Leprosy in Pre-Norman Suffolk, UK: Biomolecular and Geochemical Analysis of the Woman from Hoxne.

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ABSTRACT

Purpose. A woman's skull, exhibiting features of lepromatous leprosy (LL), was recovered from a garden in Hoxne, Suffolk. The absence of post crania and lack of formal excavation meant that diagnosis and dating was uncertain. The aim of this research was to confirm the diagnosis using biomolecular means and second, to place it in context with other British leprosy cases using SNP genotyping and radiocarbon dating.

Methodology. Bone from the skull was analysed by ancient DNA (aDNA) methods and subjected to radiocarbon dating. As a result, stable carbon and nitrogen isotope values were produced, both useful for assessing aspects of the woman's diet.

Key findings. aDNA confirmed the presence of *mycobacterium leprae* and *genotyping* demonstrated an ancestral variant of subtype 3I, the same lineage recently identified in living squirrels in the south of England. Radiocarbon dating revealed the woman lived approximately between 885-1015AD, providing evidence for endurance of this subtype in East Anglia, having been previously identified as early as the 5th-6th century (Great Chesterford) and as late as the 13th century (Ipswich).

Conclusions. The confirmation of a new pre-Norman leprosy case in East Anglia is of interest as this is where a high proportion of cases are located. Possible factors for this may include preservation and excavation biases, population density, but also connection and trade, possibly of fur, with the continent. Future research on other British LL cases should focus on exploring these aspects to advance understanding of the disease's history, here and on the continent.

INTRODUCTION and AIMS.

At some point between 1960 and 1990, a cranium and mandible with pathological changes consistent with leprosy were recovered from a garden in Hoxne, Suffolk (Anderson 1996). Unfortunately, little is known about the skull although a pre-Norman date (5th-11th century) was suggested based on the morphology of the skull [1]. With the exception of a second mandible, it is not known whether the cranium and mandible were associated with any other skeletal material. The bones are currently stored at Diss museum under accession numbers DISDS T.439.1-2. The cranium and mandible were first analysed osteologically by Sue Anderson of Suffolk County Council Archaeological Service in 1996 (see supplementary information or [1] for full report). Apart from the right side (ascending ramus) of the mandible, the skull was complete. The preservation of the material was very good. Anderson reported the skull to belong to a young to middle aged adult female [1]. Changes associated with Hansen's disease included rhino-maxillary changes, especially destruction to the nasal spine, remodelling of nasal aperture margins (see Fig. 1) and palate [2]. Although these bone changes are highly indicative of leprosy, they are not unique to the condition and can be associated with other diseases. Without the rest of the skeleton to assess for other characteristic skeletal lesions (e.g. foot and hand deformities), a definite diagnosis of leprosy could not be made from the osteological changes alone. Given the potential early date of the woman, it would therefore be of interest to confirm the diagnosis of leprosy, and if possible, assess the strain to understand more about the temporal and geographic distribution of leprosy in ancient Britain. Although many strains of leprosy exist, recent research has demonstrated that at least two distinct strains of leprosy existed in Medieval England. Type 3 strains were present from the 5th-6th century [3], while strains from the second branch (type 2F strains) are known from the 11th century [4].

Therefore, the first aim of the current research was to assess the woman's bone for the presence of *Mycobacterium leprae*, the bacteria that causes the disease, using genetic techniques. If mycobacteria were detected and the osteological leprosy diagnosis confirmed, the second aim was to assess which strain of leprosy infected the woman and how this fits into current knowledge about the disease. This required radiocarbon dating and comparison to other cases of leprosy in Britain. Overall, this research on a putatively pre-Norman case, contributes knowledge useful for understanding the nature of the leprosy epidemic that afflicted Britain and Europe in the medieval period.

Fig.1.

METHODS.

DNA sampling.

Sampling of the skull was undertaken in the museum store in Diss, Norfolk using gloves, a sterile scalpel and sampling bags. Samples of bone powder (80 mg) were taken from the crania (T439.1) from the vomer region and 45 mg was removed from the mandible (T439.2) in the vicinity of an already damaged area surrounding the right second premolar tooth.

DNA extraction.

Bone fragments were ground to a fine powder using sterilised pestles and mortars. The powders were weighed and divided into two equal amounts. One set was extracted immediately for screening using leprosy PCRs, the other was set aside for subsequent genotyping.

DNA was extracted using an in-house version of the Boom method [5]. In this procedure, 6M guanidinium thiocyanate (GUSCN, product G9020, US Biologicals, Salem, MA.) containing 1% Triton X-100 (Sigma-Aldrich, T8787) was dissolved in 1x Tris-EDTA buffer (Sigma-Aldrich, T9285) adjusted to pH 6.5 with 3M sodium acetate, pH 5.5 (Ambion,™ product 9740). Bone powder was mixed with 1ml of the GUSCN buffer on a mixing wheel for 1hr at 4C. The samples were then subjected to 3 freeze-thaw cycles to assist with DNA recovery. The bone powder was removed by centrifugation at 12,000 x r.p.m. and the supernatant buffer transferred to a fresh 1.5ml Eppendorf tube. Pre-washed silica suspension (40µl of 0.5-10µm, Sigma-Aldrich, S5631) was added and kept in contact for 3hrs to maximize recovery of fragmented DNA. After centrifugation, silica was further washed twice with 1ml

aliquots of GUSCN extraction buffer, followed by 3 washes with 75% ethanol and finally with 1ml of acetone. After thorough drying of the silica pellet, DNA residues were eluted in 60µl HPLC grade water (Sigma-Aldrich) at 55^{o}C. These were then sub-divided into 2 x 30 µl aliquots and stored in low retention plastic tubes to minimize loss of DNA through repeated freeze- thawing events.

