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Enhanced Molecular Typing of *Treponema pallidum*: Geographical Distribution of Strain Types and Association with Neurosyphilis

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Abstract

Background—Strain typing is a tool for determining diversity and epidemiology of infections.

Methods—*T. pallidum* DNA was isolated from 158 syphilis patients from the US, China, Ireland, and Madagascar and from 15 *T. pallidum* isolates. Six typing targets were assessed: 1) number of 60 bp repeats in *acidic repeat protein* gene; 2) restriction fragment length polymorphism (RFLP) analysis of *T. pallidum repeat* (*tpr*) subfamily II genes; 3) RFLP analysis of *tprC* gene; 4) determination of *tprD* allele in *tprD* gene locus; 5) presence of 51 bp insertion between *tp0126*/*tp0127*; 6) sequence analysis of 84 bp region of *tp0548*. The combination of #1 and #2 comprises the CDC *T. pallidum* subtyping method.

Results—Adding sequence analysis of *tp0548* to the CDC method yielded the most discriminating typing system. Twenty-four strain types were identified and designated as CDC subtype/*tp0548* sequence. Type 14d/f was seen in 5 of 6 locations. In Seattle, strain types changed from 1999– 2008 ($p<0.001$). Twenty-two (50%) of 44 patients infected with type 14d/f had neurosyphilis compared to 9 (23%) of 39 infected with the other types combined ($p=0.01$).

Conclusion—We describe an enhanced *T. pallidum* strain typing system that shows biological and clinical relevance.

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Keywords

syphilis; molecular typing; neurosyphilis; *Treponema pallidum*

Introduction

Strain typing is a powerful tool for determining the diversity of pathogens and epidemiology of infections. In 1998, investigators at the Centers for Disease Control and Prevention (CDC) published a molecular method to distinguish among subtypes of *Treponema pallidum* ssp. *pallidum* (hereafter termed *T. pallidum*), the causative agent of syphilis [1]. The method is based on determination of 1) the number of 60 bp repeats in the *acidic repeat protein* (*arp*) gene and 2) on sequence differences in the *T. pallidum repeat* Subfamily II genes (*tprE [tp0313], tprG [tp0317], tprJ [tp0621]*) determined by restriction fragment length polymorphism (RFLP) analysis. Subtype designation is based on the number of repeats and the RFLP pattern, for example, strain type 14d. This subtyping method has been applied to bacterial DNA recovered from chancres, condyloma lata, mouth, ear scrapings, blood, and cerebrospinal fluid and to laboratory passaged *T. pallidum* isolates from diverse geographic areas [1–8] in the hopes that molecular typing could be used to characterize an epidemic and assist in control efforts. However, a particular drawback of the published subtyping system is its inability to discriminate among the most common strain types.

We performed the work reported here to determine whether examination of additional gene targets might lead to a more discriminating typing system than the published CDC system. We reported that identification of a 51 bp insertion in the intergenic spacer between the *tp0126* and *tp0127* open reading frames could be used to discriminate among *T. pallidum* organisms [9]. Strain-to-strain sequence variations in the *T. pallidum tprC (tp0117)* and *tprD* (*tp 0131*) genes have been noted [10, 11], and sequence analysis of the *tp0548* gene has been used to distinguish among *T. pallidum* patient derived samples [12, 13]. We examined each of these targets in samples from different areas of the world and determined that sequence analysis of a short region of the *tp0548* gene, in addition to the *arp* and *tpr* Subfamily II genes, greatly improved discrimination among strains, allowing us to divide 14 CDC subtypes into 24 strain types. Using this improved strain typing system, we demonstrate geographic distributions of strain types, changes in strain types over time in a single geographic region, and association between strain type and neurosyphilis.

