International Journal of Paleopathology 27 (2019) 1–8

Contents lists available at ScienceDirect

International Journal of Paleopathology

journal homepage: www.elsevier.com/locate/ijpp

Archival, paleopathological and aDNA-based techniques in leprosy research and the case of Father Petrus Donders at the *Leprosarium* 'Batavia', Suriname

Jaap T. Van Dissel[a,*,1], Toine Pieters[b], Annemieke Geluk[a], George Maat[c], Henk E. Menke[d], Maria Tió-Coma[a], Eveline Altena[e], Jeroen F.J. Laros[e,f], Malti R. Adhin[g]

[a] Dept Infectious Diseases, Leiden University Medical Centre, Leiden, the Netherlands
[b] Freudenthal Institute for Science and Mathematics Education, Utrecht University, Utrecht, the Netherlands
[c] Dept Anatomy, Leiden University Medical Center, Leiden, the Netherlands
[d] Dermatology Service, Ministry of Health, Paramaribo, Suriname[2]
[e] Dept Human Genetics, Leiden University Medical Center, Leiden, the Netherlands
[f] Dept Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands
[g] Dept Biochemistry, Faculty of Medical Sciences, Anton de Kom Universiteit van Suriname, Paramaribo, Suriname

ARTICLE INFO

Keywords:
Mycobacterium leprae
Phylogenesis
Leper colony
Slave trade
West Africa

ABSTRACT

Objective: We assessed whether Petrus Donders (died 1887), a Dutch priest who for 27 years cared for people with leprosy in the leprosarium Batavia, Suriname, had evidence of *Mycobacterium (M.) leprae* infection. A positive finding of *M. leprae* ancient (a)DNA would contribute to the origin of leprosy in Suriname.
Materials: Skeletal remains of Father Petrus Donders; two additional skeletons excavated from the Batavia cemetery were used as controls.
Methods: Archival research, paleopathological evaluation and aDNA-based testing of skeletal remains.
Results: Neither archives nor inspection of Donders skeletal remains revealed evidence of leprosy, and aDNA-based testing for *M. leprae* was negative. We detected *M. leprae* aDNA by RLEP PCR in one control skeleton, which also displayed pathological lesions compatible with leprosy. The *M. leprae* aDNA was genotyped by Sanger sequencing as SNP type 4; the skeleton displayed mitochondrial haplogroup L3.
Conclusion: We found no evidence that Donders contracted leprosy despite years of intense leprosy contact, but we successfully isolated an archaeological *M. leprae* aDNA sample from a control skeleton from South America.
Significance: We successfully genotyped recovered aDNA to a *M. leprae* strain that likely originated in West Africa. The detected human mitochondrial haplogroup L3 is also associated with this geographical region. This suggests that slave trade contributed to leprosy in Suriname.
Limitations: A limited number of skeletons was examined.
Suggestions for further research: Broader review of skeletal collections is advised to expand on diversity of the *M. leprae* aDNA database.

1. Introduction

Leprosy, also called Hansen's disease, results from infection with *Mycobacterium* (*M.) leprae* and may affect skin, peripheral nerves, eyes and nasal mucosa. Left untreated, it can cause neurological signs, disfiguring, stigmatizing malformations of the extremities and face, including alterations of bone tissue and blindness (Britton and Lockwood, 2004). In some parts of the world, *M. lepromatosis* can cause similar disease manifestations (Han et al., 2008; Han and Silva, 2014). Moreover, *M. leprae* and *M. lepromatosis* were recently identified in red squirrels from the British Isles causing lepromatous disease in several animals (Han et al., 2014). The disease was widely feared as being contagious even before the discovery in 1873 of the etiologic bacterium *M. leprae* by Armauer Hansen; from medieval times many societies favoured preventive isolation of leprosy patients (Simons, 1948). The Dutch in their South American colony, Suriname, also took this approach, and laws concerning leprosy detection in the population and isolation of patients were strictly enforced (Snelders, 2013; Lens, 1895;

* Corresponding author.
E-mail address: j.t.van_dissel@lumc.nl (J.T. Van Dissel).
[1] Current address: Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.
[2] Previous work.

https://doi.org/10.1016/j.ijpp.2019.08.001
Received 2 January 2019; Received in revised form 2 August 2019; Accepted 3 August 2019
Available online 17 August 2019

Menke et al., 2009). At the peak of leprosy endemicity in the mid-19th century, approximately 1% of the colony's population was in government-sanctioned isolation in the remote Batavia leprosarium along the Coppename river located far from the capital Paramaribo. Batavia was in use between 1824 and 1897, and after its closure the patients with leprosy were transported to 'Groot Chatillon', along the Suriname river, closer to Paramaribo, which was used between 1897 and 1972 (Lens, 1895; Menke et al., 2009).

Untreated lepromatous patients shed millions of microorganisms daily and people closely involved in their care in leprosaria must have been exposed at a high rate. Not surprisingly, individuals who have frequent and close contact with untreated leprosy patients, like caregivers (Richardus et al., 2005), are at risk of developing the disease. Generally, the care of leprosy sufferers in colonies of Western countries was entrusted to Christian churches (Benjamins and Snelleman, 1914), thus, caregivers in the 18th and 19th centuries were frequently missionaries. One famous catholic missionary was Jozef Deveuster from Belgium, better known as Saint Damien, who worked 16 years in a leprosy colony on the Kalaupapa Peninsula of Hawaii and succumbed there to leprosy-related morbidity in 1889 (Livingstone, 1977). Also, in Suriname several missionaries contracted leprosy during their work, including Johannes Bakker, a priest born in the Netherlands, where human cases of leprosy had essentially disappeared by the 17th century. Bakker worked among leprosy patients in the Batavia leprosarium and died there in 1890 (Vernooij, 2017).