M.leprae screening methods.

Two separate PCR methods were used to screen for the RLEP element, present in 37 copies in the *M. leprae* genome. In the first of these two methods, product formation was monitored using the intercalating dye EVAGreen™ (Biotium, Fremont, CA 94538). The second method employed a FAM labelled hybridization probe. Details of these two methods have been reported previously [4].

In the present study, a novel PCR method for the REPLEP element (15 copies) was also used. The sequences of the two primers being: F-5'-TCGGGATAGGTTTTGGGCCAAC-3' and R-5'-CTTTAAAGGCCGGCAAGGTGA-3'. These amplified a 119 bp product which was reported using EvaGreen™.

Finally, we also screened for the 18-kDa antigen locus using primers 18F 5'-CTGGACATTGACATCGAACG-3' and 18R 5'- GCCAAGATCCGTTGGGTGT-3' which amplify a 155 bp product. Experience shows that positivity for this single copy method is a good indicator that SNP genotyping methods may be successful.

M.leprae SNP genotyping methods.

A series of PCR methods was used to genotype positive extracts. A number of these, used for characterising SNP type 3 strains, have been previously published [4,6].

Screening for *Mycobacterium tuberculosis* complex DNA.

As tuberculosis (TB) can also cause rhino-maxillary changes [7], and as there is significant interest in the co-infection of TB and leprosy, extracts were also tested for the presence of *Mycobacterium tuberculosis* (MTB) complex organisms using a real- time PCR method for the IS*1081* repetitive element as previously described [8].

Human aDNA.

1. Mitochondrial DNA (mtDNA).

Two PCR methods were initially used to look for evidence of human mtDNA. Extracts prepared from the mandible were tested using primers which amplify a 116 bp region of the human mitochondrial DNA hypervariable region 1 (HVR-1). The sequences of these primers were:

Forward (L15977-L15998) 5'-CCACCATTAGCACCCAAAGCTA-3' and

Reverse (H16092-H16070) 5'- ATACATAGCGGTTGTTGATGGGT-3'.

Another variant of this PCR was used with an alternative reverse primer (H16255-H16236) with the sequence 5'-CTTTGGAGTTGCAGTTGATG-3'. In combination with the forward primer, this amplifies a product of 279 bp.

2. Amelogenin.

Although morphology strongly indicated that the skull was from a female, a sex-determining PCR based on polymorphisms in the amelogenin gene was also applied. In this method, males are identified by two PCR products, one of 105 bp from the Y chromosome and another of 290 bp from the X chromosome, whereas females generate only the one product of 290 bp. The sequences of the primers used in this procedure were (F2) 5'-TGACCAGCTTGGTTCTAWCCC-3' and reverse (R1) 5'-CARATGAGRAAACCAGGGTTCCA-3' [9].

A second amelogenin method was also attempted [10]. This generates two bands from males of 106 bp and 112bp (AMELX and AMELY products respectively), and a single AMELX product of 106bp from females.

PCR Amplification details.

PCR was performed in a final volume of 15µl, using a hot start Taq kit from Qiagen (product 203445). The reactions contained 25 pmol of forward and reverse primers, each in 1µl, 7.5 µl of the kit master mix, 1.5 µl non-acetylated bovine serum albumin (BSA, 10mg/ml, Sigma B4287) and 2µl of template. The kit magnesium ion concentration of 1.5 mM per reaction was supplemented to 2 mM for PCR methods using EVAGreen™ and to 3 mM $MgCl_2$ for real-time PCR with the RLEP probe. The probe was used at a final concentration of 100 nM. The volumes were made up to 15 µl with molecular biology grade water (Sigma-Aldrich). After an initial activation step of 14 min at 95°C, 41 cycles of amplification were performed on an Mx3005P RT-PCR platform (Agilent Technologies).

The thermal profile of the amplification cycles consisted of denaturation at 95°C for 10s, annealing (range 52–60°C) for 30s and extension at 72°C for 30s. Fluorescence data was acquired during the extension step. Melt analyses was performed automatically at the end of runs monitored with EVAGreen™ and dissociation curves studied to identify likely positives.

Gel electrophoresis and automated DNA sequencing.

PCR products were run out on 3% agarose gels in a TAE buffer system alongside appropriate DNA size markers (100 bp or 50 bp DNA ladders, Promega) to confirm product identity. Positive samples for SNP or MLVA typing were bulk purified on 3% (wt/vol) low-melting-point agarose (Invitrogen); bands were excised and purified using a Geneclean DNA isolation kit (Cat.No.1001-200, mpbio.com). Templates were Sanger sequenced using both forward and reverse primers by Genewiz UK Ltd., Takeley, Essex, UK. The sequencing platform used was the Life Technologies 3730xl DNA Analyzer, a 96 capillary instrument.

