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Origin and spread of leprosy in Suriname. A historical and biomedical study

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The new world was considered free of leprosy before the arrival of Europeans. In Suriname, historical migration routes suggest that leprosy could have been introduced from West Africa by the slave trade, from Asia by indentured workers, from Europe by the colonizers, and more recently by Brazilian gold miners. Previous molecular studies on environmental and ancient samples suggested a high variability of the strains circulating in the country, possibly resulting from the various migration waves. However, a current overview of such diversity in humans still needs to be explored. The origin and spread of leprosy in Suriname are investigated from a historical point of view and by strain genotyping of *Mycobacterium leprae* from skin biopsies of 26 patients with multibacillary leprosy using PCR-genotyping and whole-genome sequencing. Moreover, molecular signs of resistance to the commonly used anti-leprosy drugs i.e. dapsone, rifampicin and ofloxacin, were investigated. Molecular detection was positive for *M. leprae* in 25 out of 26 patient samples, while *M. lepromatosis* was not found in any of the samples. The predominant *M. leprae* strain in our sample set is genotype 4P (n=8) followed by genotype 1D-2 (n=3), 4N (n=2), and 4O/P (n=1). Genotypes 4P, 4N, 4O/P are predominant in West Africa and Brazil, and could have been introduced in Suriname by the slave trade from West Africa, and more recently by gold miners from Brazil. The presence of the Asian strains 1D-2 probably reflects an introduction by contract workers from India, China and Indonesia during the late 19th and early 20th century after the abolition of slavery. There is currently no definite evidence for the occurrence of the European strain 3 in the 26 patients. Geoplotting reflects internal migration, and also shows that most patients live in and around Paramaribo. A biopsy of one patient harbored two *M. leprae* genotypes, 1D-2 and 4P, suggesting co-infection. A mutation in the dapsone resistance determining region of *folP1* was detected in two out of 13 strains for which molecular drug susceptibility was obtained, suggesting the circulation of dapsone resistant strains.

KEYWORDS

leprosy, genotypes, distribution, Suriname, dapsone resistance, *M. leprae*, historical migration

Introduction

The Americas are considered free of leprosy prior to the arrival of Europeans (1).

In Suriname, a country that is part of the Guiana Shield located in the northern part of South America, the disease was first described in the mid-18th century. From 1790 until the second half of the 20th century, leprosy patients were legally confined in leprosaria, accounting for 1% of the population in the middle of the 19th century (2, 3). At the beginning of the 20th century, 2.5-3% of the population of the capital Paramaribo suffered from leprosy (4). Dapsone (DDS) was introduced in 1946 and resulted in a sharp decline in the incidence of the disease and the closure of leprosy asylums in the 1960s and 1970s (5). Relapses after initial improvement by dapsone therapy were reported as early as 1955, hypothesizing a possible bacterial resistance, but this has never been proven to date (6). Multiple drug therapy (MDT: a combination of dapsone, clofazimine and rifampicin) was introduced in the 1980s and contributed to the rapid decrease in the number of new cases in Suriname (7). However, leprosy is still present in the country, and a steady average of 20 to 25 new cases annually in a population of around 600,000 inhabitants is reported (8, 9).

Leprosy is mainly caused by the non-cultivable pathogen *Mycobacterium leprae* and, to a lesser extent, *Mycobacterium lepromatosis* (10, 11). *M. leprae* shows low genetic variability between strains from different locations. This created the basis for the establishment of a robust genotyping system characterized by four single nucleotide polymorphism (SNP) types (1–4) and 16 SNP subtypes (A–P) used to retrace local and large scale transmission of the pathogen (1, 12, 13). The close relationship between human migration and the introduction and spread of infectious diseases, a well-documented phenomenon, is particularly evident for *M. leprae* (14, 15). Indeed, the introduction of the pathogen in the Americas is ascribed to the first Europeans who settled in the new world (1, 16).

Suriname has a rich history of migration waves that began in the early 17th century. European settlers, predominantly Dutch and Portuguese Jews, played a significant role in establishing a plantation economy (17). The workforce on the plantations consisted of enslaved West-Africans, many of whom escaped to the hinterland, establishing Maroon communities (18). Slavery was abolished in 1863, leading to the recruitment of indentured workers from China, British India (now India) and the Dutch East Indies (now Indonesia) in the period 1860s-1920s to replace the formerly enslaved West-Africans (19). The diverse origin of the population suggests that leprosy may have been introduced in the country at different times and from different countries. Previous research reported the presence of a *M. leprae* strain harboring genotype 4 in DNA extracted from the bones of a West-African adolescent buried at the former leprosarium in the 19th century. Moreover, *M. leprae* genotype 1 or 2 was identified in DNA extracted from soil samples collected around armadillo burrows in two former leprosaria in Suriname (20, 21). These findings indicate a high variability of the *M. leprae* strains circulating in the country, possibly resulting from the various immigration waves. However, no information is currently available regarding the genetic diversity of *M. leprae* strains in contemporary human samples within the country.

