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# Identification of potential biomarkers for diagnosis of syphilis from the cerebrospinal fluid based on untargeted metabolomic analysis†

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The infection rate of syphilis continues to rise globally, and the difficulty in diagnosis of neurosyphilis promptly needs to be resolved. More specific and sensitive diagnostic markers for latent syphilis and neurosyphilis should be found. Here the metabolic profiles of 88 cerebrospinal fluid samples from syphilis patients and controls were analyzed by LC/MS-based untargeted metabolomics. In total, 272 metabolites based on 3937 features obtained in ESI− mode and 252 metabolites based on 3799 features in ESI+ mode were identified. The experimental process was evaluated by principal component analysis, partial least squares discriminant analysis, and hierarchical cluster analysis. A clear separation between latent syphilis and neurosyphilis was found. Levels of lipid and linoleic acid metabolites, such as 9-oxo-octadecadienoic acid and 9,10,13-trihydroxyoctadecenoic acid, were increased in syphilis patients. In patients with neurosyphilis, significant changes in levels of 5-hydroxy-L-tryptophan (5-HTP) and acetyl-*N*-formyl-5-methoxykynurenamine (AFMK) in the tryptophan–kynurenine pathway were also detected. Only one metabolite, theophylline, differed significantly between symptomatic and asymptomatic neurosyphilis patients. Additionally, KEGG analysis revealed significant enrichment of tryptophan metabolism pathways, indicating a high correlation between tryptophan metabolism and syphilis symptoms. Levels of linoleic acid metabolites, 5-HTP, AFMK and theophylline were significantly altered in different patients. The role of these differential metabolites in the development of syphilis is worthy of further exploration. Our results may promote the development of biomarkers for diagnosis of latent syphilis from neurosyphilis, and for that of asymptomatic neurosyphilis from symptomatic neurosyphilis in the future.

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## Introduction

Syphilis is a sexually transmitted disease caused by *Treponema pallidum* (*T. pallidum*) associated with significant complications if left untreated and it can facilitate the transmission and acquisition of HIV infection. Without effective treatment, *T. pallidum* usually invades the central nervous system and causes neurosyphilis in some patients.[1] Neurosyphilis used to be a complicated disease in clinical research. With the discovery of penicillin, the prevalence of neurosyphilis and syphilis was controlled.[2] However, since 2000, the number of cases of syphilis has begun to increase again, with more than 100 000 new cases in 2018 alone; by contrast, only approximately 60 000 new cases of gonorrhoea occurred in 2018.[3] The number of patients with neurosyphilis has also risen with the resurgence of syphilis. Previous studies reported that approximately 30% of all patients developed neurosyphilis, of whom 30% were asymptomatic.[4]

Latent syphilis and neurosyphilis are difficult to distinguish when the early clinical symptoms are not obvious, which is the best treatment time. Nevertheless, diagnosis is complicated, and there is no gold standard. A neurosyphilis diagnosis is mainly based on clinical manifestations, specific and nonspecific serological tests for syphilis, abnormal cerebrospinal fluid (CSF), and occasionally neuroimaging.[1,5] Serology of neurosyphilis is usually detected by traditional methods, such as the

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nontreponemal test using the Venereal Disease Research Laboratory (VDRL) or rapid plasma reagin, and treponemal tests, such as the *T. pallidum* agglutination test, to confirm positive results.[6] Even this negative result does not entirely rule out neurosyphilis, as VDRL may be negative in 30% to 70% of cases.[7] Therefore, more specific and sensitive diagnostic markers should be found.

Metabolomics, which can directly reflect altered metabolites and their interaction with various stimulating factors, has great potential in disease screening and diagnosis biomarker identification. CSF is a biological fluid that is greatly affected by the dysfunction of the central nervous system, and analysis of CSF can well reflect changes in the nervous and biochemical states of the body.[8,9] Indeed, CSF metabolomics has been widely used in various neurodegenerative diseases and brain tumours, and studies have reported potential CSF diagnostic markers for Alzheimer's disease and malignant glioma.[10,11] Nevertheless, the use of CSF-based metabolites for a probable diagnosis of neurosyphilis remains mostly unexplored.