Measures to prevent contamination.

Separate laboratories were used for each of the three main stages of the aDNA analyses, these being extraction, amplification and post PCR analysis, such as gel electrophoresis and purification of products for sequencing. The pre- and post- PCR laboratories were physically separated and independently equipped with pipettes, fridge-freezers, mixers and bench top centrifuges, disposable plasticware, filter tips and other reagents dedicated to the project.

Surfaces and equipment in the clean "set-up" laboratory in contact with sample tubes (centrifuges, rotors, mixers, etc.) were cleaned before each assay. Two control tubes, comprising reagents less bone powder, were taken through each extraction experiment to ensure reagents were contamination free. Several template blanks were run alongside bone extracts in the PCR machine to screen for random contamination. Positive controls were not included in any of the PCR experiments.

Radiocarbon dating

A sample of bone (0.5g) was taken from the broken end of the woman's mandible for radiocarbon dating. Radiocarbon dating was undertaken at the Faculty of Mathematics and Natural Sciences at the University of Groningen, the Netherlands. The sample underwent standard chemical cleaning and collagen extraction following an improved version of Longin [11]. The collagen was combusted into CO_2. The CO_2 was cryogenically trapped using an automatic device [12], transformed into graphite, and analysed for ^{14}C by AMS [13]. The ^{14}C activities were measured relative to a standard radioactivity, corrected for isotopic fractionation using the stable isotope ratio $^{13}C/^{12}C$ to $d^{13}C$ = -25 ‰, calculated using the conventional half-life, and reported in BP [14]. Subsequently, the ^{14}C dates were calibrated into calendar ages using the internationally recommended calibration curve IntCal13 [15]. As a by-product of this analysis, stable carbon and nitrogen isotope values were produced, which are useful for assessing aspects of the woman's diet.

RESULTS

Biomolecular study.

1. *M.leprae* screening methods.

Screening methods showed that the female individual was positive for *M.leprae* DNA, with the mandible material being more strongly positive than the bone taken from the cranium (Table 1). In fact, only one of the PCR methods detected *M.leprae* DNA in the cranial sample, this being the most sensitive version of the RLEP PCR which employs the intercalating dye EVAGreen.™ This is shown in Fig. 2. In contrast, all 4 PCRs identified the pathogen in the extract prepared from the mandible (Table 1). As anticipated, the muliti-copy targets RLEP and REPLEP exhibited lower Cq values than the single copy 18-kDa locus (Table 1). Amplification profiles for the RLEP probe method and REPLEP PCR (plus associated melt curve) are shown in Supplementary material, Figs. S1 and S2 respectively. Additionally, the REPLEP product was subjected to gel electrophoresis to confirm amplicon size (Fig. S3).

(See Table 1)

Reproducibility.

Key screening PCR experiments for *M.leprae* DNA, namely for RLEP using both EVAGreen™ and specific FAM-labelled probe and for the REPLEP element were repeated several weeks after the original experiments. The results obtained (not shown) were almost identical to the original findings seen in Fig. 2 and Figs. S1 and S2 respectively. All extraction and non-template controls were negative; showing cross-contamination was not an issue.