Consequently, solid biological evidence supporting a multi-ethnic origin of leprosy in Suriname is lacking.

This paper aims to investigate the origin and spread of leprosy in Suriname by genotyping of *M. leprae* strains isolated from skin biopsies of multibacillary (MB) patients from various ethnic backgrounds. Additionally, it explores the molecular resistance to commonly used anti-leprosy drugs, namely dapsone, rifampicin and ofloxacin.

Materials and methods

Patients and clinical samples

A total of 27 formalin fixed biopsies that were collected between 2014 and 2019 from skin lesions of 26 untreated MB patients diagnosed at the Dermatology Service in Paramaribo, were recovered from the Department of Pathology of the Academic Hospital in Paramaribo. Examination of patients, clinical diagnosis and collection of epidemiological data were performed by the Dermatology Service in Paramaribo. Leprosy patients were classified according to the Ridley-Jopling classification (22). Each patient's self-reported ethnicity that had been recorded was used for the study. Biopsies were sent to Leiden University Medical Center where DNA extraction, molecular identification of *M. leprae* and *M. lepromatosis* by qPCR and strain genotyping by PCR-sequencing were performed.

DNA extraction

DNA isolation from paraffined biopsies

DNA was isolated using QIAamp UCP Pathogen Mini Kit (Qiagen) as per the manufacturer's instructions with additional modifications. The excess of paraffin surrounding the biopsies was removed using sterile surgical blades. To remove the remaining paraffin, tissues were transferred to 1,5 ml tubes (Eppendorf) and incubated in 1 ml of xylene for 30 minutes at room temperature. After centrifugation at 14,000 rpm for 5 minutes, supernatant was removed and xylene extraction was repeated as described above. Biopsy tissues were washed twice by adding 1 ml of absolute ethanol and centrifuged for 5 minutes at 14,000 rpm. To remove the ethanol, tubes were incubated with an opened cap at 37°C for 15 minutes. The biopsies were cut into thin slices using sterile surgical blades and transferred to new microtubes (Sarstedt) containing 400 µl buffer ATL (provided in Qiagen kit) and three sterile 3 mm glass beads (VWR). Samples were homogenized twice in a Precellys 24 tissue homogenizer (Bertin) at 5000 rpm for 45 seconds with 5 minutes of incubation on ice in between. Next, 20 µl of proteinase K (provided in the Qiagen kit) was added and tubes were incubated at 55°C with continuous stirring at 1300 rpm for 2 hours. After centrifugation for 1 minute at 14,000 rpm supernatants were transferred to new microtubes containing 250 µl of 0,1 mm

Zirconia beads (Biospec) and homogenized in the tissue homogenizer as described above. To remove the beads, tubes were centrifuged at 14,000 rpm for 1 minute and supernatants were transferred to new microtubes. Subsequently, 20 µl of proteinase K was added prior to incubation at 55°C with constant stirring at 1,300 rpm for 30 minutes. Then 200 µl APL2 buffer (provided in the kit) was added and tubes were incubated at 70°C with constant stirring at 1300 rpm for 10 minutes. After absolute ethanol precipitation (300 µl), column extraction was performed according to the manufacturer's instructions.

Molecular identification of *M. leprae* and *M. lepromatosis*

RLEP qPCR was performed on isolated DNA samples as previously described (23). The mix included 12.5 µl TaqMan Universal Master Mix II (Applied Biosystems, Foster City, CA), 0.5 µl (25 µM) forward and reverse primers, 0.5 µl (10 µM) TaqMan probe and 5 µl template DNA or water (negative control) or *M. leprae* DNA (positive control provided by BEI resources, Manassas, VA, strains Br4923 and Thai-53 DNA) were mixed in a final volume of 25 µl. DNA was amplified using the following protocol: 2 min at 50°C and 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C with a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems). Presence of *M. leprae* DNA was considered if a sample was positive for RLEP qPCR with a cycle threshold (Ct) lower than 37.5.

