The Institute of Medical Science
The University of Tokyo

Epidemic of Treponema pallidum Strains in Japan and Biomarkers for Molecular Epidemiology

Eisuke Adachi[1] Wakana Sato [1] Ayako Sedohara[1] Hirona Ichimura[2] Hiroshi Yotuyanagi[1], Yasutohi Kido[2]

[21]IMSUT Hospital of The Institute of Medical Science, The University of Tokyo,Japan, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan

BACKGROUND

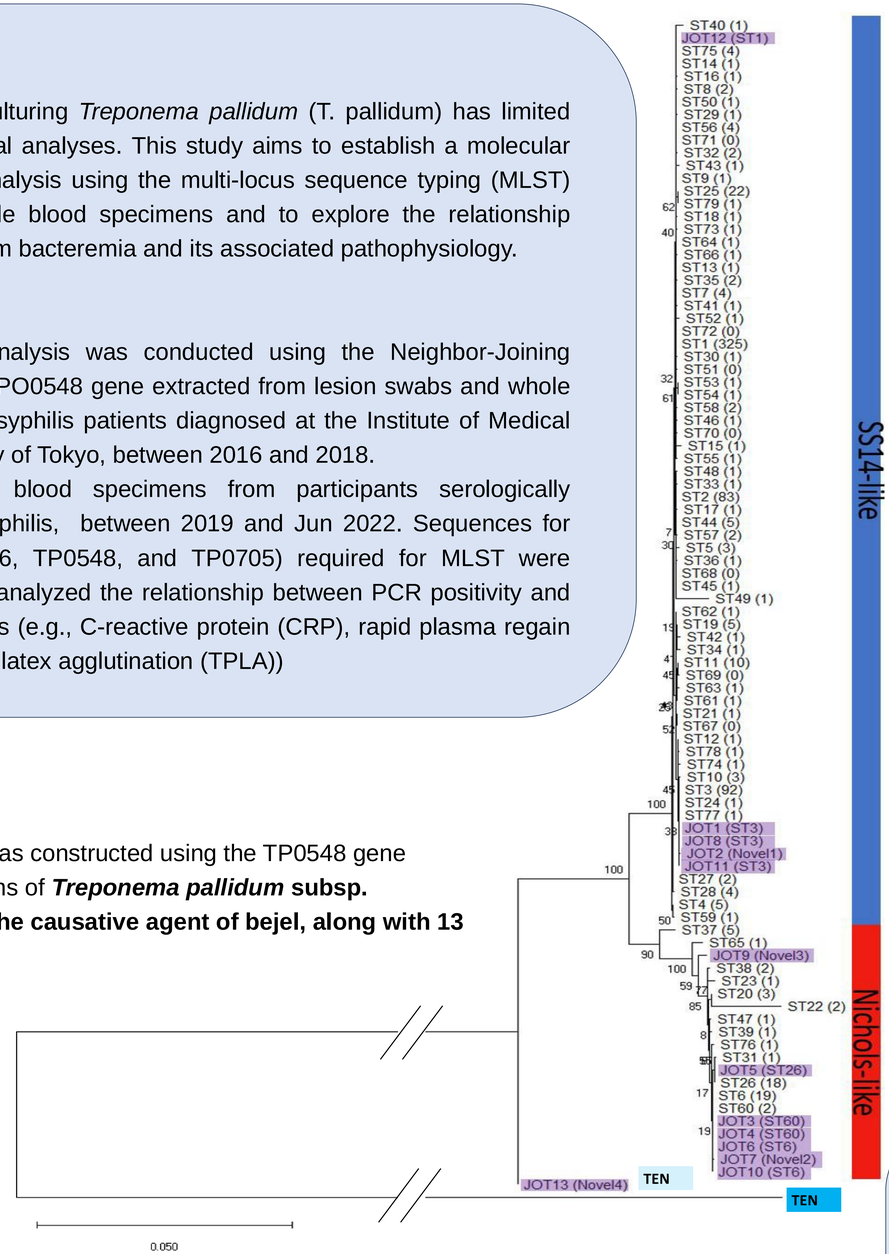
- The difficulty in culturing *Treponema pallidum* (T. pallidum) has limited molecular-biological analyses. This study aims to establish a molecular epidemiological analysis using the multi-locus sequence typing (MLST) scheme with whole blood specimens and to explore the relationship between T. pallidum bacteremia and its associated pathophysiology.