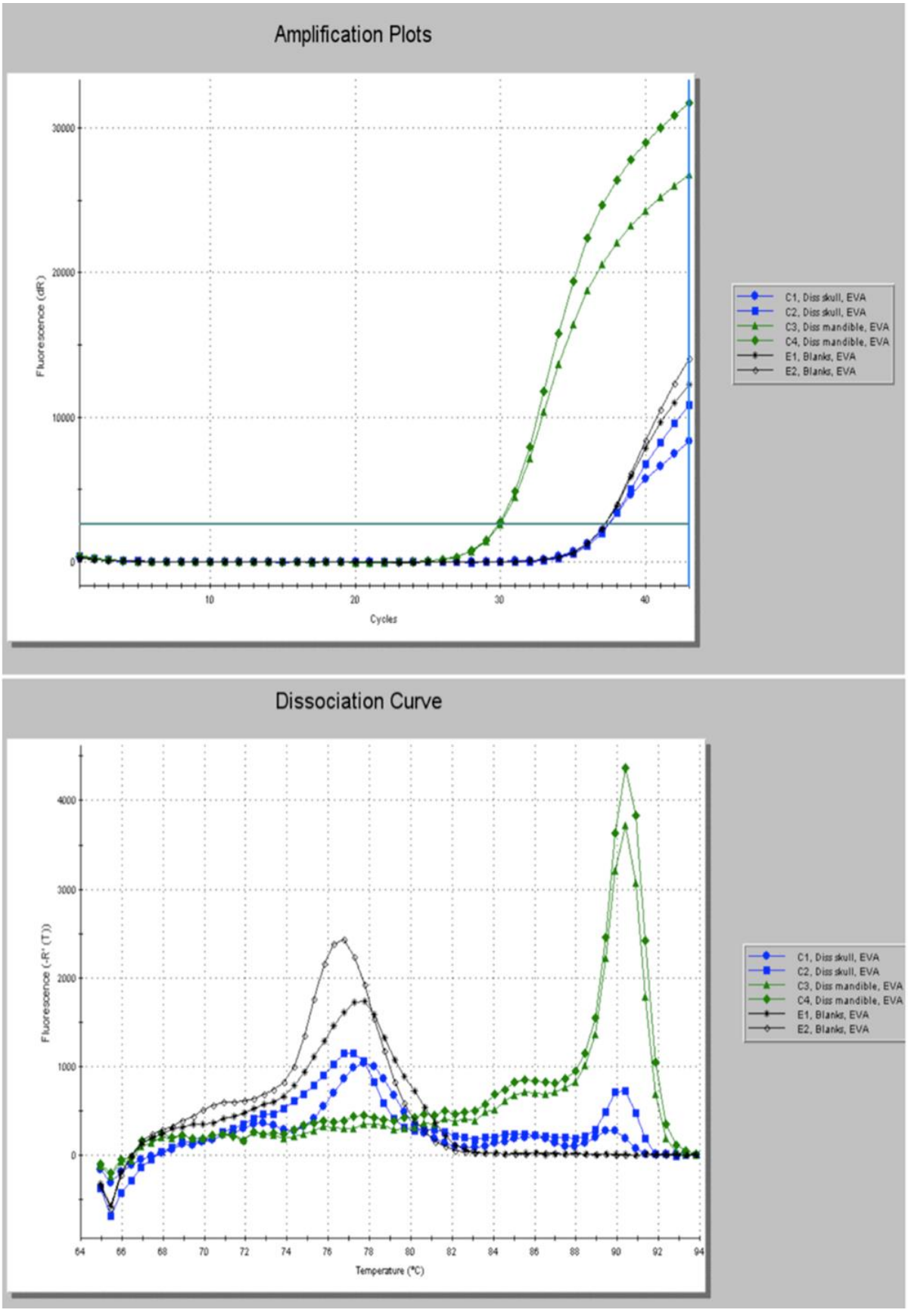
Amplification Plots
Fluorescence (dR')
Cycles
C1, Diss skull, EVA
C2, Diss skull, EVA
C3, Diss mandible, EVA
C4, Diss mandible, EVA
E1, Blanks, EVA
E2, Blanks, EVA
Dissociation Curve
Fluorescence (-R' (T))
Temperature (°C)
C1, Diss skull, EVA
C2, Diss skull, EVA
C3, Diss mandible, EVA
C4, Diss mandible, EVA
E1, Blanks, EVA
E2, Blanks, EVA

Fig.2.

2. SNP genotyping of *M. leprae*.

SNP genotyping methods were applied to aDNA prepared from the mandible. The Hoxne case was found to be SNP type 3, based on the 3 main loci described by Monot and colleagues [16]. These results are shown in Fig. 3, panels a-c. Further subtyping showed the strain of leprosy belonged to the 3I lineage [17,18] (Fig.3, panels d-f). The full genotyping findings are summarised in Table 2. Two SNP loci failed to amplify. These were the polymorphic loci at nucleotide positions 403,902 and 1,527,056. The latter is useful to further sub-type 3I strains into either 3I-1 or 3I-2. We therefore cannot distinguish between these two alternatives, although the 3I-1 is more likely in archaeological material (see [3, 4]).