In this study, to understand the pathogenesis of syphilis better, UPLC-Q Exactive-MS was conducted to analyze the metabolic profiles of CSF from different patients. By evaluating the original data by multistatistical analysis, significant differences in the levels of CSF metabolites between syphilis and neurosyphilis were detected. Our results could probably improve the diagnosis of latent syphilis from neurosyphilis, and early period of neurosyphilis which symptoms are not apparent from symptomatic neurosyphilis in the future.

# Methods

### Participants

Informed consent was obtained from all subjects in this study. All experiments were performed following the approved guidelines. All the participants with syphilis based on positive treponemal test and the toluidine red unheated serum test (TRUST) results underwent physical examinations, blood testing, and lumber puncture, in which blood and CSF samples were kept at −80 °C.[12,13] Furthermore, the patients recruited in this study had no metabolic issues, such as diabetes, diuretic use, excessive caffeine or other consumption (tea, specific herbs). Non-syphilis control was enrolled from patients with negative treponemol pallidum serological tests. HIV-positive patients and all other infective causes of CSF pleocytosis were excluded as well.

### Sample preparation

Serum and CSF samples were collected from participants. Serological test (TRUST and TPPA) and CSF tests (Cell counts, Differential, Microscopic exam; CSF Chemical tests and VDRL) were carried out. Approximately 1 mL of CSF was transported on ice immediately, and CSF metabolites were prepared within six hours after CSF collection and stored at −80 °C until use. In detail, CSF samples were thawed, and 100 μL of sample and 300 μL of methanol were transferred to a 1.5 mL Eppendorf tube and vortexed for 30 s. All samples were kept at −40 °C for 1 h, vortexed for 30 s and centrifuged at 12 000 rpm and 4 °C for 15 mins. Next, 200 μL supernatant and 5 μL DL-*o*-chlorophenylalanine (internal reference, 140 μg $mL^{-1}$) were transferred to vials for HPLC-MS analysis.

### LC/MS-based untargeted metabolomics

The HPLC-MS analysis was performed on an Ultimate 3000 UPLC system combined with Q-Exactive Orbitrap-MS (Thermo, Waltham, MA, USA). The LC system is comprised of an ACQUITY UPLC HSS T3 (100 × 2.1 mm 1.8 μm) with Ultimate 3000LC. QC samples were also injected at regular intervals (every ten samples) throughout the analytical run.[14] All of the raw metabolomic data have been deposited into the publicly accessible database PeptideAtlas and now are available under dataset Identifier PASS01659 (**https://www.peptideatlas.org/PASS/PASS01659**).

### MS data processing and statistical analysis

Raw data were acquired and aligned using Compound Discover (version 3.0, ThermoFisher Scientific, Waltham, MA, USA) according to the *m*/*z* value and the retention time of the ion signals. Normalized data were imported into the SIMCA-P program (version 14.1, Umetric, Umea, Sweden) for principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and the calculation of variable importance in projection (VIP). All data from differential compounds by fold change (FC) analysis and independent-sample t-test statistics were used to build PCA models. The quality of the models was described by relevant R2 and Q2 values. The cut-off criteria for screening differential metabolites were FC > 2, VIP > 1.5, and $P$ value < 0.05. According to databases, the chemical structures of important metabolites were then identified, such as the Human Metabolome Database (**https://www.hmdb.ca**), using accurate mass and MS/MS fragment data.

Hierarchical cluster analysis (HCA) was performed and visualized by using the embedded module of MetaboAnalyst 4.0.[15] Based on the differential metabolites, metabolic pathway and metabolite biofunction analyses were performed using the network database (KEGG pathway **https://www.genome.jp/kegg/**) to investigate the bioprocesses affected by *T. pallidum* infection. The enrichment level was calculated by the *t*-test, and metabolic pathways with $P$ values less than 0.05 were considered statistically significant.

# Results

### Clinical information for the study participants

According to the Centers for Disease Control guidelines, 33 patients were diagnosed with symptomatic neurosyphilis who were defined as CSF abnormalities (reactive CSF-VDRL or CSF-WBCs (white blood cells) > 5/μl) with neurological symptom; nine patients were diagnosed with asymptomatic neurosyphilis through CSF examination who were defined as a reactive CSF-VDRL or CSF-WBCs > 5/μl and absence of neurological symptom; and there were 39 latent syphilis patients without any symptom and with normal cerebrospinal fluid test results.