PCR to detect *M. lepromatosis* was performed as described previously (24). Briefly, the 244 bp *hemN* sequence (primers LPM244) (25) was amplified in 50 µl of reaction by addition of 10 µl 5x Gotaq® Flexi buffer (Promega, Madison, WI), 5 µl MgCl2 (25 mM), 2 µl dNTP mix (5 mM), 0.25 µl Gotaq® G2 Flexi DNA Polymerase (5 u/µl), 5 µl (2 µM) forward and reverse primers and 5 µl template DNA, water (negative control) or *M. lepromatosis* DNA (kindly provided by Dr Han, Clinical Microbiology Laboratory, the University of Texas M. D. Anderson Cancer Center, Houston, TX, USA) as a positive control. PCR mixes were subjected to 2 min at 95°C followed by 40 cycles of 30 s at 95°C, 30 s at 53°C and 30 s at 72°C and a final extension of 10 min at 72°C. PCR products (15µl) were used for electrophoresis in a 3.5% agarose gel at 130V. Amplified DNA was visualized by Midori Green Advance staining (Nippon Genetics Europe, Dueren, Germany) using iBright™ FL1000 Imaging System (Invitrogen, Carlsbad, CA).

Genotyping

To determine the genotype (1, 2, 3 or 4) of *M. leprae*, SNP-14676 (locus 1), SNP-1642875 (locus 2) and SNP-2935685 (locus 3) were amplified and sequenced as described with minor modifications (12): PCRs were performed with 5 µl of template DNA using the aforementioned PCR mixes and forward and reverse primers for loci 1-3 in a final volume of 50 µl. DNA was denatured for 2 minutes at 95°C, following 45 cycles of 30 s at 95°C, 30 s at 58°C and 30 s at 72°C and a final extension cycle of 10 min at 72°C. PCR products were resolved by agarose gel electrophoresis as explained above. PCR products showing a band were purified prior to sequencing using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI). Sequencing was performed on the ABI3730xl system (Applied Biosystems, Foster City, CA) using the BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA).

Two set of primers covering the position 978589 and 1476525 (16) were used to differentiate between the genotypes 4N, 4O and 4P. For the PCR reaction, a total of 3 µl of each purified DNA sample, or the positive control (Thai53, BEI resources NR-19352) or the negative control (water), was added to a total PCR reaction volume of 50 µl containing 25 µl of DreamTaq Green PCR master Mix 2X (Thermofisher), and 200 nM of each forward and reverse primers. The reaction mixtures were submitted to an amplification started with an initial denaturation step of 1 min at 95°C, followed by 40 cycles of 15s at 95°C, 15s at 55°C-62°C, and 1min at 72°C followed by a final extension at 72°C for 5min. Amplification was checked on agarose gel 1% and sent for sequencing through Genewiz company. Alignment of amplicon was performed using Codon code aligner v9.0.2 underfree access.

The RLEP positive *M. leprae* DNA samples were then transferred to the Department of Microbiology, Pathology and Immunology, Colorado State University, for whole-genome sequencing and further genotyping. DNA was quantified using the Qubit Fluorometer instrument (Thermofisher).

Library preparation

Because of the low quality of DNA extracted from FFPE, DNA libraries were prepared without fragmentation using 1 to 100ng of starting material in 50µl of Tris-HCl 10mM. The 50µl of each extract was used for library preparation using the Kapa Hyper Prep kit (Roche, Switzerland) as per the KAPA HyperCap Workflow (v3.2). UDI primer mixes (Roche) were used to index each library as recommended in the protocol.

M. leprae enrichment of libraries

No bacterial DNA enrichment was performed during DNA extraction, so enrichment for *M. leprae* DNA was approached using bait capture. Based on our previous investigations, all samples with Cts around 25 were considered for capture (24).

To obtain enough *M. leprae* coverage for DNA extract from FFPE samples, libraries were target enriched for the *M. leprae* genome using the KAPA HyperExplore Max 3Mb custom bait capture kit (Roche, IRN 1000013073, *M. leprae* Br4923). Libraries were pooled with one to three other libraries with a similar RLEP-qPCR Ct for a total amount of 1.5µg of DNA library according to the KAPA HyperCap Workflow (v3.2). Each enrichment was followed by an amplification step using the Kapa library amplification kit following manufacturer recommendations and a purification step using AMPure beads. Libraries were quantified using the Qubit dsDNA BR (Thermofisher) kit and the quality was

assessed on a TapeStation 4150 using the D1000 screen tape (Agilent). Libraries were pooled and sequenced single-end on NextSeq 500.

Genomic data analysis

Raw reads were processed as described elsewhere (26). Briefly, reads were adapter- and quality-trimmed with Trimmomatic v0.35 (27). The quality settings were "SLIDINGWINDOW:5:15 MINLEN:40". Preprocessed reads were mapped onto the *M. leprae* TN reference genome (GenBank AL450380.1) with Bowtie2 v2.3.4.2 (Langmead 20120). SNP calling was done using VarScan v2.3.9 (28). To avoid false-positive SNP calls, duplicate reads were omitted from downstream analyses and the following cutoffs were applied for VarScan: minimum overall coverage of five non-duplicated reads, minimum of three non-duplicated reads supporting the SNP, mapping quality score >8, base quality score >15, and a SNP frequency above 80%. All VCF files were analyzed using snpEff v4.3 (29). Heterozygous sites (20-80% SNP frequency), repetitive regions and ribosomal RNA genes in the reference sequence were omitted. Indel calling was done using Platypus v0.8.1 followed by manual curation (30). Putative unique variants of the strains associated with genotyping and drug resistance determining regions of *rpoB, folP1* and *gyrA* were manually checked and visualized using the Integrative Genomics Viewer (31).