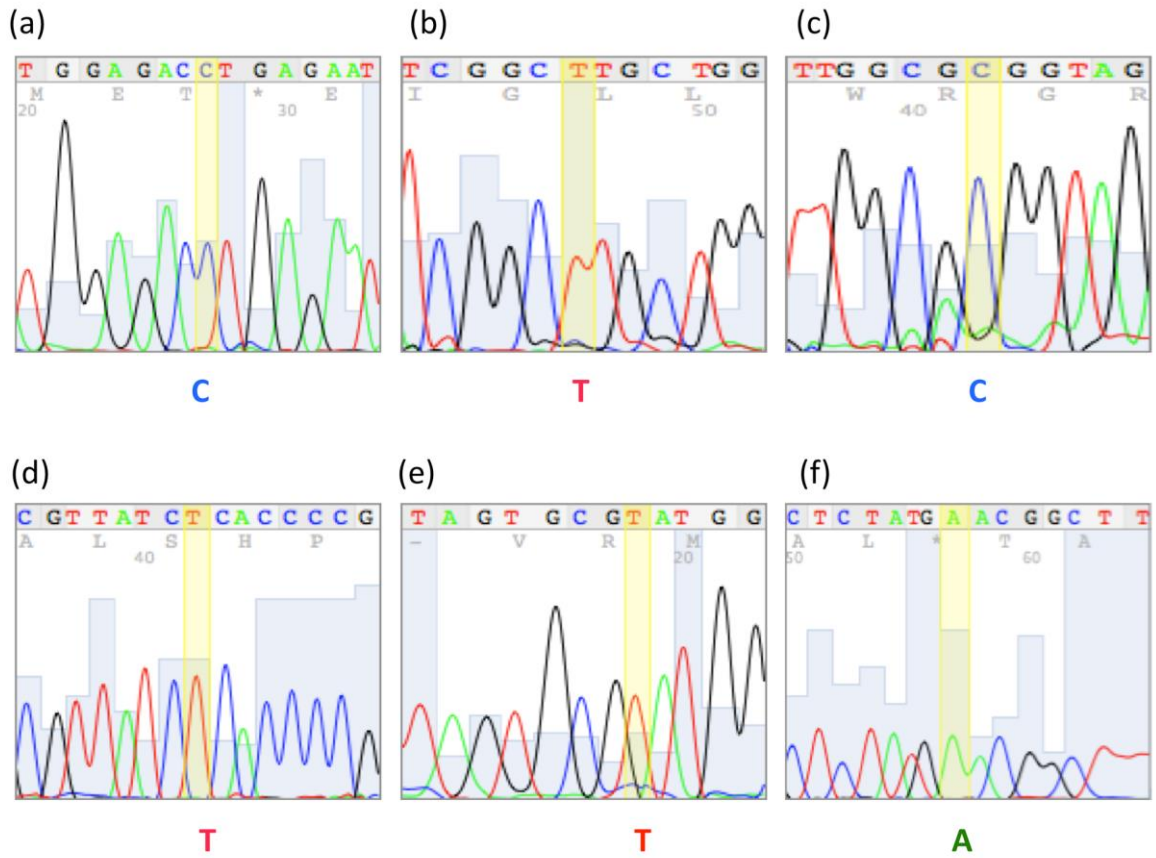

Fig.3.

(See Table 2)

3. VNTR typing.

Neither the AGA(20) nor the GTA(9) loci amplified, so the only variable repeat which was successful was the 21-3 (ML0058) region, of which 2 copies were present. Failure of the triplet repeat loci may indicate a strain with multiple copies of these, with DNA fragmentation taking template survival below the cut-off point for amplification.

4. *M.tuberculosis* complex DNA.

No evidence was found for *M.tuberculosis* complex DNA in the Hoxne case.

Human DNA.

1. mtDNA.

A PCR product was obtained with the primer pair (L15977-L15998) and (H16092-H16070) which generate a 116 bp product (Fig. S4 in Supplementary material). However, attempts to generate the longer amplicon of 279 bp with primer pair (L15977-L15998) and (H16255-H16236) were unsuccessful.

2. Amelogenin.

No PCR products were obtained with either version of the amelogenin methods we used, which probably reflects the extremely fragmented nature of DNA in this skeleton.

Radiocarbon dating and stable isotopes.

Analysis of the sample (GrA-66655) demonstrated that the quality of the bone collagen was good with C/N ratio of 3.3. In addition, it appeared from the carbon and nitrogen stable isotope results that there was no reservoir effect affecting the results with δ13C (‰) of 19.78 and δ15N (‰) of 11.03. The uncalibrated date was 1105+/-30BP. The calibrated dating revealed that the woman likely lived sometime between 885-1015 AD (2-sigma), confirming the pre-Norman date.

As part of the radiocarbon dating of the jaw bone, stable isotope values for carbon and nitrogen were produced, as mentioned above. Although there has been limited isotopic research in this region for the 10th and 11th centuries, some information is available. In a study of East Anglian Anglo-Saxon diet, presented values for three sites of a similar date to the woman from Hoxne: Caistor-by Yarmouth, Burgh Castle and South Acre [19]. The values for the woman from Hoxne fit well with these sites.

The values for carbon suggests that the woman was likely to have been eating a diet based largely on c3 terrestrial plants, which considering the time period, is likely to consist of wheat, barley, pottage. The nitrogen isotope values suggest that she is likely to have consumed some animal protein.

DISCUSSION.

The aim of this research was to confirm the macroscopic diagnosis of leprosy in the woman found in Hoxne through the detection of the mycobacteria responsible for the disease. In addition, it was desirable to know which strain of the disease the woman was suffering from and when she had lived. The results indicate that she was infected with leprosy, of which the strain belongs to the 3I branch of the *M.leprae* phylogenetic tree. Modern 3I-1 isolates display T and G bases at nucleotide positions 7,614 and 1,113,926 respectively. In the Hoxne skull, the SNPs were T and A respectively. This appears to be an intermediate genotype between what would be expected from other genotypes (including type 3 strains other than 3I) namely C and A and implies that this woman was infected with a strain which may have been ancestral to modern 3I exemplars. We have previously found the same combination at these loci in a case of leprosy from Great Chesterford [3]. The Great Chesterford case was earlier, with a calibrated radiocarbon date of 415-545 AD, whereas the present skull was dated between 885-1015 AD. Therefore, very similar strains of leprosy persisted for several hundred years in this part of Britain.

The strain type has little bearing on the pathogenesis or severity of disease, as this is dictated by the individual's immune response to *M. leprae*, but rather assists in understanding the origin of disease in the Anglo-Saxon period. Other type 3I cases have been reported from medieval Britain (Winchester and Ipswich), Denmark and Sweden [4, 20]. Bearing in mind the location of the latter two, a Scandinavian origin for this lineage remains one possibility, given the proximity of the Anglo-Saxon tribal homelands in Northern Germany with Denmark, and the significant population movements that took place between Britain and this region in the Anglo-Saxon period.