Table 1 Clinical information for the study participants

| Variable | Asymptomatic neurosyphilis patients (A) | Symptomatic neurosyphilis patients (S) | Latent syphilis patients (L) | Non-syphilis controls (N) | *P*-value | All |
|---|---|---|---|---|---|---|
| *n* | 9 | 33 | 39 | 7 | | 88 |
| Age (years) | 42.89[47] | 48.39[49] | 35.49[32] | 46.57[47] | 0.534 | 41.97[42.5] |
| Sex | | | | | 0.361 | |
| Male *n* (%) | 3(33.33%) | 24(72.73%) | 26(66.67%) | 4(57.14%) | | 44(50.00%) |
| Female *n* (%) | 6(66.67%) | 9(27.27%) | 13(33.33%) | 3(42.86%) | | 44(50.00%) |
| Serological test | + | + | + | − | | |
| CSF TRUST | + | + | + | − | | |
| Clinical symptoms | No obvious neurological symptoms | General paresis (19/33); tales dosalis (5/33); meningovascular syphilis (5/33); ocular syphilis and cranial neuritis *et al* (4/33) | No obvious clinical manifestations | — | | |
| Treatment | Penicillin injection followed by benzathine penicillin intramuscular injection | | Benzathine penicillin intramuscular injection | No penicillin | | |

CSF TRUST, cerebrospinal fluid toluidine red unheated serum test.

Of majority of the symptomatic neurosyphilis patients were general paresis (19/33); tales dosalis (5/33); meningovascular syphilis (5/33); others including ocular syphilis and cranial neuritis *et al.* were 4 of 33 symptomatic neurosyphilis. Because CSF samples from healthy people are challenging to obtain, the CSF samples collected from these seven patients in control groups were aim to exclude other neurologic diseases with negative treponemol pallidum serological test results.

Furthermore, patients with latent syphilis were treated with benzathine penicillin intramuscular injection (2.4 million U, once a week) for a total of 3 times. Patients with asymptomatic neurosyphilis and symptomatic neurosyphilis were treated with Penicillin injection (18–24 million U per d, 3 million to 4 million U per 4h, intravenous infusion or continuous intravenous infusion) for consecutive 10–14 days, followed by benzathine penicillin intramuscular injection (2.4 million U, once a week) for a total of 3 times. No penicillin was used in non-syphilis participants. The clinical characteristics of the patients are summarized in Table 1.

### Quality control and overall metabolic profile

Data were subjected to a data integrity check before subsequent analysis, and no missing values were detected. The relative standard deviation (RSD) calculated from the ion features of the quality control (QC) samples showed RSD values mostly less than 30%, indicating that the analysis program was reliable and could be used for subsequent sample analysis. The %RSD distribution is presented in Fig. 1A and B. The base peak intensity chromatograms of the sample are illustrated in Fig. 1C and D. Among samples, 3799 and 3937 features were obtained in ESI+ and ESI− modes, respectively.

PCA was carried out using the molecular features of all the groups, and the distribution of metabolic profiles for the test samples in PCA was shown in Fig. 2A. The samples in each group were tightly clustered, but the difference between the groups was not large enough for a clear distinction. To eliminate any nonspecific effects of the technique and confirm the biomarkers for differentiating the metabolite profiles of non-syphilis controls and other syphilis patients (including latent syphilis and neurosyphilis patients), a supervised PLS-DA model was established focused on the actual class discriminating variation. As depicted in Fig. 2B, due to the individual differences of the control samples, the distribution in the larger picture was scattered, but it was separated from the patient samples, with a clear distinction; R2Y was calculated to be 0.986 and Q2Y 0.632, which are greater than 0.4. To investigate dynamic changes in CSF metabolic profiles from syphilis to neurosyphilis, metabolomic data for the 39 latent syphilis and 42 neurosyphilis samples were analyzed by PLS-DA. As shown in Fig. 2C, we observed a clear separation between the two groups. Specifically, R2 and Q2 were 0.992 and 0.873 for differentiating syphilis patients and neurosyphilis controls, respectively. In addition to the obvious clinical manifestations, there was a significant difference in the metabolic spectrum between asymptomatic neurosyphilis and symptomatic neurosyphilis. According to the PLS-DA results (Fig. 2D), there was a significant separation between the two groups with Q2Y = 0.818 and R2Y = 0.995.