Here, we present the findings from our investigation of Petrus Donders who served as a priest at Batavia and had intensive exposure to leprosy patients under basic conditions for almost 27 years (Vernooij, 2017; Anonymous, 1894). We combined archival research with a macroscopic paleopathological examination and DNA-based testing of his skeletal remains, more than 125 years after his death, to establish evidence on whether he had been infected with *M. leprae*.

As a control, we investigated the remains of two individuals excavated at the cemetery of Batavia where Petrus Donders had been buried initially before being reburied in the Paramaribo basilica. One of these skeletons showed characteristic macroscopic signs of leprosy, whereas the other did not. A positive ancient (a)DNA finding in remains of Petrus Donders or in the control skeletons would represent the first archaeological sample of *M. leprae* isolated in South America. Positive finding of *M. leprae* aDNA and subsequent phylogenetic analysis would contribute to the pressing question of the spread of leprosy worldwide (as raised by, e.g., Monot et al., 2005) and more specifically, the origin of leprosy in Suriname.

2. Materials and methods

2.1. Ethical clearance

We obtained ethical clearance for the excavation and analysis of the skeletal remains of Petrus Donders and the controls from the church authorities (represented by Monsignor De Bekker, Bishop of Paramaribo) who have authority over both the Paramaribo basilica as well as Batavia, from the Suriname government (ref#012-036-3414, represented by Mr Punwasi, Attorney General's Office at the Court of Justice), and from the regional government authorities (ref#1801/13, represented by the District commissioner of the Saramacca district). An announcement of our intention to investigate the physical remains of Petrus Donders for leprosy was published in the magazine of the Friends of Petrus Donders Foundation (March 2012 issue, page 18; Tilburg, the Netherlands); we received no negative reactions. Moreover, the living next of kin of Petrus Donders was contacted and did not object to the research and publication. With respect to the individuals buried at Batavia in the nineteenth century, no formal organization of offspring of leprosy patients of Batavia is registered in Suriname; the Catholic church has ownership of the Batavia premises.

2.2. Archival research

We collaborated with historians at the Anton de Kom University of Suriname in an ongoing historiographic investigation in which the available literature and archival documents on Petrus Donders, present in the Netherlands and Suriname, were systematically studied (Jagdew and Vernooij, 2017). Personal letters have been archived and published separately (Van Dongen and Peeters, 2009). Together with Joop Vernooij, one of us (HEM, dermatologist) systematically screened these letters along with available literature and archival records for issues related to health; in particular for symptoms and signs compatible with leprosy. These were summarized and cross-checked against available reviews (Anonymous, 1894; Van Grinsven, 1923), including ethnohistoric accounts of Petrus Donders collected in the context of his beatification in 1982 (Govers, 1933).

2.3. Paleopathological examination

On 28 September 2013, Petrus Donders' marble sarcophagus in the left wing of the Saint Peter and Paul basilica of Paramaribo was opened and the physical remains were examined on site. Of note, Petrus Donders had initially been buried at the leprosarium, and the removal of his skeletal remains from the cemetery of Batavia and subsequent placement in the Paramaribo basilica has been documented (see Supplement S1). On 26 September 2013, three graves were excavated on the site of the former cemetery of Batavia along the Coppename river (see Supplement Fig. 1). These were the graves of Father Bakker and two adjacent anonymous graves. Father Bakker's tiled grave was marked by a gravestone and excavated to ground water level at 115 cm below the surface; no skeleton was recovered. However, two skeletons were revealed by extending the excavation to the south side of Father Bakker's grave. These unmarked graves each contained one skeleton in a supine and extended position with the arms along the body and the hands on the pelvis and were designated SK1 and SK2. To assess whether or not the skeletons (Petrus Donders, SK1, SK2) showed signs of leprosy infection, we inspected the remains for paleopathological indicators characteristic for leprosy. These included the specific leprosy-induced "rhinomaxillary syndrome", i.e., bilateral symmetrical resorption of the maxillary alveolar process, progressing to loss of the front teeth and a wide nasal aperture. It also included noting subperiosteal new bone disposition of long bones, in particular the distal lower limbs, e.g., tibiae, which occurs in about 70% of leprosy cases (Ortner, 2008; Möller-Christensen, 1961; Zimmermann and Kelly, 1982). Moreover, proximal phalanges, metacarpals and metatarsals of hands and feet were evaluated for pathological changes that included a tapered appearance caused by trauma and infection linked to secondary destruction associated with loss of sensation. Sex was estimated according to the improved recommendations of the Workshop of European Anthropologists (Maat et al., 1980, 2012), making use of anatomical distinctions of the pre-auricular sulcus, greater sciatic notch, pubic angle, overall pelvic form, obturator foramen, ischial body, iliac crest, iliac fossa and pelvic inlet. Age at death was estimated based on skeletal growth (dental eruption, epiphyseal fusion) and by closure of the sphenobasilar synchondrosis, pubic symphyseal face changes, texture changes of the cancellous tissue in the head of the humerus and femur, and closure of endocranial sutures (Maat et al., 1980, 2012).

2.4. Samples of skeletal remains for DNA-based testing

For all three investigated individuals (Petrus Donders, SK1, SK2), samples for DNA-based testing were collected under forensic guidelines to avoid contamination with exogenous DNA. In general, preferential samples for investigating *M. leprae* are bones affected by related lesions. For investigating the human genome, teeth or the petrous bone are preferred. Petrus Donders lost his dentition antemortem and considering the status of his skeleton, sampling of his petrous bone was not

a feasible option because of the risk of severely damaging the skull. The most common locations of *M. leprae*-related bone lesions are the long bones, hands, feet and face (Ortner, 2008; Roffey et al., 2017). Considering the available remains and to ensure the best chance to detect possible persistent remains of *M. leprae*, we selected a proximal hand phalanx and metacarpal bones for analysis from Petrus Donders, SK1 and SK2. Teeth, a facial cranial fragment, and part of an affected tibia, were selected from SK2 who exhibited pathological lesions associated with leprosy.