Comparative genomics

The phylogenetic analysis was performed using a concatenated SNP alignment. Maximum parsimony (MP) trees were constructed in MEGA11 (32) with the 12 new genomes from this study (Table 1) and 312 previously published genomes using 500 bootstrap replicates and *M. lepromatosis* as an outgroup (24, 33). Sites with missing data were partially deleted (arbitrary 80% coverage cutoff), resulting in 5625 variable sites used for the tree calculation.

Geoplotting

The geomap (Figure 1) was constructed using residence and ethnicity of the patients and the results of *M. leprae* genotyping, as presented in Tables 1 and 2.

TABLE 1 List of the 16 newly sequenced genomes – sequencing information, genotyping, molecular drug resistance and SAM numbers wt: sequence wild-type.

Strain name	Total number of reads	Percentage of reads mapping against the reference TN (AL450380) (%)	Genome coverage (no duplicates)	Genotype	Molecular drug susceptibility			Accession number
					folP1	*rpoB*	*gyrA*	
Su-01	25883766	98.1	26.5	1D-2	Wt	wt	wt	SAMN35774743
Su-02	18466578	97.7	12.5	4P	Wt	wt	wt	SAMN35774744
Su-03	60711226	95.8	10.0	4N	Wt	wt	wt	SAMN35774745
Su-05	22956510	96.4	10.3	4P	Wt	wt	wt	SAMN35774746
Su-06	3687802	93.5	15.3	4P	Wt	wt	wt	SAMN35774747
Su-07	3770746	95.5	9.0	4P	Wt	wt	wt	SAMN35774748
Su-13	14156114	25.8	3.5	4P	not covered	not covered	wt	SAMN35774749
Su-14	2603173	94.5	4.9	4O/P	Wt	wt	wt	SAMN35774750
Su-15	14945860	95.1	45.1	4N	Wt	wt	wt	SAMN35774751
Su-17	63576250	97.5	3.8	4P	Wt	wt	wt	SAMN35774752
Su-19	47935707	11.7	2.6	1D-2	not covered	not covered	wt	SAMN35774753
Su-20	28006348	96.7	66.4	1D-2/4P	wt/ P55S (25%)	wt	wt	SAMN35774754
Su-21	5343543	96.4	3.8	4P	not covered	not covered	wt	SAMN35774755
Su-23	10043178	89	33.7	4P	P55R	wt	wt	SAMN35774756
Su-25	8067409	83.4	4.8	1D-2	Wt	wt	wt	SAMN35774757
Su-26	15301368	28.2	4.8	4P	Wt	wt	wt	SAMN35774758

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TABLE 2 Data of patients and samples analyzed in this study.