Methods

Sample Sources and DNA Extraction

Samples from 173 individual patients from different geographical sites were examined in this study: 1) 72 *T. pallidum* isolates derived from blood, cerebrospinal fluid, or lesion exudates in Seattle WA collected in 1981, 1983, 1987, and from 1999–2008; 2) 11 historical *T. pallidum* isolates, including the reference Nichols strain, which was derived from CSF in 1912; 3) 16 blood samples collected in Seattle between 1999–2008; and 4) randomly selected swab samples from primary or secondary lesion exudates collected in: Antananarivo, Toamasina, and Mahajanga in Madagascar between 2003–2008 (n=20) [14]; San Francisco CA between 2001–2007 (n=19)[15]; Baltimore, MD between 1999–2001 (n=15); Nanning city of Guangxi Autonomous Region of China between 2006–2007 (n=10), and Dublin, Ireland in 2002 (n=10) [16]. The study protocol was reviewed and approved by local Institutional Review Boards, and human experimentation guidelines were followed in the conduct of this research. Written informed consent was obtained from all participants.

The relationship between strain types and neurosyphilis was examined in a subset of 84 patients enrolled in an ongoing study of CSF abnormalities in patients with syphilis conducted at the University of Washington, Seattle WA between 1999 and 2008 [17]. Briefly, individuals were eligible for enrollment if they had clinical or serological evidence of syphilis, and were deemed by the referring provider as possibly having neurosyphilis. All participants underwent a structured history and neurological examination, lumbar puncture and venipuncture.

DNA was extracted from swabs and *T. pallidum* suspensions stored in 10mM Tris-HCl, 0.1M EDTA, and 0.5% SDS using a QIAamp DNA Mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. DNA was extracted from blood stored in 20mM Tris-HCl, 0.2M EDTA, and 1% SDS using a QIAamp DNA Blood Midi kit (Qiagen, Valencia, CA) according to manufacturer's instructions.

Determination of Number of 60 Base Pair Repeats in the *arp* Gene

Ten ul of DNA was amplified using 2.5 units GoTaq Flexi DNA polymerase (Promega, Madison, WI) in a 50 ul reaction with 200 nM dNTPs, 0.6 uM primers, 1.5 mM $MgCl_2$ and 1X GoTaq Flexi Buffer. Primer sequences were: sense, 5′AGCGTGATCCTCTGTCATCC3′ and antisense, 5′TATGCTGAGAAGCGACCTCA3′. Cycling conditions were: 94°C for 4 min, then 40 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, followed by 72°C for 7 min. The PCR products were separated by agarose gel electrophoresis. The size of all PCR products was determined by comparison with a 1 Kb DNA ladder (Invitrogen, Carlsbad, CA) using Quantity One software (Biorad, Hercules, CA), and the number of repeats established by comparing the size of the amplified product with the product amplified from the Nichols strain, which contains 14 repeats [1].

RFLP Analysis of *tprE*, *tprG*, *tprJ* Genes

Five ul DNA was amplified using 2.5 units of GoTaq Flexi DNA polymerase in a 50 ul reaction with 200 nM dNTPs, 0.6 uM primers, 2.5 mM $MgCl_2$ and 1X GoTaq Flexi Buffer. Primers sequences were: sense, 5′CAGGTTTTGCCGTTAAGC3′ and antisense, 5′AATCAAGGGAGAATACCGTC3′. Cycling conditions were: 94°C for 4 min, then 40 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, followed by 72°C for 7 min.

The *tprE*, *tprG*, *tprJ* amplicons were digested according to published methods with minor modifications. Digestion products were separated by agarose gel electrophoresis and banding patterns were compared to published data [1].

RFLP Analysis of the *tprC* Gene

Five ul of DNA was amplified using 1.25 units of GoTaq Flexi DNA polymerase in a 50 ul reaction with 200nM dNTPs, 2.5mM $MgCl_2$, 0.6 uM primers and 1X GoTaq Flexi Buffer. Primer sequences were: sense, 5′GTACATCCCCCTCAC CTAC3′; antisense, 5′AAAAATGGTACAGGCGTTGC3′. Cycling conditions were: 95°C for 2 min, then 40 cycles of 95°C for 1 min, 60°C for 30 sec, and 72°C for 1 min, followed by 72°C for 7 min.