2.5. DNA extraction

Sample preparation for DNA analysis was conducted at the department of Human Genetics of the Leiden University Medical Center (LUMC). All steps prior to DNA amplification were performed in a laboratory dedicated to aDNA analysis. Extensive precautions were taken during the laboratory work and the analysis and interpretation of the results to avoid and track contamination. First, to remove dirt, the skeletal samples were cleaned with sterile wipes (Microtek). Next, to minimize possible contaminating DNA on the samples, they were placed under UV-C twice for 45 min in two distinct positions. For optimal DNA extraction, the samples were ground into a fine powder using the Mixer Mill 400 (Retsch) in containers with a lining of zirconium oxide and a zirconium oxide bullet. The containers including sample were placed in liquid nitrogen for 20 min. Next, the samples were ground in several 10-second rounds at an intensity of 30 Hz, depending on the size of the sample, with a 20-minute break in liquid nitrogen after the first three rounds. For DNA isolation, up to 0.4 gr powder was used per sample, if available, to which 1.0 ml of 0.5 M EDTA pH 8.0 (Invitrogen) with 5% sarcosyl (Sigma-Aldrich) and 90 µl Proteinase K (Qiagen) was added. This was mixed overnight in a thermal mixer at 1000 rpm and 56 °C. The samples were then centrifuged at 13,000 rpm for three minutes. The supernatant was purified with the QIAquick PCR Purification kit® (Qiagen), following the manufacturer's centrifuge protocol. The purified aDNA was finally eluted in 40–80 µl of nuclease- free water (Qiagen), depending on the input amount of sample. During this process, extraction blanks were also created using only the reagents and eluted in 40 µl of water and processed in an identical manner as the samples.

2.6. PCR amplification of M. leprae RLEP

To detect the presence of *M. leprae* aDNA in samples from the remains, a PCR amplifying an *M. leprae*-specific repetitive sequence was performed as described (Tió-Coma et al., 2019; Donoghue HD, et al., 2001). This was completed by PCR amplification of a 129 bp sequence of *M. leprae* repetitive element (RLEP) (see Supplement Table 2 and Supplement S3.1). PCR to detect *M. lepromatosis* DNA (LPM244) was completed following the protocol reported by Tió-Coma et al. (2019) and Donoghue et al. (2001).

2.7. Genotyping M. leprae DNA

To determine the SNP type (1, 2, 3, or 4) of *M. leprae*, nucleotide positions 14,676 (Locus 1), 1,642,879 (Locus 2) and 2,935,693 (Locus 3) were amplified and sequenced as previously described with minor modifications (Tió-Coma et al., 2019; Donoghue et al., 2001; Monot et al., 2009) (see Supplement 3.2).

2.8. Quality assessment of human DNA and autosomal microsatellite and mitochondrial SNP typing

To assess the quality of the human aDNA in the samples and to determine the sex of the individuals, we measured the concentration of human aDNA including Y chromosomal aDNA. We also typed 15 autosomal short tandem repeats (STRs) and a marker for the amelogenin gene, and mitochondrial single nucleotide polymorphisms. The concentration of human aDNA, including the Y chromosome, was tested with the Quantifiler® Duo kit (Applied Biosystems) according to the manufacturer's instructions with 2 µl aDNA extract. Based on the results, the input volume was determined for further PCRs. The amelogenin gene and 15 autosomal Short Tandem Repeat (STR) markers were typed with the PowerPlex® ESX 16 and/or PowerPlex® ESI 16 systems (Promega). The PCRs were performed according to the manufacturer's directions, but with half the volumes for reagents and 5 µl aDNA extract. At each PCR run, one positive control and at least one negative control were included. The PCRs were carried out on a GeneAmp® PCR System 9700 (Applied Biosystems). The PCR products were analysed on an ABI PRISM® 3100 or 3500 XL Genetic Analyzer (Applied Biosystems) and with GeneMarker® software, version 1.75 or 2.4.0 (SoftGenetics LLC®).

An individual is considered as male when the presence of a Y chromosome is observed with the Quantifiler® Duo kit and/or the PowerPlex® ESX 16 or PowerPlex® ESI 16 systems. In the absence of a Y chromosome, an individual is interpreted as female, but it is also possible that the Y chromosome is too degraded to be observed. Therefore, if alleles are obtained with a PowerPlex® ESX 16 or PowerPlex® ESI 16 PCR for less than five markers, and there is no indication of the presence of the Y chromosome, sex is not determined, due to possible degradation issues. An individual is regarded as female when there are no indications for the presence of a Y chromosome, and at least for one PCR with the PowerPlex® ESX 16 or PowerPlex® ESI 16 systems alleles are obtained for at least five markers. It is possible that a deletion on the male amelogenin gene can be mistaken for the shorter female variant. However, the prevalence of such a deletion is usually very low, e.g. 0-0.018% in European men (Steinlechner et al., 2002).

Mitochondrial haplogroup reconstruction was based on single nucleotide polymorphisms (SNPs) that were typed in 42 fragments on the mitochondrial genome (see Supplemental Table 1) (Van Der Gaag et al., 2016). PCR products were sequenced on a MiSeq instrument (Illumina) and raw data was analysed with FDSTools with the following settings: minimum 10 reads per sequence, minimal 10% of the highest allele per marker and minimal 5 reads in both orientations (Hoogenboom et al., 2017). Haplogroups were defined with Haplogrep 2.0 software, based on Phylotree v17. SNPs are compared to the revised Cambridge Reference Sequence (rCRS) (Kloss-Brandstätter et al., 2011; Andrews et al., 1999).