### Identification of differential CSF metabolites and pathway analysis

Based on the database search, 272 metabolites were identified in ESI− mode and 252 in ESI+ mode (Table S1, ESI†). Metabolites with VIP greater than 1.5 were included in t-tests to assess significant differences in CSF metabolites between different patients. A total of 20 metabolites obtained from the metabolic spectrum in both modes were filtered out compared to syphilis and non-syphilis control group, including ascorbic acid, acetaminophen, and fucose, which play an essential role in the immune system or nervous system. Next, five significantly different metabolites (including nicotine, trihydroxycoprostanoic acid, 4-hydroxybenzoic acid (4-HBA), 5-hydroxy-L-tryptophan (5-HTP), and acetyl-*N*-formyl-5-methoxykynurenamine (AFMK) were found between patients with latent syphilis and neurosyphilis. However, only theophylline showed a significant difference in comparison of the metabolic profiles of symptomatic neurosyphilis and asymptomatic neurosyphilis. Differences in expression of 5-HTP and AFMK between the two types of patients were also found, which with no significant differences ($0.05 < P < 0.1$). Levels of

 

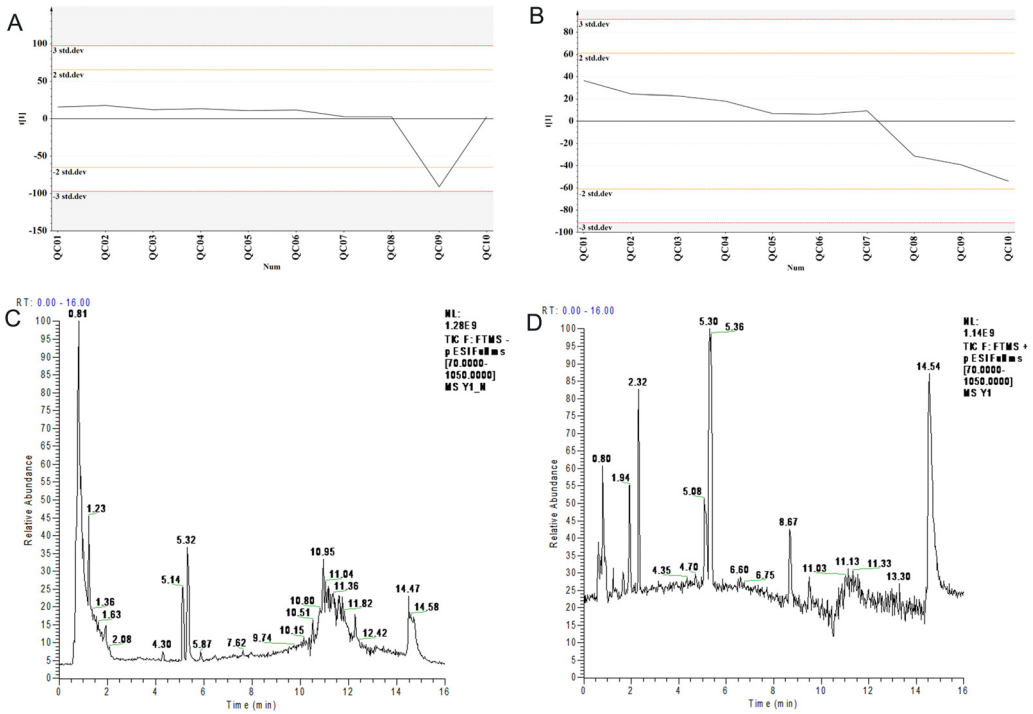


Fig. 1 (A) The score plot of the quality control (QC) samples in ESI− mode; (B) the score plot of the quality control (QC) samples in ESI+ mode. The *X*-axis indicates the number of QC samples; the *Y*-axis indicates the range of relative standard deviation (RSD). (C) Base peak intensity chromatograms of the sample in ESI− mode. (D) Base peak intensity chromatograms of the sample in ESI+ mode.

both metabolites were up-regulated more than three times in the samples from patients with symptomatic neurosyphilis. It is worth noting that the level of 5-HTP differed between the non-syphilis control group and latent syphilis, symptomatic neurosyphilis, and asymptomatic neurosyphilis groups. The significant differential metabolite components are described in Table 2. According to the differential metabolites screened, a heat map of HCA was generated based on Euclidean distance and the average clustering algorithm, as shown in Fig. 3.