Amplified products were digested using 5 units each of BsiEI and BsiHKAI enzymes (New England Biolabs, Ipswich, MA) for 2 hours at 60°C and then 2 hours at 65°C. Products of the restriction digestion were separated by agarose gel electrophoresis. The size of the largest band (276bp, 249bp or 203bp) was used to distinguish between organisms.

Analysis of *tprD* Gene Locus

Five ul of DNA was amplified using 1.25 units of GoTaq Flexi DNA polymerase in a 50 ul reaction with 200 nM dNTPs, 3.0 mM $MgCl_2$, 0.6 uM primers and 1X GoTaq Flexi Buffer.

Primer sequences were: sense, 5′AGTACCAGGTGGGACTGACG3′; antisense, 5′GAACGGGTCTCCACACTCAC3′. Cycling conditions were as follows: 95°C for 2 min, then 40 cycles of 95°C for 1 min, 65°C for 30 sec, and 72°C for 1 min, followed by 72°C for 5 min.

Amplified products were digested using 10 units of MluI enzyme (New England Biolabs, Ipswich, MA) at 37°C for two hours. Products of the restriction digestion were separated by agarose gel electrophoresis. The size of the products identified the *tprC/D* gene (232bp and 167bp) or the *tprD2* gene (399bp).

Sequence Analysis of the *tp0548* Gene

Two sense and three antisense primers were used to amplify DNA from bacterial isolates (sense #1 and antisense #1), lesion exudates (sense #2 and antisense #2) and blood (sense #2 and antisense #3 (Table 1). Different primer sets were used for the three sample types to optimize analytic sensitivity. For amplification from isolates, 5 ul of DNA was amplified using 2.5 units of GoTaq Flexi DNA polymerase in a 50 ul reaction with 200 nM dNTPs, 0.8 uM primers, 1.5 mM $MgCl_2$ and 1X GoTaq Flexi Buffer. Cycling conditions were: 95°C for 2 min, then 40 cycles of 95°C for 1 min, 60°C for 2 min, and 72°C for 1 min, followed by 72°C for 10 min. For amplification from swabs or blood, methods were identical except that 0.6 uM of each primer was used and the annealing step was at 62°C for 75 sec. PCR products were purified using a QIAquick PCR purification kit (Qiagen, Valencia, CA), and sequenced using standard methods. Variability in a portion of the gene 131 bp downstream from the start codon was noted. Eight distinct sequence groups were observed and designated as a–h (Figure 1).

Cloning

Amplicons were ligated into the TOPO TA vector (Invitrogen, Carlsbad, CA) according to manufacturer's instructions and clones sequenced according to standard methods.

Clinical Laboratory Methods

CSF Venereal Disease Research Laboratory (CSF-VDRL) test, and measurement of CSF WBCs were performed in Clinical Laboratory Improvement Amendments (CLIA)-approved laboratories. Serum Rapid Plasma Reagin (RPR) test was performed in a single research laboratory according to standard methods [18].

Statistical Methods

Associations between categorical variables were determined by Chi-square or Fisher's exact test. P values < 0.05 were considered to be statistically significant.

Results

Determination of the Optimal Typing System

We first analyzed 84 samples using all six targets to determine which combination provided the highest level of discrimination. Among these samples, the CDC method identified 12 subtypes, most frequently 14a (n=10), 14d (n=51) and 15d (n=9). Compared to the CDC method, determination of the presence or absence of the *tp0126*/*tp0127* insertion and RFLP analysis of *tprC* did not improve discrimination. Sequence analysis of *tp0548* divided the most common CDC subtype, 14d, into three separate groups, and subtypes 12a and 14a could each be divided into two groups. Addition of the *tprD/D2* analysis to that provided by *tp0548* only incrementally improved discriminatory ability, allowing us to divide one additional CDC subtype, 10d, into two groups.

Based upon practical considerations of cost versus increased discriminating ability, we propose a new *T. pallidum* typing method that includes the published CDC method plus sequence analysis of bases 131 to 215 of the *tp0548* open reading frame. From here forward we refer to the results of the revised method as defining a "strain type," to distinguish it from the CDC method, which has been termed a "subtype." We express strain type as CDC subtype/*tp0548* sequence type, for example 14d/f. Using this method, we subsequently determined strain type for all 173 samples.