No	Age	Gender	Ethnicity	District	results of the *M. leprae* qPCR detection	qPCR Ct	SNP type	SNP subtype	Clin type	Hist type	Smear BI (range)
Su-1	20	F	Maroon	Commewijne	pos	23.08	1	1D	n.s	LL	n.p
Su-2	65	F	Maroon	Wanica	pos	23.26	4	4P	BL-LL	BL	2+(2-3+)
Su-3	double 32	F	Maroon	Wanica	pos	23.69	4	4N	BL-LL	BL-LL	n.p
Su-4	double 32	F	Maroon	Wanica	pos	25.96	4	neg	BL-LL	BL	n.p
Su-5	29	M	Maroon	Paramaribo	pos	23.33	4	4P	BL-LL	BL	n.p
Su-6	71	F	Maroon	Sipaliwini	pos	20.11	4	4P	BT	LL	3+(1-4+)
Su-7	12	F	Maroon	Para	pos	25.14	4	4P	BT	BL-LL	3+(2-4+)
Su-8	30	M	Maroon	Sipaliwini	pos	29.39	2, 3 or 4	n.p	BL-LL	LL	3+(2-5+)
Su-9	33	M	Maroon	Marowijne	pos	23.05	3 or 4	n.p	BT-BB	BL-LL	2+(1-3+)
Su-10	65	M	Maroon	Paramaribo	pos	26.43	4	ud	BL-LL	BL-LL	4+(2-4+)
Su-11	48	M	Maroon	Sipaliwini	pos	37.69	neg	n.p	BL-LL	n.p.	1+(1-2+)
Su-12	51	M	Maroon	Paramaribo	pos	29.92	3 or 4	n.p	BL	BT	1+(0-2+)
Su-13	24	F	Creole/ mixed	Paramaribo	pos	24.34	4	4P	BT	BB-BL	1+(1-2+)
Su-14	23	M	Creole/ mixed	Wanica	pos	23.59	4	4O/P	BL-LL	LL	3+(1-3+)
Su-15	47	M	Creole/ mixed	Paramaribo	pos	28.11	4	4N	LL	histoid	2+(1-3+)
Su-16	26	F	Creole/ Mixed	Paramaribo	pos	28.23	2, 3 or 4	n.p	BB-BL	BB-BL	2+(1-3+)
Su-17	49	M	Creole/ Mixed	Paramaribo	pos	25.36	4	4P	LL	LL	1+(0-1+)
Su-18	26	M	Creole/ mixed	Wanica	pos	30.22	1	neg	BL-LL	LL	2+(1-4+)
Su-19	46	M	Hindustani	Paramaribo	pos	25.39	1	1D	BL-LL	BL-LL	3+(2-3+)
Su-20 DR	21	F	Hindustani	Nickerie	pos	25.23	1+4	1D+4P	LL	LL	4+(3-4+)
Su-21	55	M	Hindustani	Para	pos	21.69	4	4P	BB-BL	LL	2+(1-3+)
Su-22	21	F	Hindustani	Paramaribo	neg	neg	ud	ud	BB	LL	2+(0-1+)
Su-23 DR	20	M	Javanese	Paramaribo	pos	30.41	4	4P	BL-LL	LL	4+(1-6+)
Su-24	29	M	Javanese	Wanica	pos	25.41	1	neg	BL-LL	LL	3+(2-4+)
Su-25	33	M	Javanese	Wanica	pos	25.81	1	1C(1D)	LL	LL	1+(0-2+)

(Continued)

TABLE 2 Continued

No	Age	Gender	Ethnicity	District	results of the *M. leprae* qPCR detection	qPCR Ct	SNP type	SNP subtype	Clin type	Hist type	Smear BI (range)
Su-26	43	M	Indigenous	Para	pos	23.96	4	4P	BL-LL	LL	3+(1-5+)
Su-27	53	M	Chinese	Paramaribo	pos	26.66	4	4O or 4P	BL	BB-BL	2+(1-3+)

neg: qPCR negative, pos: qPCR positive, DR: dapsone resistance, Ct: cycle threshold, BI: median value bacteriological index, n.p: not performed, n.s: not specified, double: sample collected twice from the same patient. Ud, undetermined; neg, negative; pos, positive; n.s., not specified; n.p., not performed; DR, Dapsone Resistant; BI, median value of bacterial Index; Double, two samples of the same patient; Ct, cycle threshold; Mixed, Mixed ethnicity.

Results

Patients, ethnic origin and identification of *M. leprae*

The 26 patients (9 females and 17 males; age ranging from 12 to 71, mean 37,4 years) included in this retrospective study belong to six ethnic groups: Maroons = African origin (n=11); Creoles/mixed (n=6); Hindustani= predominantly from the northeastern part of India (n=4), Javanese origin= from the Indonesian island of Java (n=3) and Chinese origin (n=1); Indigenous = autochthonous people (n=1) (Table 2, Figure 1). The Ridley-Jopling classification as well as the histopathological classification are given in Table 2. The 27 biopsies presented a median bacillary index of 2 (Table 2).