Next, we performed a KEGG analysis of the differential metabolites to examine the effect of *T. pallidum* infection on metabolic pathways. The results showed that these differential metabolites were enriched in amino acids and metabolic intermediates of various acids. The metabolic pathways of enrichment are mainly related to amino acid metabolism, energy metabolism, and phosphatidic acid metabolism, such as the tricarboxylic acid cycle. Fig. 4 shows that there were marginally significant differences ($0.01 < P < 0.1$) between the groups: the citric acid cycle and gluconeogenesis pathway (syphilis *vs.* non-syphilis), ubiquinone biosynthesis pathway (latent syphilis *vs.* neurosyphilis), tryptophan metabolism and caffeine metabolism pathway (symptomatic *vs.* asymptomatic neurosyphilis). In particular, tryptophan metabolism was significantly different ($P < 0.01$) between neurosyphilis and latent syphilis groups.

## Discussion

*T. pallidum* could invade the nervous system during early primary syphilis[16,17] Previously, Yang's team conducted a metabolomic analysis of serum and CSF samples from patients with syphilis and neurosyphilis.[18] Several significant metabolites, *i.e.*, L-gulono-gamma-lactone, *D*-mannose, *N*-acetyl-L-tyrosine, hypoxanthine and *S*-methyl-5′-thioadenosine, were identified in CSF and trimethylamine N-oxide in serum from neurosyphilis patients. Previous studies performed in only syphilis patients, or neurosyphilis patients, or both were provided and compared in Table S2 (ESI†). In this study, a more detailed metabolomic analysis was carried out on 88 CSF samples from patients with neurosyphilis (symptomatic and asymptomatic) and latent syphilis as well as those without syphilis. The metabolic analysis detected more than 400 metabolites, of which 20 were significantly different between syphilis and non-syphilis control group and five between neurosyphilis and latent syphilis patients. Only theophylline was significantly different between symptomatic and asymptomatic neurosyphilis patients. KEGG pathway enrichment analysis revealed that tryptophan metabolism pathways were significantly enriched, indicating a high correlation between tryptophan metabolism and syphilis symptoms.

Significant differences in metabolites and pathways were found between the syphilis group and the non-syphilis control group. The levels of 9,10,13-trihydroxyoctadecenoic acid (9,10,13-TriHOME), 9-oxo-octadecadienoic acid (9-OxoODE), azelaic acid, and phosphatidylserine (PS) (16 : 0/14 : 0) were found to up-regulated in the syphilis group, but 2-hemoglobin (2-HB) and guanosine diphosphate (GDP) were significantly down-regulated. Among the up-regulated metabolites, 9,10,13-TriHOME, 9-OxoODE, and PS (16 : 0/14 : 0) are lipid metabolites. PS is an active substance on the cell membrane, especially in brain cells, and its main function is to improve the function of