Figure 2 shows the relationship between CDC subtype and strain type. Analysis of all 173 samples identified fourteen CDC subtypes, which could be further divided into 24 strain types. Specifically, subtypes 10d, 11d, 12a, 14a, 14d, 14e, and 15d could all be subdivided using the revised strain typing system, attesting to its improved discrimination. Types 14d/f, 14d/g, and 15d/f were the most common strain types seen. Table 2 shows a summary of the newly defined strain types and tested samples.

Stability of Strain Type Within Isolates

Pillay and colleagues showed that the CDC subtype of the Nichols strain did not change with repeated rabbit passages [1]. We confirmed this finding. Five clones of the Nichols strain 22 passages after infection and five clones of the original inoculum showed identical strain type (14a/a). We examined strain type stability for two other *T. pallidum* isolates. The strain type of the Sea 81-4 isolate (14e/b) remained the same at rabbit passages 12 and 22. Previous work showed that the Chicago C isolate rapidly acquires diversity of the *tprK* gene sequence with serial rabbit passage [19]. Even after 12 passes of Chicago C in rabbits, this isolate demonstrated strain identity with the original inoculum (14a/a).

Geographic Distribution of Strains

Nineteen of the 24 strain types were identified in Baltimore, San Francisco, China, Dublin, Madagascar, and in the subjects enrolled at the University of Washington in Seattle (Figure 3, Table 2). More than one type was seen in each of the 6 locations. Types 14d/c, 14d/f, 14d/g, 15d/f and 16a/e were seen in at least two different locations, with type 14d/f seen everywhere except Madagascar. Three of the four California strain types were also represented in Seattle, perhaps suggesting a sexual connection between the San Francisco and Seattle cohorts.

Characterization of the 84 Patients from the University of Washington, Seattle

The characteristics of the 84 Seattle study participants are shown in Table 3. Most patients were white men who have sex with men (MSM). There was a trend toward a difference in strain type by HIV status (p=0.05).

Strain type was identified in three subjects who returned during our study with a second episode of syphilis. Subject 1's first episode of syphilis was in July 2003, when he was treated for secondary syphilis. He returned with secondary syphilis in January 2004. Strain type 13d/d was identified in each episode, suggesting reinfection from a member of the same sexual network or treatment failure. Subject 2's first episode of syphilis was in April 2006 when he was treated for secondary syphilis. He returned with early latent syphilis in July 2008. Strain type 14d/f was identified in the first episode and type 14d/g in the second episode, indicating reinfection by a new strain. Subject 3 presented in January 2003, when he was treated for secondary syphilis and neurosyphilis. Serum RPR titer declined from 1:512 to 1:64 seven months after treatment. He returned with early latent syphilis in January 2009. Strain type 14d/f was identified in the first episode and type 14d/g in the second episode, again demonstrating reinfection by a new strain.

Temporal distribution of strain types in Seattle

Typing of a large number of samples collected over 10 years in Seattle enabled us to examine the temporal distribution of strain types in a single community. Figure 4 shows the 7 Seattle strain types identified by year of collection. Type 14d/f predominated from 1999–2003 and remained as a minor strain through 2007. Type 15d/f appeared in 2002, and was not seen after 2005. Type 14d/g was first seen in 2004 and was identified through 2008. It is notable that the introduction of type 14d/g coincided with increasing incidence of syphilis in Seattle <http://www.kingcounty.gov/healthservices/health/communicable/std/statistics.aspx>. Type 16a/e was seen in two African American heterosexual partners in 2004, a single patient with type 15d/g was seen in 2004, and a single patient with type 15e/e was seen in 2006.

For purposes of analysis, year of sample collection was divided into three epochs (1999–2002, 2003–2005, 2006–2008). There was a significant change in strain type over the three time periods ($p<0.001$). We also determined strain type for four isolates collected in Seattle in 1981, 1983 and 1987. Two isolates (collected in 1983 and 1987) were type 14d/f, the most common strain type identified in Seattle between 1999 and 2008. In contrast, one isolate collected in 1981 was type 14e/b and another collected in 1983 was type 14a/a. Neither of these types was seen in Seattle samples collected in 1999–2008.