3. Results

3.1. Archival research

Within a few years after his death, Redemptorist colleagues of Petrus Donders began to document his life and work. They collected the primary source documents concerning his work at Batavia and other places in Suriname that were subsequently published in the early twentieth century, and reissued in the context of his beatification in 1982 (Van Grinsven, 1923; Govers, 1933). Petrus Donders' personal letters and correspondence were also published (Van Dongen and Peeters, 2009; Govers, 1933). A comprehensive and systematic historiographic review of the completeness of these materials has been performed (Jagdew and Vernooij, 2017). Screening the primary source documents, we found no indication nor references of possible manifestations of leprosy (e.g., skin discoloration, anaesthesia of skin, etc.) in his personal letters, church archives, and biographies celebrating his life. In one of the publications, his colleagues in Paramaribo summarized his condition during his time in Batavia as follows, "His health is excellent. His physical constitution is of a wonderful toughness, resistant to any sacred foolishness. He fasts regularly three times a week. He is a person small in shape, very skinny, with white hair, without teeth, and bent a little. His mental power is rather mediocre, his memory is good, his judgment is correct and clear." (Govers, 1933).

Fig. 1. Content of the casket from the marble sarcophagus in the burial chapel in the left wing of the Paramaribo basilica. The upper image displays two metal boxes in the casket housing the remains of Donders' Redemptorist gown (left), and documents (right) detailing prior reburials and previous inspections of his remains. The lower image shows the skull with closed and atrophied alveolar sockets, indicating ante mortem tooth loss.

Furthermore, it is documented that Petrus Donders had yellow fever in 1851, and when elderly, he had lost all his teeth and likely suffered from recurring kidney infections (Jagdew and Vernooij, 2017; Govers, 1933). Additional background information on Petrus Donders, his burial in Batavia in 1887, the subsequent exhumation of his physical remains in 1900, and placement in the basilica in Paramaribo is provided in the Supplement S1.

3.2. Paleopathological examination

A wooden casket about 80 by 40 cm from inside his tomb in the basilica contained an almost complete skeleton (Fig. 1); two metal boxes in the casket housed the remains of his Redemptorist gown and documents detailing prior reburials (in 1900, 1921 and 1982) and the inspection of his remains completed in 2010. The sex assessment of the pelvis and skull associated with Petrus Donders yielded a score of +0.1 (slightly masculine; hyper masculine = +2.0) and –0.2 (slightly feminine, hyperfeminine = –2.0), respectively. Overall, this describes an uncertain sex. Age at death could only be assessed by scoring the degree of closure of the ectocranial sutures due to the preservation of the remains. All were nearly or completely closed, indicating a moderate to old age at death.

The skeleton did not show paleopathological characteristics of leprosy as detailed under the Methods section above. All alveolar sockets were closed and atrophied, confirming complete ante mortem tooth loss (Fig. 1). More details on the paleopathological examination are provided in the Supplemental Materials S2. The skeleton of Petrus Donders, which had been recovered and cleaned in 1900 AD (13 years after his burial) was almost complete, intact and well preserved. The SK1 and SK2 skeletons were also complete and intact, but very fragile, so sex and age at death scoring was performed in situ. With respect to the two skeletons excavated and inspected at Batavia (Fig. 2), skeleton SK1 was a child about 4 (± 1) years of age at time of death, according to the phase of dental eruption and non-fusion status of the neuro-central

Fig. 2. Two skeletons excavated from the cemetery of the leprosarium Batavia in Suriname. A) SK1 designated as "I" in the photograph and SK2 designated as "2 II" in the photograph; B) details of both tibia and left fibula of SK2; open arrows point to pitting and the irregular surface of these long bones; C) portion of the left tibia of SK2 displaying the pitting and irregularity of the surface with fine longitudinal striated subperiosteal bone deposition; D) cranium of SK2. Bone lesions in image (C) may be associated with chronic infectious diseases like tuberculosis but are compatible, as well, with advanced leprosy. Both the tibial bone fragment shown in (C) as well as a maxillary sample from the fragile skull (inset D) tested positive for *M. leprae* aDNA.