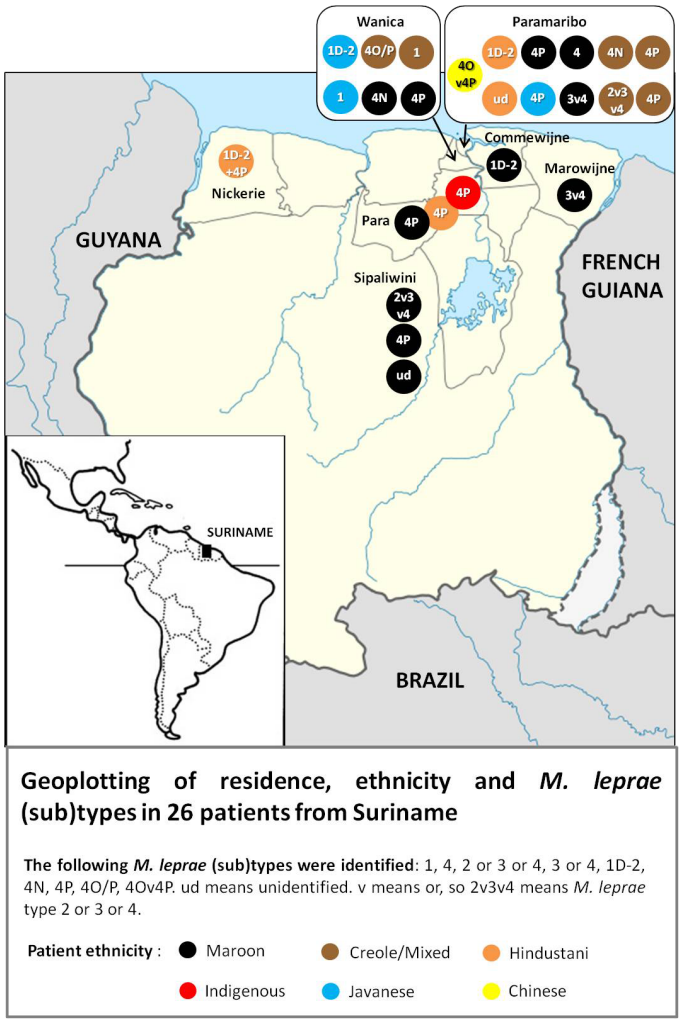

FIGURE 1
Geoplot of residence, ethnicity and *M.Leprae* subtypes.

A total of 26 out of the 27 DNA extracts were positive for *M. leprae* by RLEP qPCR with a Ct ranging from 21.7 to 37.7 (Table 2). All samples were negative for *M. lepromatosis* in qPCR.

Genotyping and comparative genomics

Library preparation was performed on 19 samples (Ct ranging from 21.69 to 28.11, Table 2). Three samples did not pass the quality control, and 17 samples were submitted to bait enrichment and Illumina sequencing. A total of 12 genomes were obtained with coverage sufficient for comparative genomics (>4) (Table 1). The four other sequenced genomes had a coverage between 2.5 and 4 which was sufficient for manual checking of the genotype using the Integrative Genome Viewer (v2.8.13) (Table 3). For all other strains, genotyping was determined by PCR-sequencing (Table 2).

Out of the 26 positive DNA extracts, genotyping was undetermined for two samples (Su-11 and Su-22), and inconclusive (no SNP type determined) for four samples (Su-08, Su-09, Su-12, Su-16). Partial genotyping (SNP-type alone or inconclusive SNP-subtype) was obtained for five samples (Su-4, Su-10, Su-18, Su-24, and, Su-27), while full genotyping was obtained for 16 strains (Table 2).

Among the 16 full or partial genomes available, three belong to the 1D-2 genotype commonly found in Asia (India, Nepal, Bangladesh, Japan) (24, 26) (Table 3; Figure 2A), two belong to genotype 4N, one to genotype 4O/P and twelve harbor the genotype 4P mostly found in West Africa and Brazil (Table 3; Figure 2B) (36). One sample, Su-20 presents with two strains, one from genotype 1D-2 (around 25% of the reads) and one with genotype 4P (around 75% of the reads) (Table 3).

A mutation in the resistance determining region of *folP1* was detected in the sample with two strains Su-20, (P55S, 25% of the reads) and Su-23 (P55R), suggesting that the strains are resistant to dapsone (Table 1).

Geoplotting

The geoplotting (Figure 1) shows that most of the patients in this study appear to live in Paramaribo or south of this city, in districts in the catchment area along the Suriname River. Data from the Dermatology Service shows that most leprosy patients registered in recent decades also live in these areas (5). The plot in Figure 1 therefore seems to be a good reflection of the actual distribution of leprosy across Suriname

Genotyping of *M. leprae* strains was correlated with the ethnic group of each patient. Six of the 11 Maroons carried type 4; 3 of the 6 Creoles/Mixed carried type 4; 2 of the 4 Hindustani patients and 2 out of 3 of Javanese origin carried type 1; the only indigenous patient carried type 4 and the only Chinese type 4 (Figure 1 and Tables 1 and 2). The patient presenting with the mixed infection (type 1 and 4) is of Hindustani ethnicity.

Discussion

The origin of leprosy in Suriname has been discussed since colonial times, with blame and associated stigma being apportioned to different ethnic groups, primarily those of African and Jewish descent. This led to stigmatizing labels such as 'nengre siki' ('disease of the blacks') and 'yu-siekie' ('disease of the Jews') (2, 3). Historical migration routes suggest that leprosy could have been introduced in Suriname through various means. It may have arrived from West Africa through the slave trade, from Asia by indentured workers, from Europe by the European colonizers and Portuguese Jews, and

TABLE 3 Single nucleotide polymorphisms manually checked to decipher the *M. leprae* genotypes in the samples with insufficient coverage by genome sequencing and compared to already published genomes from the different genotypes (in grey).