Association of strain type with neurosyphilis

A sensitive and discriminating typing method would be particularly useful if strain type could be associated with clinical findings. The Seattle samples were obtained in an ongoing study of neurosyphilis, and we examined whether any particular strain type was more commonly associated with this disease. Twenty-two (50%) of 44 patients infected with type 14d/f had neurosyphilis (defined as CSF WBC > 20/ul or a reactive CSF-VDRL) compared to 9 (23%) of 39 infected with the other 6 strains combined ($p=0.01$). Similarly, 16 (36%) of 44 patients infected with type 14d/f had a reactive CSF-VDRL, compared to 4 (10%) of 39 infected with the other 6 strains combined ($p=0.006$). There was no difference in the proportion of patients who had neurosyphilis over the 10 year period of observation, and type 14d/f was equally common in HIV-infected and HIV-uninfected patients.

Discussion

Several previous studies have used the CDC method to investigate *T. pallidum* subtype prevalence among communities. For example, Sutton and colleagues determined CDC subtypes from *T. pallidum* DNA isolated from blood and genital ulcer swabs during an outbreak of syphilis in Phoenix, AZ [2]. About half of the samples were CDC subtype 14f. Nine other types were identified, including 14d. Subtypes 14d and 14f have been identified in diverse geographic settings, including North and South Carolina [4], Lisbon [7], Scotland [8], South Africa [1, 3] and China [20]. In samples examined in our study, CDC subtype 14d was most common, and we did not identify any subtype 14f samples. By enhancing the CDC subtyping method with sequence analysis of a small region of the *tp0548* gene, we were able to separate 14 CDC subtypes into 24 different strain types. Importantly, we were able to separate the subtypes with 14 *arp* repeats into 8 individual strain types.

We show a change in circulating strain types in Seattle, Washington, between 1999 and 2008, with introduction and disappearance of strain types in the region during that period. Retrospectively, we also saw disappearance of two strains that were evident in Seattle in the 1980s compared to the strains identified between 1999 and 2008. The expansion of the introduced types within our recent, predominantly MSM, cohort is consistent with one or more overlapping sexual networks in the MSM community in Seattle. Moreover, we saw a

strong trend toward an association between strain type and HIV status. This finding further suggests that there are separate networks within MSMs in our community and is consistent with serosorting such that patients choose sexual partners based on their HIV status. We identified an unusual type (type 16a/e) in only two individuals in 2004, without subsequent detection in other patients. These two individuals were African American heterosexual partners. In addition, a single African American patient was infected with a type 15e/e strain. The finding of these unique strains in African American patients in the setting of a predominantly white MSM epidemic provides additional evidence for separate sexual networks.

Many of the samples examined in this study have also been tested for 23S rDNA mutations associated with macrolide resistance in *T. pallidum* [14, 21, 22], and we considered including either or both of these mutations in our typing scheme. Although these markers were able to distinguish among strains within some types, we were uncertain about the long term stability of this marker within a strain, particularly in light of studies that have shown the association of the A2058G mutation with prior exposure to macrolides [9, 15]. Similarly, in a small number of samples, we examined two other molecular targets that proved to be unsuitable for strain typing: sequence analysis of the *tp0136* gene [12], and determination of the number of 24 bp repeats in the *tp0470* gene. Sequence analysis of the *tp0136* gene lacked discriminatory ability, and the number of repeats in the *tp0470* gene varied within a single bacterial isolate (data not shown).

The association between strain type and neurosyphilis is particularly notable. Although literature from the first part of the 1900s discusses the existence of “neuroinvasive” or “neurotropic” strains of *T. pallidum*, this concept has not been rigorously studied in humans. In a rabbit model, we showed that the clinical phenotype of disease differed depending on the inoculating strain [23]. In that study, rabbits were infected with three different strain types: type 14a/a, 14e/b and 14d/f. Animals infected with one type 14a/a strain and one type 14d/f strain had the greatest degree of neuroinvasion. In the study reported here, we found that patients with neurosyphilis were most commonly infected with type 14d/f, consistent with the rabbit studies. We cannot comment on the neuroinvasive potential of type 14e/b in our human study, as none of the Seattle patients was infected with this strain. *T. pallidum* type 14d/f strains may be more neuroinvasive, or may be better able to evade host immune responses in the central nervous system (CNS) than other strain types.