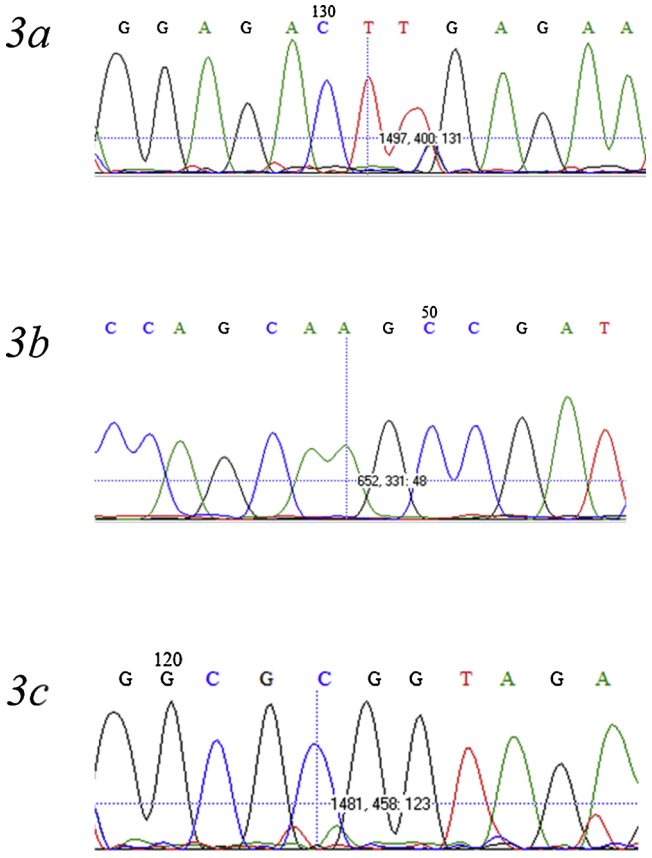

Fig. 3. The three relevant SNP loci to genotype *M. leprae*: 3a) Locus 1 SNP14676 (in Genbank Br2493 14672): GGAGAC**T**TGAGAA, with size of 194 bp; 3b) Locus 2 SNP1642875 (in Genbank Br2493 1642808): ATCGGC**T**TGCTGG, showing in reverse: CCAGCA**A**GCCGAT, with size of 122 bp; 3c) Locus 3 SNP2935685 (in Genbank Br2493 2935583): GGCG**C**GGTAGA, with size of 180 bp.The findings demonstrate that the SNP type of *M. leprae* of SK2 is genotype 4 (i.e., TTC).

junction of its vertebrae. There were no signs of skeletal changes associated with leprosy. Skeleton SK2 was an adolescent with an in situ assessed developmental age of about 15–18 years based on the non-fused epiphyseal discs of the tibias and of the medial ends of the clavicles, fusing vertebral endplate epiphyses and a fully erupted dentition. Probably due to bioturbation the third molars seemed to be in the occlusal plane, since their roots had only developed to half their length (the latter indicated a developmental age of 12–18 years) (Ubelaker, 1989). Other age assessment methods could not be applied due to the fragility of the bones.

SK2 bone lesions were compatible with advanced leprosy, i.e., subperiosteal new bone disposition of long bones, in particular the distal lower limbs (Fig. 2C). The reactive bone formation was designated macroscopically as woven bone; no microscopic examinations were completed. Moreover, there was partial bone resorption of the inferior margin of the nasal aperture and the turbinate bone tissue in the sino-maxillary region, and porotic changes with resorption of some of the distal ends of the pedal metatarsals and phalanges.

3.3. DNA analysis amelogenin marker

For Petrus Donders and SK1, no human aDNA could be measured with the quantification method, and for SK2 the maximum was 6 pg per µl. For Petrus Donders, only three of 15 autosomal STRs alleles could be typed (D16S539, D18S51 and TH01) and no results were obtained for the amelogenin marker. For SK1 and SK2, alleles for all 15 STRs and the amelogenin marker could be typed. Based on these findings, it can be concluded that the human aDNA was poorly preserved in the analysed remains of Petrus Donders when compared with SK1 and SK2. Moreover, SK1′s and SK2′s remains were interpreted as female.

3.4. M. leprae aDNA and genotyping

Of the assessed samples from the three skeletal remains, only SK2 tested positive in the RLEP PCR for *M. leprae*. None of the samples tested positive for *M. lepromatosis* aDNA. We used aDNA of SK2 that was confirmed as PCR-positive for *M. leprae*-specific repetitive element (RLEP) to determine SNP type. The diagnostic loci for determining the SNP-type according to those described by Monot and colleagues (Monot et al. 2004), namely nucleotide positions 14,676 (Locus 1), 1,642,879 (Locus 2) and 2,935,693 (Locus 3) were analysed by stringent

individual PCR and Sanger sequencing of the PCR products. For SK2, the SNPs were found to be T, T and C, respectively (Fig. 3), which is the classical pattern of an *M. leprae* SNP type 4 occurring in West Africa and currently in South America (Monot et al., 2005; Monot et al., 2009).

Considering this outcome, we also sought to define the mitochondrial haplogroup of skeleton SK2 to obtain some indication of the genetic ancestry (Supplemental Table 2). SK2 yielded mitochondrial haplogroup L3 (based on the SNPs 1438 G, 2706 G, 8701 G, 10873C, 11719A, 12705 T, 14766 T compared to the revised Cambridge reference sequence (Andrews et al., 1999), Haplogroup 2 rank: 1), which is common in modern indigenous populations in Africa and the Middle East.

4. Discussion

By combining archival research, paleopathological inspection of excavated skeletal remains, and aDNA-based testing of bone samples, we did not find any evidence that the priest Petrus Donders suffered from leprosy, despite his almost 27-year stay among leprosy patients at the leprosarium Batavia in Suriname during the late 19th century. However, in one of the control samples from another skeleton in the cemetery, we uncovered evidence of the presence of *M. leprae* aDNA through specific RLEP PCR and were able to increase the resolution to SNP type via PCR and sequencing. The genotype suggests that it evolved in West Africa, consistent with the hypothesis that the slave trade with West Africa contributed to leprosy in Suriname.

To assess whether our approach was sensitive enough to detect *M. leprae* aDNA in skeletal remains and to put our findings on Petrus Donders in context, we examined and sampled two skeletons as controls from the same cemetery of Batavia where Petrus Donders initially had been buried. SK2 displayed characteristic paleopathological lesions consistent with leprosy; aDNA-based testing of bone samples by RLEP PCR unequivocally confirmed the presence of *M. leprae* aDNA. Stringent PCRs of SNP loci and Sanger sequencing of the PCR products were applied to type the *M. leprae* strain as genotype 4. SK1 lacked bone lesions compatible with leprosy and tested negative in the aDNA-based testing, as had the bone samples from Petrus Donders. These findings demonstrate that the combined approach of macroscopic inspection and aDNA-based testing was sensitive and specific for identifying *M. leprae* in the skeletal remains. The lack of evidence of *M. leprae* aDNA in the remains of Petrus Donders should not be taken as definite proof that his samples were truly negative since the poor preservation of the human aDNA in his skeletal remains cannot exclude the possibility that *M. leprae* aDNA had degraded, preventing detection. Degraded aDNA fragments may be short in comparison to the length of the PCR products, which require > 100 bp. On the other hand, it has often been observed that mycobacterial aDNA persists longer and is more resilient to degradation than human aDNA (Monot et al., 2005; Bos et al., 2014). Other possible explanations for potentially false negative findings are that there was not enough aDNA available in the tested samples to be detected by the methods we applied, or that a suboptimal skeletal element was chosen for sampling, or any combination of the above.