METHODS

- Epidemiological analysis was conducted using the Neighbor-Joining method with the TPO0548 gene extracted from lesion swabs and whole blood samples of syphilis patients diagnosed at the Institute of Medical Science, University of Tokyo, between 2016 and 2018.
- We used whole blood specimens from participants serologically diagnosed with syphilis, between 2019 and Jun 2022. Sequences for three loci (TP0136, TP0548, and TP0705) required for MLST were obtained, and we analyzed the relationship between PCR positivity and associated markers (e.g., C-reactive protein (CRP), rapid plasma regain (RPR), T. pallidum latex agglutination (TPLA))

RESULTS

- A phylogenetic tree was constructed using the TP0548 gene sequences from strains of ***Treponema pallidum* subsp. *endemicum* (TEN), the causative agent of bejel, along with 13 isolates**



- **From 2019 to 2021, of the 71 whole blood samples tested, 26 (36.6%) were PCR-positive for TP0136. Complete sequences for the three loci required for MLST were successfully obtained from 22 samples (31.0%)**
- The most frequently detected sequence type (ST) was ST3(1.1.8) (n=9), followed by ST6 (3.2.3) (n=6). Phylogenetic analysis showed that 12 samples belonged to the SS14-like genetic group (12/20, 60%), while 8 samples were classified within the Nichols-like group (8/20, 40%).

- A new allele was identified in TP0136**. Notably, TEN, was detected in three samples (12%, 3/26).**
- The Cochran-Armitage test for trend also failed to reveal an increase in TP0136 detection as TPLA levels increased (p = 0.056, Table 3). Similarly, the TP0136 detection rate did not exhibit a significant association with the RPR cut-offs of 10 RU (p = 0.40) or 100 RU (p = 0.21).
- The trend test did not show an increase in TP0136 detected as the RPR increased (p = 0.12).
- In contrast, positive CRP levels were significantly associated with TP0136 detection at cut-offs of 0.1 mg/dL (p = 0.0007) or 0.5 mg/dL (p < 0.0001).
- **For CRP ≥ 0.5 mg/dL, 77.0% were positive for TP0136. A trend test further revealed that TP0136 detection significantly increased as CRP levels increased (p < 0.0001)**

Table Characteristics of cases that TP0136 was detected.

		All cases	TP0136 not detected	TP0136 detected	P value
Number of cases		71	45	26	
Stage of syphilis, n (%)	Primary syphilis	7 (9.9)	3 (6.6)	4 (15)	0.41‡
	Secondary syphilis	23 (32)	12 (27)	11 (42)	0.19
	Early asymptomatic syphilis	35 (49)	26 (58)	9 (35)	0.085‡
	Neurosyphilis*	6 (8.5)	2 (4.4)	4 (15)	0.18‡
	Unknown	6 (8.5)	4 (8.9)	2 (7.8)	0.99‡
Serum TPLA titer,	Median, U/mL (IQR)	6571 (2582 - 15857)	5209 (2042 - 9858)	11262 (4527 - 23081)	0.0071†
	<1000 U/mL, n	7	6	1	0.056§
	1000 - 5000 U/mL, n	23	16	6	
	> 5000 U/mL, n	41	23	19	
Serum RPR titer	Median, RU (IQR)	75.7 (34.4 -188.5)	66.9 (27.3 -136)	115 (52.5 -317)	0.016†
	<10 RU, n	6	5	1	0.12§
	10 - 100 RU, n	36	25	12	
	> 100 RU, n	29	15	13	
CRP	Median, mg/mL (IQR)	0.23 (0.09 - 0.65)	0.13 (0.06 - 0.31)	0.865 (0.575 - 1.29)	<0.0001†
	<0.1 mg/dL, n	19	18	1	<0.0001§
	0.1 -0.5 mg/dL, n	26	21	5	
	> 0.5 mg/dL, n	26	6	20	

TPLA, Treponema pallidum latex agglutination; RPR, Rapid Plasma Reagin; CRP, C-reactive protein * All symptoms were indicative of secondary syphilis and were categorized as such † Mann-Whitney test, ‡ Fisher's exact test, § Cochran-Armitage test for trend

CONCLUSIONS

Our study unveiled the dissemination of SS14-like TPA in Japan, with the concurrent detection of Nichols-like strains. CRP emerged as a potential marker for evaluating PCR performance. Individuals diagnosed with syphilis may include those with bejel, and TEN could have been sexually transmitted among Japanese MSM. The MLST scheme, particularly when utilizing blood samples, proves to be valuable for broader research endeavors.

ADDITIONAL KEY INFORMATION

Additional Resources; https://doi.org/10.1186/s12981-024-00663-y

Author Contact Information: Eisuke Adachi, e-adachi@ims.u-tokyo.ac.jp

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