The ability to identify the infecting strain types in patients with syphilis could have important clinical implications. Controversy abounds regarding which patients with syphilis should undergo lumbar puncture to evaluate the possibility of neurosyphilis. Currently, the best indicator of risk is serum RPR titer ≥ 1:32, but that criterion leads to some false positives [17, 24]. If strain type could be determined in a blood sample, it could help to identify those patients who are at greatest risk for neurosyphilis. These individuals, particularly those with RPR titer ≥ 1:32, could be then targeted for lumbar puncture or empiric neurosyphilis treatment. Our data suggest that future larger investigations of the correlation between strain type and the clinical or laboratory markers that indicate increased risk of neurosyphilis are warranted. In addition, the association between specific strain type and neurosyphilis could lead a new understanding of the molecular mechanisms underlying neuroinvasion.

The enhanced typing method that we describe shows biological and clinical relevance, as well as epidemiological utility. It represents a significant advance in our ability to study the molecular epidemiology of syphilis, and it offers the potential to learn more about the pathogenesis of CNS disease. Future epidemiological studies that combine social network analysis and strain typing data are required to determine the ultimate utility of this new

typing method for syphilis investigation and control. Similarly, continued study of risks for neurosyphilis is required to determine the ultimate role of strain typing as part of risk assessment.

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Figure 1.
Typing groups determined by sequence of bp 131 to 215 in the *tp0548* open reading frame.

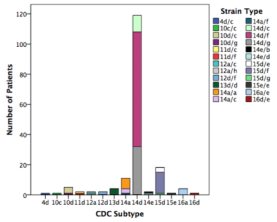

Figure 2.

Comparison of CDC subtypes to strain types as defined by addition of sequence analysis of *tp0548* to the CDC method. Fourteen CDC subtypes could be divided into 24 strain types. Importantly, the 3 subtypes containing 14 *arp* repeats could be separated into 8 separate types using the enhanced typing scheme.