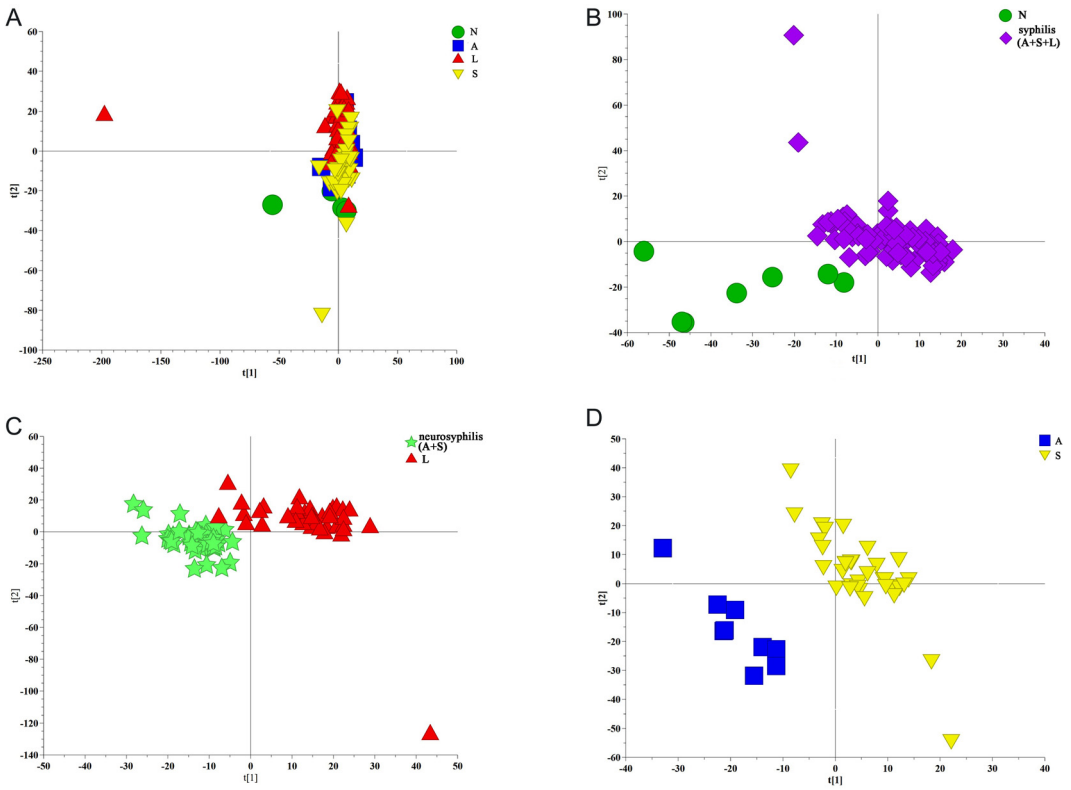


Fig. 2 (A) The scores scatter plot of principal components analysis (PCA) model, R2X = 0.422. (B–D) The scores scatter plot of partial least squares discriminant analysis (PLS-DA) model. (B) The PLS-DA model based on non-syphilis controls and syphilis patients; (C) the PLS-DA model based on latent syphilis and neurosyphilis patients; (D) the PLS-DA model based on asymptomatic and symptomatic neurosyphilis patients. L: latent syphilis; N: non-syphilis controls; S: symptomatic neurosyphilis; A: asymptomatic neurosyphilis.

Table 2 List of differential cerebrospinal fluid metabolites in comparisons of different study groups. The cut-off criteria for screening differential metabolites were FC > 2, VIP > 1.5, and *P* value < 0.05

| Comparison | Metabolite | RT [min] | Monoisotopic mass | VIP | FC | *P* value |
|---|---|---|---|---|---|---|
| Syphilis *vs.* non-syphilis | 2-Hydroxybutyric acid | 1.626 | 104.046 | 2.881 | 0.490 | <0.001 |
| | Azelaic acid | 2.739 | 188.104 | 1.535 | 2.020 | 0.027 |
| | 9-OxoODE | 4.994 | 294.219 | 1.897 | 4.422 | 0.009 |
| | 9,10,13-TriHOME | 5.026 | 330.241 | 2.222 | 5.125 | 0.001 |
| | Guanosine diphosphate | 1.579 | 443.012 | 2.340 | 0.105 | 0.001 |
| | PS (16 : 0/14 : 0) | 4.116 | 707.466 | 1.763 | 3.049 | 0.015 |
| Latent syphilis *vs.* neurosyphilis | 4-Hydroxybenzoic acid | 2.323 | 138.032 | 2.039 | 4.177 | <0.001 |
| | 5-Hydroxy-L-tryptophan | 1.518 | 220.084 | 1.797 | 4.674 | 0.0003 |
| | Acetyl-*N*-formyl-5-methoxykynurenamine | 2.320 | 264.111 | 1.850 | 3.882 | 0.0002 |
| | Nicotine | 0.822 | 162.115 | 2.037 | 2.927 | <0.001 |
| | Trihydroxycoprostanoic acid | 10.682 | 464.361 | 2.298 | 2.672 | <0.001 |
| Asymptomatic *vs.* symptomatic neurosyphilis | Theophylline | 1.918 | 180.065 | 3.091 | 9.718 | 0.001 |

RT for retention time; VIP for variable importance in projection; FC for fold change; 9-oxoODE for 9-oxo-octadecadienoic acid; 9,10,13-TriHOME for 9,10,13-trihydroxyoctadecenoic acid; PS for phosphatidylserine.