Thus, although we were unable to conclude definitely that Donders' samples were negative, just how significant would it be if the skeletal remains of Petrus Donders lacked evidence of leprosy? Under circumstances of relatively short (e.g., a few years) exposure, missionaries' risk of acquiring leprosy in a leprosarium were relatively small, e.g., about 1.3% as reported in the mid-20th century (Gray and Dreisbach, 1961) and the recognizable spread of infection to the bones often does not allow for documentation long after death (Britton and Lockwood, 2004). However, this low percentage of disease incidence may be different in circumstances of extended and intense contact. In a recent analysis of 28 reports on *M. leprae,* which explored the transmissibility from an affected individual to his or her spouse and offspring before antibiotic treatment became standard therapy for patients, 2.5–46% of the children in a household and 0.3–32% of partners developed manifest signs of leprosy infection (Joyce, 2012; Bechelli and Guinto, 1970; Worth, 1972). The situation may well have been worse – and incidence rates higher – in previous centuries, before a transmissible microbial agent as the cause of disease was identified and hygienic practices to reduce exposure were put into effect. Unaware of the route of transmission and contagiousness of various manifestations of leprosy, primary caregivers were particularly at risk. Indeed, a number of priests, including Bakker, and nuns, who worked at the leprosaria of Suriname from 1830 onwards and who were closely involved in the care of leprosy patients, developed leprosy (Vernooij, 2017). Petrus Donders, who lived in close contact with leprosy patients at Batavia alongside priest Bakker for many years, undoubtedly exposed himself to *M. leprae*, but this did not result in manifest leprosy or post-mortem molecular evidence of *M. leprae* infection in his bones. It is well known that the host immune system determines the clinical outcome of leprosy, and that even when exposed and infected, a strong cell-mediated response may lead to the elimination of *M. leprae* from soft tissues, leading to recovery or a relatively mild condition of tuberculoid leprosy (Britton and Lockwood, 2004). Unfortunately, the low coverage of human aDNA based on shotgun sequencing results from Petrus Donders remains did not allow further exploration of possible host immune factors involved in host resistance to *M. leprae*, e.g., of HLA susceptibility loci (Krause-Kyora et al., 2018).

Our findings on the phylogenetic analysis of the first *M. leprae* aDNA from skeletal remains from South America have consequences beyond use as a contextual and positive control. Genotyping *M. leprae* aDNA in bones of SK2 is relevant to address the on-going debate on the origin of leprosy in Suriname, and revealed the likely geographical origin of the strain (Benjamins and Snelleman, 1914; Monot et al., 2005, 2009). Using genome scale comparison, *M. leprae* has shown no major variations in the past 1000 years (Schuenemann et al., 2013). Phylogenetic analysis of *M. leprae* shows a good correlation between genotypes of *M. leprae* and the geographical origin of the patients (Pushpendra and Cole, 2011; Reibel et al., 2015; Benjak et al., 2018). Earlier, just four phylogenetic groups, SNP-types 1 to 4, each subdivided into three to five subtypes, were distinguished and these lineages have been linked to specific global regions (Roffey et al., 2017; Monot et al., 2009; Benjak et al., 2018). Recently, however, a lineage "5″ (in fact, the new branch or SNP type 3I is called branch 0 or SNP type 3I) was separated out of the other four lineages already defined (Schuenemann et al., 2017; Mendum et al., 2018), thus expanding Monot's (2009) original classification. Current residence and human migration in the past (including the slave trade) have been tied to the evolution of *M. leprae* genotypes (Roffey et al., 2017; Monot et al., 2009; Donoghue et al., 2015; Grimm, 2005). Leprosy in the Americas has been found to be of European origin (Schuenemann et al., 2013), but earlier, Monot et al. (2005) showed that leprosy in the French West Indies and Brazil is also of Asian and African origin. Some hold that leprosy was introduced into Suriname by European immigrants; many of them were Portuguese Jews who had lived in Brazil before coming to Suriname; others hold that leprosy was introduced in Suriname through the slave trade from West Africa (Benjamins and Snelleman, 1914). The population of Suriname in the 18th century included indigenous people and individuals of European (Dutch, Portuguese, German, English and French) and African origin. Chinese immigrants arrived in Suriname in 1853, and after the abolition of slavery in 1863, migrants from India and Indonesia entered the country as contract laborers (Benjamins and Snelleman, 1914). The leprosarium Batavia was not closed until 1897. In the 1880s, colonial reports mentioned that sufferers of leprosy from Chinese and British Indian migrant groups had been admitted to Batavia (Snelders, 2017). Indeed, late 19th century maps of Batavia display the presence of dedicated leprosy barracks assigned to British Indian residents (Map of leprosarium Batavia, St Agatha, nr 9301). Genotyping of the *M. leprae* aDNA from SK2, based on the most relevant SNPs, was consistent with a branch 4 strain, which includes type 4 strains. These strains have been found in West Africa (e.g., S13 (4 N) and S14 (4O) from Mali) and