Genotype	1/2 or 3/4	1D	1D-2	4	4N	4P	Genotype
Sample	A73G	C3016895A	C953582G	C14676T	ins978589	1476525del	
TN	A	C	C	C	wt	wt	1A
NHDP-63	**G**	C	C	C	wt	wt	3I
Airaku-3	A	**A**	**G**	C	wt	wt	1D-2
Ng14-35	A	**A**	C	C	wt	wt	1D-1
S13	**G**	C	C	**T**	**wt**	wt	4N
Fio3	**G**	C	C	**T**	ins	**del**	4P
Su-19	nc	**A (3)**	**G (1)**	C (1)	wt (2)	wt (5)	**1D-2**
Su-13	**G (5)**	C (2)	C (1)	**T (3)**	ins (3)	**del (3)**	**4P**
Su-21	**G (4)**	nc	C (5)	**T (5)**	ins (3)	**del (2)**	**4P**
Su-20	21%A - 79%G	27% **A** - 73% C	18% **G** / 82% C	22% C - 78% **T**	39% wt - 69% ins	27% wt - 63% **del**	**1D-2 / 4P**
Su-17	**G(2)**	nc	C(4)	**T (5)**	nc	**del (2)**	**4P**

The coverage of each position for the strains from Suriname are shown in parenthesis. Positions are informative positions described by Monot and colleagues (1).
Value in parenthesis represents the number of reads at the position, Nc, not covered. Previously sequenced strains (in grey) were used as a reference for each genotype (1, 34, 35).

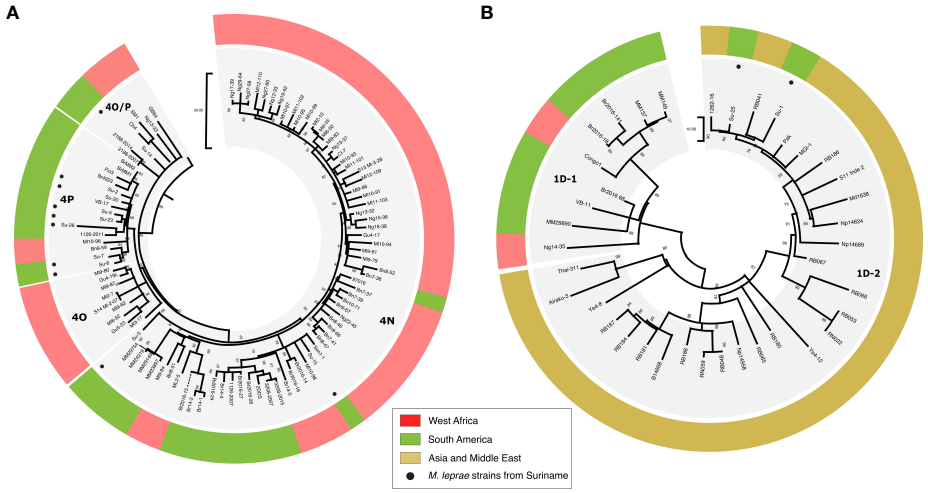

FIGURE 2
Maximum parsimony tree of 317 *M. leprae* genomes highlighting the new genomes from Suriname harboring the genotypes 4 **(A)** and 1D **(B)**. The tree was initially constructed using 317 genomes, 500 bootstrap replicates, and *M. lepromatosis* as an outgroup. Sites with missing data were partially deleted (80% coverage cutoff), resulting in 5625 variable sites used for the tree calculation. Subtrees corresponding to branches were then retrieved in MEGA. Corresponding genotypes are indicated on the side of each subtree. Newly sequenced genomes are shown with circles, and genomes are colored-code based on the region where the isolate was identified. The branch length represents the number of SNPs. RB, Bangladesh; Np/Md, Nepal; Br or MM, Brazil; VB, Venezuela; Pak, Pakistan; 1262-16/S11/MGI-1, India; Ng, Niger; Ye, Yemen; Airaku-3, Japan; Thai, Thailand; Number (e.g, 20H02900), French Guyana; Bn, Benin; Ml, Mali.

more recently from Brazil, by wildcat gold miners (37, 38). Previous studies on ancient remains (19th century) and soil samples collected from different sites in the country have indicated the co-existence of multiple *M. leprae* strains in Suriname, possibly originating from different sources (20, 21).