Although the total evidence from the early medieval period is limited to around a score of cases, at present it does seem that the 3I genotype was one of two predominant lineages associated with the rise in disease in Britain in the early and high medieval periods. There is also evidence to suggest that the 3I lineage was present in Britain much later in the timeline of European leprosy, which had begun to decline by the 13th century [21]. An earlier study found this lineage in a case from 13th-16th Suffolk [6, 22]. This lineage is now found in southern states of the United States of America and it was likely taken to the New World by early European settlers. Given persistence of the 3I lineage over nearly 800 years, it seems unlikely that a change in genetic makeup of the bacillus was responsible for the decline in European leprosy: an inference confirmed by comparison of present day 3I whole genomes with those recovered from both Winchester and Scandinavia [23] which revealed remarkably high degrees of conservation amongst the ancient and modern strains.

Although indigenous human leprosy has been absent from the British Isles for over 200 years, a recent study demonstrated *M.leprae* infection in red squirrels on Brownsea Island, Dorset, UK. Interestingly, sequencing of the red squirrel *M. leprae* strain showed it to be most closely related to an *ancient M. leprae* that was detected in medieval human skeletal remains (SK2, Sk7 and Sk19) from Winchester, UK [23, 24]. These strains were Type 3I, similar to that detected here in the woman from Hoxne. An attractive theory is that *leprosy* is, in part, partially a zoonotic infection that can be passed from human to human, between armadillos and humans [18] and also from squirrel to human. Historically, this route of transmission is made viable by the common usage of squirrel for fur and meat in the medieval period and it is known that squirrel fur was imported into the East Anglia from Scandinavia and the Baltic region [25]. However, it is questionable how long the bacteria could survive in meat or fur to be transmitted, but it is notable that squirrels were occasionally kept as pets. Thus, while contact with squirrels has declined and human disease has been eliminated in the UK, a reservoir of *M. leprae* remains in the red squirrel. It is also of note that the British red squirrel population also harbours another leprosy causing bacterium, *M. lepromatosis*, which has been shown to cause human disease predominantly in Mexico [26]. No modern or ancient human leprosy in Europe has yet been demonstrated to be caused by *M. lepromatosis* but it remains possible that this bacterium may also be an aetiologic agent of leprosy in the British Isles.

There is a long existence of leprosy in the east of Britain, which is evidenced by the foundation of many leper hospitals or *leprosaria* in East Anglia from the 11th century onwards. Examples close to Hoxne include the suburban examples at Eye, 4 miles to the southeast, Eccles 20 miles to the north east (both dedicated to St. Mary Magdalene) and Little Snoring some 45 miles to the north. Norwich itself was known to have at least four houses dedicated to the care of those with leprosy, including the still-standing example at St. Margaret in Sprowston to the north east of the medieval town. There were several hospitals just outside the city gates, such as St. Giles and St. Benedict's to the west and St. Leonard's and St. Mary Magdalen and St. Clements' to the north. Other East Anglian towns with *leprosaria* included Dunwich, Ipswich, King's Lynn, Sudbury and Great Yarmouth. Indeed, some of the later foundations, that is after 1350 AD, were in this part of Britain including Walsingham, founded pre 1486 [21].

Significantly, the confirmation of leprosy causing mycobacteria in the woman from Hoxne adds to a growing number of pre-Norman and early Norman cases (see Table 3 and Fig. 4) in Britain. When putting the case in context with others prior to the widespread foundation of hospitals, a number of trends can be noted. First, it appears that the earliest cases come from the south west of the country, although it would be useful to subject these to radiocarbon dating and aDNA analyses. In the early Anglo-Saxon period, with the exceptions of Beckford and Cannington in the south west, the other cases are in the east, especially East Anglia, with

five cases being found along the route of the Icknield Way, an important travel route partly linking the south west, where the earliest cases are, and the east (see Fig. 4). In the Middle Saxon period cases can be found at eight sites, including the Hoxne case. Half are still found in East Anglia with one in nearby Northamptonshire. Cases are also found at two sites in Yorkshire and in the Scilly Isles. This appearance of later cases in a more northerly location has already been highlighted by others [27].

(See Table 3)

