of the Skull*. Odense, Denmark: Odense University Press; 1978.
19. Andersen JG, Manchester K. The rhinomaxillary syndrome in leprosy: a clinical, radiological and palaeopathological study. *Int J Osteoarchaeol*. 1992;2:121-129.
20. WHO. Global leprosy situation September 1999. *Wkly Epidemiol Rec*. 1999;74:313-320.
21. Pääbo S. Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proc Natl Acad Sci U S A*. 1989;86:1939-1943.
22. Taylor GM, Rutland P, Molleson T. A sensitive polymerase chain reaction method for the detection of *Plasmodium* species DNA in ancient human remains. *Ancient Biomolecules*. 1997;1:193-203.
23. Guhl F, Jaramillo C, Vallejo GA, et al. Isolation of *Trypanosoma cruzi* DNA in 4,000-year-old mummified human tissue from Northern Chile. *Am J Phys Anthropol*. 1999;108:401-407.
24. Drancourt M, Aboudharam G, Signoli M, et al. Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. *Proc Natl Acad Sci U S A*. 1998;95:12637-12640.
25. Haack, K. Screening auf aDNA-Sequenzen von über 300 bp Länge [abstract]. *HOMO*. 1998;49(suppl):40.
26. Lassen C, Hummel S, Herrmann B. Comparison of DNA extraction and amplification from ancient human bone and mummified soft tissue. *Int J Legal Med*. 1994;107:152-155.