Our study confirms this hypothesis and provides a better understanding of the different introductions of the disease into Suriname. Genotyping of modern human *M. leprae* strains circulating in the Surinamese population suggests multiple introductions of *M. leprae* from West Africa, Asia and Brazil. The predominant strain in our sample set is represented by genotype 4P, followed by two strains harboring genotype 4N and one harboring genotype 4O/P. These genotypes are predominant in West Africa and Brazil. Comparative genomics analysis revealed that the Surinamese strains are clustering with strains from West Africa (Su-6, Su-7, Su-15), from Brazil (Su-5, Su-26, Su-23), in a broader cluster including strains from Brazil and Venezuela (Su-20, Su-2) or are branching alone in the three (Su-3) (Figure 2A). These data suggest that the *M. leprae* genotypes were introduced during the slave trade into South America between the 17th century and the abolishment of Dutch colonial slavery in 1863, over which period approximately 550,000 enslaved West-Africans were transported to Suriname. These genotypes could also have been introduced in the 17th century from Brazil when the Dutch, along with the Portuguese Jewish plantation owners and their enslaved West-African workers, were expelled and moved to Suriname. They could also have been introduced in recent decades by Brazilian gold seekers (garimpeiros) who have been working in gold mines in the interior of Suriname.

One notable genotype encountered is genotype 1D, which is separated in three different clusters in the *M. leprae* phylogeny and found in different regions worldwide. One cluster, called 1D-Malagasy is found predominantly in Madagascar and the Comoros, the second cluster called 1D-1 is mostly found in Brazil and Venezuela, while the third one, called 1D-2, is mostly observed in Asian countries such as India, Bangladesh, Nepal, Thailand, and Japan (13). Some might expect that the genotype 1D circulating in Suriname would belong to the 1D-1 cluster due to the proximity with Brazil. However, all 1D strains belong to 1D-2 cluster (n=3), suggesting that the 1D strains were introduced during the 19th century and early 20th century following the large migration of Asian contract workers (India, Indonesia and China) to Suriname.

A conspicuous observation is the absence of the genotype 3I in the samples analyzed. This genotype is predominant in North and Central America and has also been reported in the north of Brazil, Colombia and in Venezuela (13, 26). Previous ancient DNA studies have hypothesized that the first European settlers brought leprosy with genotype 3I into the new world. The absence of this genotype in our biopsy samples suggests that if present, the genotype 3I is not predominant.

To get deeper into the transmission pattern in Suriname, we also seek to correlate patients' ancestry with the strain's genotype. Geo-plotting showed that the majority of the patients live in and around Paramaribo. Enslaved West-Africans fled from the plantations to the hinterland, establishing Maroon communities along the Suriname River (and other rivers) upstream, where they were safer from the authorities. In recent times, these Maroons have moved to the capital Paramaribo, where the majority of patients is also diagnosed. The Javanese and Indian-Hindustani migrants mainly settled on plantations in the coastal area. Strain type 4 was not only found in people of West-African descent and type 1 not only in Asians, suggesting that the current distribution of strains is not linked with ancestry, and interethnic contacts in

daily life and personal relationships, including marriages between Surinamese people of different ethnicity probably explain it.

In an earlier report, 3 out of 28 samples taken at the entrance of armadillo holes in former leprosaria in Suriname were positive for *M. leprae* DNA (21). The positive soil samples from armadillo burrows may also indicate that armadillos play a role in the persistence of *M. leprae* in the environment in Suriname (39, 40). Also, armadillos are hunted and their meat is part of the food consumption of inhabitants in the interior of Suriname, especially among Maroons. Additionally, during a field trip into the interior, leprosy was diagnosed in an armadillo hunter (unpublished data). It has been suggested that contact with armadillos or environmental shedding may be risk factors in acquiring leprosy (41, 42).

A mutation in the dapsone resistance determining region of *folP1* was detected in sample Su-20 with two strains (P55S, 25% of the reads) and in Su-23 (P55R). The mutation P55S is rarely reported and to our knowledge, only one *M. leprae* strain in Korea was identified with this mutation (43). Based on the number of reads mapping both strains, we suspect that the mutation is carried by the strain 1D-2. Both patients were new cases and showed a BI 4+ at diagnosis. They were positive for *M. leprae* DNA after prolonged MDT and did not show a relapse, although the follow-up period was short in both cases, in one case due to the Covid pandemic, while the second patient died while still on extended treatment. These data suggest primary transmission of dapsone-resistant cases in a high percentage of the infected population (2/13 screened) (44). These data call for additional molecular surveillance in the population to avoid transmitting resistant strains.

Overall, our data unraveled an unprecedented diversity of *M. leprae* circulating in Suriname directly linked with the various migration waves into the country.

Data availability statement

The datasets generated for this study can be found in the NCBI Sequence Read Archive (SRA) under accession number PRJNA984678.

Ethics statement

The studies involving humans were approved by ethics committee of the Academic Hospital Paramaribo. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

WF: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft. KS: Investigation, Methodology, Writing – review & editing. HM: Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. CA: Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Software, Writing – review & editing. AG: Data curation, Funding acquisition, Investigation, Methodology, Software, Writing – review & editing. EV: Investigation, Methodology, Writing – review & editing. MTC: Investigation, Methodology, Writing – review & editing. MC: Investigation, Writing – review & editing. TP: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) CA declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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