countries linked to this region by the slave trade (e.g., Brazilian 4 P strain) (Schuenemann et al., 2013; Benjak et al., 2018; Donoghue et al., 2015). The mitochondrial haplogroup L3 of the skeleton from which *M. leprae* was isolated revealed that her genetic ancestry at least partly rested within the indigenous populations of Africa and/or the Middle East, which is consistent with that of the majority of individuals with leprosy living in the leprosarium Batavia (Lens, 1895) and suggests an ancestry of SK2 related to these regions (Harich et al., 2010). Obviously, our findings from one single skeleton – as far as we know the first *M. leprae* aDNA isolated from skeletal remains in South America – cannot resolve the debate of the various routes by which *M. leprae* may have reached Suriname. However, the presence of SNP type 4 aDNA identified in a skeleton of over a hundred years old from a former Surinamese leprosarium and the reported finding of SNP type 1 or 2 *M. leprae* DNA, i.e. of strains that occur predominantly in Asia and East Africa, in Suriname soil samples (Tió-Coma et al., 2019), suggests the co-existence in Suriname of multiple *M. leprae* strains originating from various geographical areas over the past centuries.

In conclusion, by taking a tripartite approach, we found no evidence that the priest Petrus Donders, beatified by the Roman Catholic Church, suffered from leprosy, despite having worked among leprosy patients at the leprosarium of Batavia in Suriname for almost 27 years. Moreover, we identified *M. leprae* aDNA from a skeleton with macroscopic signs of leprosy infection and buried at the leprosarium, a first in South America, and provided evidence that suggests at least some of the leprosy cases in Suriname can be linked to the slave trade with West Africa. Our findings show how aDNA-based testing on archaeological human remains can provide important information beyond traditional paleopathological research, allowing investigators to reconstruct the evolution of past pathogens like *M. leprae* and to address the association between the presence of macroscopic lesions associated with leprosy and recovered aDNA.

Author contributions

JTvD, TP and HEM initiated the study; JTvD and TP supervised the excavation, did the overall analysis and wrote the first version of the manuscript; GM, HEM and MRA helped perform the excavation and commented on the manuscript versions; AG, MTC, EA and JFJL did the molecular experiments on bone samples, the bioinformatics analysis, and commented on the manuscript versions.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors have no Competing Interests to declare.

Acknowledgements

We are grateful to Julia Challinor for her English manuscript correction service. We thank Boris Everts for the photographs of the Batavia excavation.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijpp.2019.08.001.