nerve cells, sooth vascular smooth muscle cells and increase blood supply to the brain.[19] It has been confirmed that the membrane protein of *T. pallidum* contains phosphatidylserine lipoprotein, which may explain the increase in PS.[20] This might distinguish syphilis from false-positive VDRL caused by antiphospholipid syndrome due to antiphospholipid antibodies. In general, 9,10,13-TriHOME and 9-OxoODE are related to the oxidative metabolism of linoleic acid.[21,22] TriHOMEs are the end product of linoleic acid oxidation, which have a physiological role in maintaining the water-skin barrier, though other physiological effects are unclear.[23] Linoleic acid has a neuroprotective effect, and its deficiency has been found in patients with mild neurological disorders and Alzheimer's disease.[24] This may also explain the increase in linoleic acid metabolites in syphilis patients. Downregulated 2-HB is associated with dyslipidemia, involved in glutathione oxidative stress, and elevated in patients with depression.[25]

 

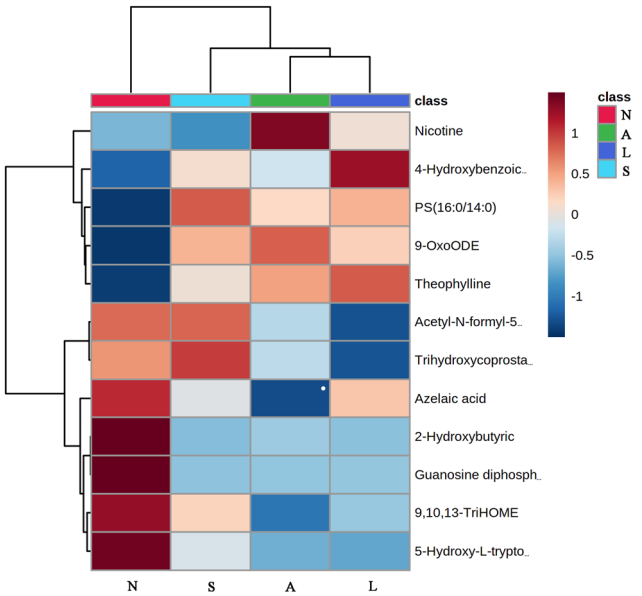


Fig. 3 Heat map based on differential metabolites of latent syphilis (L), asymptomatic neurosyphilis (A), symptomatic neurosyphilis (S) and non-syphilis controls (N).

Significant differences in 5-HTP and AFMK were found in neurosyphilis patients compared with latent syphilis, and it may constitute a marker of whether *T. pallidum* has invaded the nervous system. In some lymph node tumour cells, expression of indoleamine-2,3-dioxygenase is increased by activating regulatory T cells (Tregs) that escape the host immune system.[26,27] This enzyme is involved in the breakdown of indoleamine (tryptophan, 5-hydroxytryptamine, and melatonin). AFMK participates in the kynurenine pathway, one of the three main metabolic pathways of melatonin and has potent antioxidant and anti-inflammatory abilities.[28] The high levels of AFMK observed in this study may result from melatonin production by local immune cells. 5-HTP is decarboxylated by aromatic L-amino acid decarboxylase with vitamin B6 as a cofactor to form serotonin (5-hydroxytryptophan, the precursor of melatonin). 5-HTP contributes to serotonin production, promoting emotional and nervous system health. Both melatonin and serotonin are important metabolites in regulating emotion. It has been suggested that the tryptophan–kynurenine pathway may be involved in depression by mediating the inflammatory response.[25] This may explain why some symptoms of neurosyphilis are consistent with depression; it also suggests that more attention should be paid to the mental state and emotional counselling of patients during treatment for neurosyphilis.