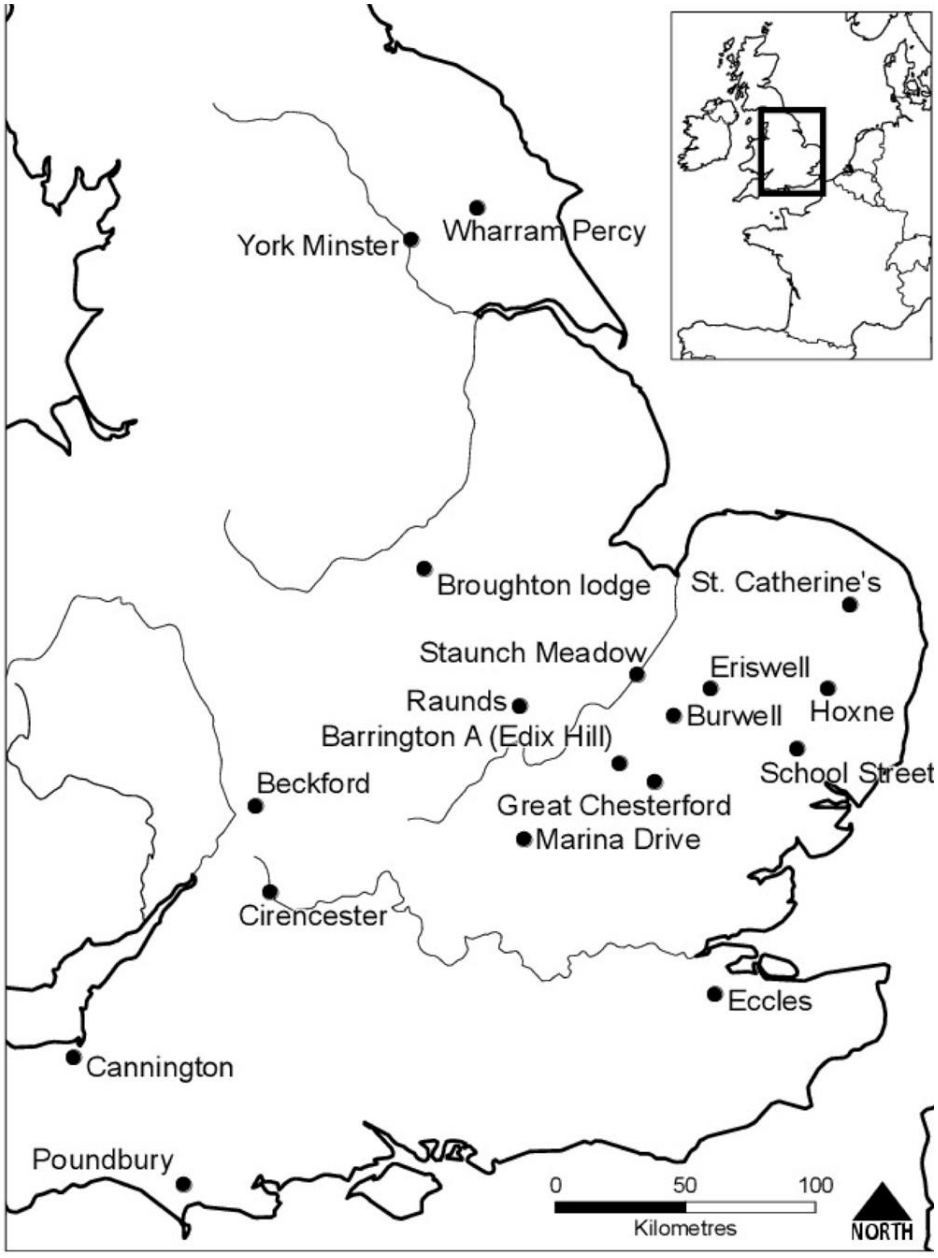

Fig.4.

The appearance of so many leprosy cases in the (East) Anglia region is noteworthy as it could potentially suggest that the disease was endemic in this region earlier than other parts of the country. There are many problems with determining the prevalence of a disease from archaeological material, as is cautioned by Roberts [27], so there could be a number of explanations for this trend, some of which could be interconnected. While it is not the purpose of this paper to fully explore them here, and much radiocarbon dating and aDNA research is required, some themes can be highlighted. First, it is important to consider that there is a general excavation bias in the region of analysis. In relation, it is possible that this trend is a result of archaeological excavation bias due to the development of places with early medieval precursors. However, one may expect cases from locations near cities that have had significant development and/or have rapidly expanded beyond their early medieval borders into the hinterland, such as London, Nottingham and Bristol.

A further factor may relate to preservation. In particular, the sites yielding cases in the East Anglia region are places dominated by chalk. This leads to very good skeletal preservation, and it could be argued that the number of cases could be related to their survival to discovery. However, there are other parts of Britain that have similar geology, for example Hampshire and Dorset, and while many early Anglo-Saxon cemeteries have been excavated (e.g. Alton, Worthy Park and Appledown) no leprosy cases have been recovered at present.

It is also pertinent to consider population densities at the time when leprosy was becoming endemic in Britain. East Anglia had many of the most densely populated areas, including Norwich, Ipswich, Thetford and Lincoln. Although leprosy is mostly a disease of the countryside today [28], it can also have a high urban presence [29]. It is therefore possible that increasing population density, and/or interconnectedness between rising towns and the rural hinterland, may have provided opportunities for the disease to survive and spread in this region. However, if purely related to density, one would expect more cases from other regions with high density, although the later cases from York, a region also with high population density in the medieval period, could support this idea.

If the trends in the current evidence remain true, and as it appears that the strain identified here came from the Scandinavia region via the Anglo-Saxon homelands and/or later Viking activities, some explanation may lay in the significant movement in and between East Anglia and the continent. In addition, strong trade connections existed between the two regions. Even more intriguing is the fact that King's Lynn and Yarmouth became very significant for fur import, including Scandinavian and Baltic squirrel, in the Medieval period [25]. Perhaps then it is the prolonged connection between these two regions that is important in the disease's history in the UK. In addition, as potentially three of the earliest case are actually in the west

of England, it would be very interesting to see if they have the same strain, which may point to a first appearance of the disease in the west. Further research confirming or refuting these trends, as well as the role of the fur trade could be highly enlightening and exciting.

CONCLUSION.