References

Andrews, R., et al., 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat. Genet. 23, 147–149.
Anonymous, 1894. In: Donders, P., Romme, J.B. (Eds.), Twee missionarissen onder de melaatschen en indianen. Drukkers van Z.H. den Paus en van het Bisdom, Roermond Romen JJ & Zonen.
Bechelli, L.M., Guinto, R.S., 1970. Some recent laboratory findings on *Mycobacterium leprae*. Implications for the therapy, epidemiology and control of leprosy. Bull. WHO 43, 559–569.
Benjak, A., et al., 2018. Phylogenomics and antimicrobial resistance of the leprosy bacillus Mycobacterium leprae. Nat. Commun. 9, 352. https://doi.org/10.1038/s41467-017-02576-z.
Benjamins, H.D., Snelleman, J.F. (Eds.), 1914. Encyclopaedie van Nederlands West-Indie. Nijhof M,' s Gravenhage, and Brill EJ, Leiden.
Bos, K.I., et al., 2014. Pre-columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. Nature 514, 494–497.
Britton, W.J., Lockwood, D.N., 2004. Leprosy. Lancet 363, 1209–1219.
Donoghue, H.D., et al., 2001. PCR primers that can detect low levels of *Mycobacterium leprae* DNA. J. Med. Microbiol. 50, 177–182.
Donoghue, H.D., et al., 2015. A migration-driven model for the historical spread of leprosy in medieval Eastern and Central Europe. Infect. Genes Evol. 31, 250–256.
Govers, N., 1933. Leven van den eerbiedwaardigen dienaar Gods Petrus Donders C.ss.R.: apostel der melaatschen in Suriname. Publisher: Secretariaat van de missie van Suriname, Redemptoristen.
Gray, H.H., Dreisbach, J.A., 1961. Leprosy among foreign missionaries in northern Nigeria. Int. J. Leprosy 29, 279–290.
Grimm, D., 2005. Global spread of leprosy tied to human migration. Science 308, 936–937.
Han, X.Y., et al., 2008. A new Mycobacterium species causing diffuse lepromatous leprosy. Am. J. Clin. Pathol. 130, 856–864.
Han, X.Y., Silva, F.J., 2014. On the age of leprosy. PLoS Negl. Trop. Dis. 8, e2544. https://doi.org/10.1371/journal.pntd.0002544.
Han, X.Y., et al., 2014. Analysis of the leprosy agents *Mycobacterium leprae* and *Mycobacterium lepromatosis* in four countries. Am. J. Clin. Pathol. 142, 524–532. https://doi.org/10.1309/ajcp1glcbe5cdzrm.
Harich, N., et al., 2010. The trans-Saharan slave trade; clues from interpolation analyses and high-resolution characterization of mitochondrial DNA lineages. BMC Evol. Biol. 10, 138–142.
Hoogenboom, J., et al., 2017. FDS Tools: a software package for analysis of massively parallel sequencing data with the ability to recognise and correct STR stutter and other PCR or sequencing noise. Forensic Sci. Int. 27, 27–40 Genetics.
Jagdew, E., Vernooij, J., 2017. Peerke Donders en Batavia. Een historiografisch overzicht, 1842-2016. Acad. J. Suriname 8, 733–754.
Joyce, M.P., 2012. Historic Aspects of Human Susceptibility to Leprosy and the Risk of Conjugal Transmission 107. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, pp. 17–21.
Kloss-Brandstätter, A., et al., 2011. HaploGrep: a fast and reliable algorithm for automatic classification of mitochondrial DNA haplogroups. Hum. Mutation 32, 25–32.
Krause-Kyora, B., et al., 2018. Ancient DNA study reveals HLA susceptibility locus for leprosy in medieval Europeans. Nat. Commun. 9, 15–69.
Lens, Th., 1895. Lepra in Suriname. Elsevier's Illustrated Monthly, pp. 521–552.
Livingstone, E.A., 1977. The Concise Oxford Dictionary of the Christian Church, third edition. Oxford University Press.
Maat, G.J.R., et al., 1980. Manual for the physical anthropological report. Barge's Anthropologica nr 6, 7th ed., Leiden. from: Workshop by European Anthropologists for age and sex diagnosis of skeletons. J. Hum. Evol. 9, 517–549.
Maat, G.J.R., et al., 2012. Manual for the Physical Anthropological Report. Barge's Anthropologica Nr. 6. 7th ed. Leiden, Amsterdam.
Map of the leprosarium Batavia, 2019. Archive: Heritage Collection of Dutch Monastery Life 'St Agatha'. Inventory Nr. 9301.
Mendum, T.A., et al., 2018. The genome sequence of a SNP type 3K strain of *Mycobacterium leprae* isolated from a 7th century Hungarian case of lepromatous leprosy. Int. J. Osteoarchaeol. 28, 439–447.
Menke, H., et al., 2009. Coping with leprosy in the Dutch West Indies in the 19th century; opposing but meaningful views from Suriname. Studium 2, 65–77.
Möller-Christensen, V., 1961. Bone Changes in Leprosy. Munksgaard, Copenhagen.
Monot, M., et al., 2005. On the origin of leprosy. Science 308, 1040–1042.
Monot, M., et al., 2009. Comparative genomic and phylogeographic analysis of Mycobacterium leprae. Nat. Genet. 41, 1282–1289.
Ortner, D., 2008. Skeletal manifestations of leprosy. In: Magilton, J., Lee, F., Boylston, A. (Eds.), Lepers outside the Gate: Excavations at the Cemetery of the Leper Hospital of St James and St Mary Magdalene, Chichester, 1986-1993. CBA Research Report No. 158. Council for British Archeology, York, pp. 198–207.
Pushpendra, S., Cole, S.T., 2011. *Mycobacterium leprae*: genes, pseudogenes and genetic diversity. Future Microbiol. 6, 57–71.
Reibel, F., et al., 2015. New insights into the geographic distribution of *Mycobacterium leprae* SNP genotypes determined for isolates from leprosy cases diagnosed in metropolitan France and French territories. PLoS Negl. Trop. Dis. 9, 0004141. https://doi.org/10.1371/journal.pntd.0004141.
Richardus, J.H., et al., 2005. Close contacts with leprosy in newly diagnosed leprosy patients in a high and low endemic area: comparison between Bangladesh and Thailand. Int. J. Lepr. Other Mycobact. Dis. 73, 249–257.
Roffey, S., et al., 2017. Investigation of a medieval pilgrim burial excavated from the leprosarium of St Mary Magdalen Winchester, UK. PLoS Negl. Trop. Dis. 11, e0005186. https://doi.org/10.1371/journal.pntd.0005186.
Schuenemann, V.J., et al., 2013. Genome-wide comparison of medieval and modern Mycobacterium leprae. Science 341, 179–183.
Schuenemann, V.J., et al., 2017. Ancient genomes reveal a high diversity of *Mycobacterium leprae* in medieval Europe. PLoS Pathog. 14, e1006997.
Simons, R.D.G., 1948. Modern conceptions concerning the compulsory isolation of lepers.

Proc. R. Soc. Med. 41, 751–754.
Snelders, S., 2013. Leprosy and slavery in Suriname: godfried Schilling and the framing of a racial pathology in the eighteenth century. Soc. Hist. Med. 26, 432–450.
Snelders, S., 2017. Leprosy and Colonialism, Suriname under Dutch Rule, 1750-1950. Manchester University Press, Manchester.
Steinlechner, M., et al., 2002. Rare failures in the amelogenin sex test. Int. J. Legal Med. 116, 117–120.
Tió-Coma, M., et al., 2019. Detection of *Mycobacterium leprae* DNA in soil: multiple needles in the haystack. Sci. Rep. 9, 3165–3168. https://doi.org/10.1038/s41598-019-39746-6.
Ubelaker, D.H., 1989. Human Skeletal Remains. Excavation, Analysis Interpretation. Taraxacum, Washington.
Van Der Gaag, K., et al., 2016. Genotyping Y-chromosomal and mitochondrial single nucleotide polymorphisms in degraded DNA samples using massive parallel sequencing. Momento mori. Een archeologische opgraving rondom de St. Plechelmuskerk, Oldenzaal. Deel II. ADC Monografie 21 / ADC Rapport 3919.
Van Dongen, A., Peeters, R., 2009. Peerke Donders, een leven in brieven. Stadsmuseum Tilburg, Tilburg. .
Van Grinsven, C.fr, 1923. Portretten van den Eerb. Petrus Donders. Rooms Leven.
Vernooij, J., 2017. Lepra en Katholieke kerk in Suriname (19e eeuw). Een korte historie over de relatie lepra en de Rooms-Katholieke kerk in Suriname. Acad. J. Suriname 8, 762–770.
Worth, R.M., 1972. The risk of transmission from lepromatous leprosy cases under therapy. Bull. WHO 46, 853–854.
Zimmermann, M.R., Kelly, M.A., 1982. Atlas of Human Paleopathology. Praeger, New York.