Only one metabolite was found to differ between symptomatic and asymptomatic neurosyphilis patients, which may be due to the different organs involved in symptomatic neurosyphilis. Theophylline is a metabolite of caffeine that can increase CSF secretion to regulate intracranial pressure and relieve headaches.[29,30] Theophylline also reduces smooth muscle tension, promotes endogenous epinephrine and norepinephrine release, inhibits calcium release from the endoplasmic reticulum of smooth muscle, and decreases intracellular calcium concentrations for respiratory tract dilation.[31–33]

Due to the difficulty of collecting CSF samples in healthy individuals, this study enrolled few control samples from non-syphilis patients who underwent lumbar puncture for excluding neurological diseases. Only preliminary conclusions were obtained in this study, and the sample size of non-syphilis controls needs to be expanded for follow-up research. Nonetheless, some interesting conclusions were obtained. In the comparison between syphilis patients and non-syphilis control group, significant differences in lipids and derivatives were detected, indicating that lipid metabolism plays a vital role in syphilis. Thus, liposomics may be used as a further research direction. As majority of symptomatic neurosyphilis is usually the result of the late development of untreated syphilis, it is important to promptly distinguish neurosyphilis from latent syphilis, especially from sero-fast syphilis patients. Both 5-HTP and AFMK are important metabolites for maintaining the health of the nervous system. Although differences in other comparisons were found for 5-HTP, they were not significant. The findings suggest that the mental state and emotional change of patients should be paid more attention to screen neurosyphilis. The metabolites identified should be further studied to determine their relationship with *T. pallidum* infection.

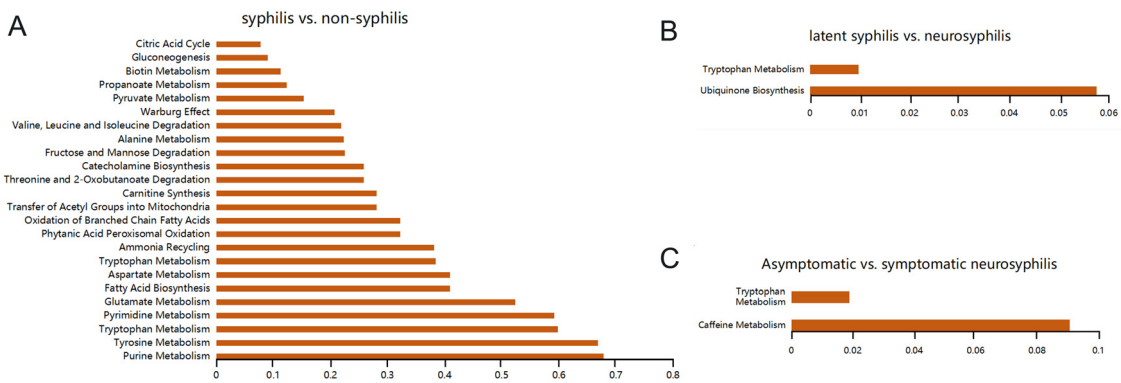


Fig. 4 The bar chart of enriched KEGG pathways of the differential metabolite components in the comparisons. The *X*-axis indicates the *p*-value of each pathway; *Y*-axis indicates the name of the pathway.

## Conclusions

In conclusion, we performed a metabolomics analysis of CSF samples from patients with neurosyphilis (including both of symptomatic and asymptomatic neurosyphilis) and latent syphilis. And the limitation of the present study was that only two small cohorts of non-syphilis and asymptomatic neurosyphilis were provided in the present study. No conclusion can be draw with the two small cohorts (non-syphilis and asymptomatic) due to their size, and only for speculation and perspectives could be made. Through comparative analysis, we found that levels of linoleic acid metabolites were up-regulated in patients with syphilis. 5-HTP and AFMK in the tryptophan metabolism pathway were also significantly altered in neurosyphilis. Theophylline levels were significantly up-regulated in patients with symptomatic neurosyphilis. The role of these differential metabolites in the development of neurosyphilis is worthy of further exploration. This analysis could improve the diagnosis of latent syphilis from neurosyphilis, and early period of neurosyphilis which symptoms are not apparent and virtually absent from symptomatic neurosyphilis in the future.

## Author contributions

LL and DX performed the experiments and carried out data analysis. FC, SC and JW designed experimental procedures. JD and FC performed the experiments. LL and JZ conceived the study and wrote the manuscript. JZ, QJ and WL supervised this study. All authors read and approved the submitted version.

## Data availability

All of the raw metabolomic data have been deposited into the publicly accessible database PeptideAtlas and now are available under dataset Identifier PASS01659 (**https://www.peptideatlas.org/PASS/PASS01659**).

## Conflicts of interest

The authors have declared no conflicts of interests.

## Acknowledgements

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