The aim of this research was to confirm the macroscopic diagnosis of leprosy in a female individual excavated from a garden in Hoxne, Norfolk. In addition, it was desirable to know which strain of the disease she had, and how this fits into our current knowledge of the disease in Britain and beyond. Genetic analysis detected *M. leprae* in the cranium and mandible. SNP typing demonstrated that the strain was from the third branch of the phylogenetic tree, subtype I. This (sub)type has also been identified at Great Chesterford in a 5th to 6th century man. Radiocarbon dating demonstrated that the woman from Hoxne lived later, sometime between 885-1015AD, demonstrating the persistence of this strain in the region. In addition, her discovery adds to the growing number of pre-Norman and Norman leprosy cases in Britain of which over half are in East Anglia or surrounding regions. It is possible that this apparent clustering could relate to Anglo-Saxon and Viking movements, trade and/or the high population density that existed in this region during the Anglo-Saxon period, although excavation and preservation factors could be compounding the picture. Further research should focus on exploring these possibilities and their interconnectedness to improve our understanding of the origins and spread of the disease in Britain and its connected regions.

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Conflicts of interests: The authors have declared that no competing interests exist.

Ethical Statement: The date of the human skeletal remains means that it is not subject to the Human Tissue Act 2004. The skull was disturbed accidently on private land. As such no permissions for excavation was required.

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TABLES.

533 **Table 1.**

M.leprae PCR locus (reporter).	Amplicon Size (bp)	Skull T439.1 Result (Cq)	Mandible T439.2 Result (Cq)
RLEP (EvaGreen™)	111	± (37.22)	++ (29.75)
RLEP (FAM probe)	78	- (no ct)	++ (30)
REPLEP (EvaGreen™)	119	ND	++ (30.1)
18-kDa (EvaGreen™)	155	- (No ct)	+ (34.49)

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535 **Table 2.**

Locus	**Amplicon Size (bp)**	**Nucleotide Base.**	**SNP typing inference.**
SNP 1 14,676	136	C	type 3
SNP 2 1,642,879	122	T	
SNP 3 2,935,693	107	C	
SNP 4 413,902	120	Fail	
SNP 5 591,857	107	C	subtypes I-L
SNP 6 1,133,495	121	T	subtype I
SNP 7 2,312,066	120	C	subtypes I or J
SNP 8 7,614	109	T	subtype I
SNP 9 1,113,926	117	A	
SNP10 1,104,235	117	G	type 3
SNP 12 1,527,056	101	Fail	
Indel 17915 11 bp repeat	120	1 copy*	subtype I
Overall			**3I**

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541 **Table 3.**

Site	Century AD	Number of cases	Source
Poundbury, Dorset	4th	1	30
Cirencester, Gloucestershire	Roman	1	28
Great Chesterford, Essex	5th	1	3
Cannington, Somerset	5th	1	31
Beckford, Gloucestershire	6th	1	32,33
Eriswell, Suffolk	5th-7th	4	34
Broughton lodge, Nottinghamshire	5-7th	1	35
Barrington A (Edix Hill), Cambridgeshire	6th-7th	1	36
Burwell, Cambridgeshire	7th	1	37
Marina Drive, Dunstable	7th	1	38
Eccles, Kent	7th	1	39
Tean, Scilly Isles	7th-8th	2	40
Staunch Meadow, Brandon, Suffolk	Middle Saxon	1	41
Hoxne, Norfolk	9th-10th	1	This study
Raunds, Northamptonshire	10th-12th c	3	42
School Street, Ipswich	10th-11th	2	43
St. Catherine's, Thorpe, Norwich	Late Saxon	1	44
York Minster	8th onwards	2	45
Wharram Percy, Yorkshire	10-12th	1	46

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LEGENDS TO TABLES.

Table 1. Results of PCR screening methods for *M.leprae* DNA.

- = PCR negative; + = Weak positive; + = Positive; ++ = Strong positive

All extracts were tested in duplicate and the mean Cq (cycle of quantitation) values are shown in parentheses.

Table 2. SNP genotyping of Hoxne case.

Table 3. Cases of pre-Norman and early Norman cases of leprosy in the east region of Britain.

LEGENDS TO FIGURES.

Fig.1. Left, remodelling of the nasal aperture and spine in cranium T439.1. Right, frontal view of the skull.

Fig.2. Upper panel: RLEP PCR method showing amplification profile for the Hoxne skull (blue traces) and mandible (green traces). Lower panel shows the dissociation or melt curves for these samples. Note primer-dimer generation is seen in the water blanks (black traces) but this product melts at a far lower temperature (77C) compared to the specific RLEP amplicon (91C). Sequencing confirmed identity of the RLEP amplicon.

Fig. 3. Sequencing of phylogenetically informative loci from the strain of *M.leprae* amplified from the Hoxne mandible. Panels a-c show C at nucleotide position 14,676, T at 1,642,879 and C at 2,935,693 respectively, indicating a main SNP type 3. Panels d-f show T at position 1,133,495, T at 7,614 and A at 1,113,926, further indicating a sub-type I. In each case, SNPs of interest are highlighted with a yellow bar in each panel. Nucleotide positions refer to the Tamil Nadu (TN) reference strain of *M.leprae*.

Fig. 4. Map demonstrating the distribution of pre-Norman and early Norman cases of Leprosy in Britain. Note: Tean cases not shown.