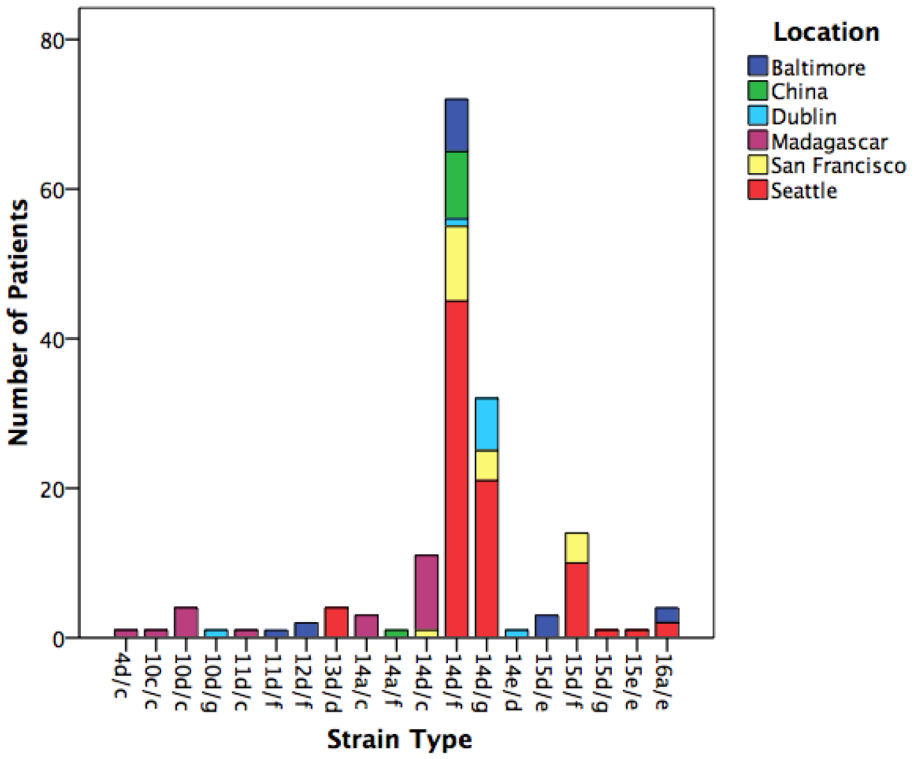

Figure 3.
Strain types identified in six geographic regions. More than one strain type was seen in all locations, and Type 14d/f was identified in all sites other than Madagascar. Several strain types were seen in only a single location.

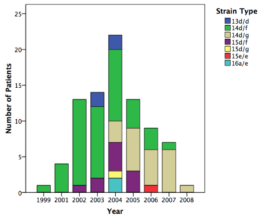

Figure 4.

Strain types identified in each year from 1999 through 2008 in patients from Seattle, WA, showing introduction and loss of some strain types during that period. There was significant change in type over time ($p<0.001$).

Table 1

Primers Used to Amplify the *tp0548* Gene

Primer Designation	Primer Sequence
Sense #1	5′GCGTGGTGGTGAGTTCTTCT3′
Antisense #1	5′ACGGCAGGCTAGTTGAGAAT3′
Sense #2	5′GGTCCCTATGATATCGTGTTCG3′
Antisense #2	5′GTCATGGATCTGCGAGTGG3′
Antisense #3	5′CGTTTCGGT GTGTGAGTCAT3′

Table 2

Strain Types and Sample Sources

Strain Type	Geographic Site (number of samples)	Representative Isolate
4d/c	Madagascar (1)	NA
10c/c	Madagascar (1)	NA
10d/c	Madagascar (4)	NA
10d/g	Dublin (1)	NA
11d/c	Madagascar (1)	NA
11d/f	Baltimore (1)	NA
12a/c	—	Bal 8
12a/h	—	Bal 9
12d/f	Baltimore (2)	NA
13d/d	Seattle (4)	UW279
14a/a	—	Nichols
14a/c	Madagascar (3)	NA
14a/f	China (1)	NA
14d/c	San Francisco (1), Madagascar (10)	NA
14d/f	San Francisco (10), China (9), Dublin (1), Baltimore (7), Seattle (45)	Street 14
14d/g	San Francisco (4), Dublin (7), Seattle (21)	UW249
14e/b	—	Seattle 81-4
14e/d	Dublin (1)	NA
15d/e	Baltimore (3)	NA
15d/f	San Francisco (4), Seattle (10)	UW116
15d/g	Seattle (1)	NA
15e/e	Seattle (1)	NA
16a/e	Baltimore (2), Seattle (2)	UW284
16d/e	—	Mexico

“—” indicates that the strain type was not seen in any of the six geographic sites.

NA, *T. pallidum* isolate not available because these strain types were identified from patient samples only.

Table 3

Characteristics of 84 Subjects Enrolled at the University of Washington, Seattle, WA

Characteristic	Number (Percent)
Male	82/84 (98%)
Race	
White	72/84 (86%)
African American	8/84 (10%)
Other	4/84 (4%)
Sexual Identification	
MSM	73/84 (87%)
Bisexual	6/84 (7%)
Heterosexual	4/84 (5%)
Transgender	1 (1%)
HIV	70/84 (83%)
Injection drug use ever	19/84 (23%)
Syphilis	
Primary, secondary, early latent	78/84 (93%)
Late latent or unknown duration	6/84 (7%)
1/Serum RPR titer	128 (64–256)[1]
CSF WBC > 20/ul or reactive CSF-VDRL	31/83 (37%)[2]
Reactive CSF-VDRL	20/83 (24%)[2]

[1] Median (IQR)

[2] CSF-VDRL was missing for one participant

MSM, men who have sex with men; RPR, Rapid Plasma Reagin test; VDRL, Venereal Disease Research Laboratory Test