

Vaginal homeostasis features of Vulvovaginal Candidiasis through vaginal metabolic profiling

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Abstract

Vulvovaginal candidiasis (VVC) is an inflammatory disease primarily caused by candidiasis albicans infection. Metabolomics has been applied to research a variety of inflammatory diseases. In the present study, the vaginal metabolic profiles of VVC patients and healthy populations were explored by a non-targeted metabolomics approach. In total, 211 differential metabolites were identified, with the VVC group having 128 over-expressed and 83 under-expressed metabolites compared with healthy individuals. Functional analysis showed that these metabolites were mainly involved in amino acid metabolism and lipid metabolism. In addition, network software analysis indicated that the differential metabolites were associated with MAPK signaling and NF- κ B signaling. Further molecular docking suggested that linoleic acid can bind to the ACSL1 protein, which has been shown to be associated with multiple inflammatory diseases and is an upstream regulator of the MAPK and NF- κ B signaling pathways that mediate inflammation. Therefore, our preliminary analysis results suggest that VVC has a unique metabolic profile. Linoleic acid, a significantly elevated unsaturated fatty acid in the VVC group, may promote VVC development through the ACSL1/MAPK and ACSL1/NF- κ B signaling pathways. This study's findings contribute to further exploring the mechanism of VVC infection and providing new perspectives for the treatment of Candida albicans vaginal infection.

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Abstract

Vulvovaginal candidiasis (VVC) is an inflammatory disease primarily caused by candidiasis albicans infection. The condition has good short-term treatment effects, high recurrence, and seriously affects the quality of life of women. Metabolomics has been applied to research a variety of inflammatory diseases. In the present

study, the vaginal metabolic profiles of VVC patients and healthy populations (CTL) were explored by a non-targeted metabolomics approach. In total, 211 differential metabolites were identified, with the VVC group having 128 over-expressed and 83 under-expressed metabolites compared with healthy individuals. Functional analysis showed that these metabolites were mainly involved in amino acid metabolism and lipid metabolism. In addition, network software analysis indicated that the differential metabolites were associated with MAPK signaling and NF- κ B signaling. Further molecular docking suggested that linoleic acid can bind to the ACSL1 protein, which has been shown to be associated with multiple inflammatory diseases and is an upstream regulator of the MAPK and NF- κ B signaling pathways that mediate inflammation. Therefore, our preliminary analysis results suggest that VVC has a unique metabolic profile. Linoleic acid, a significantly elevated unsaturated fatty acid in the VVC group, may promote VVC development through the ACSL1/MAPK and ACSL1/NF- κ B signaling pathways. This study's findings contribute to further exploring the mechanism of VVC infection and providing new perspectives for the treatment of *Candida albicans* vaginal infection.

Keywords

Vulvovaginal candidiasis (VVC), Metabolomics, Linoleic acid, ACSL1, Protein spatial conformation

Introduction

Vulvovaginal candidiasis (VVC) is an exceedingly common infection of female vulvovaginal inflammations[1]. The disease is estimated to affect 75% of women at least once during their lives, and approximately 8% of women develop recurrent VVC (RVVC)[2, 3]. The most common clinical symptoms of VVC are increased vaginal discharge that resembles bean curd residue accompanied by vulval itching and burning, leading to dyspareunia and dysuria in more severe cases[4]. While VVC is non-lethal, the morbidity associated with VVC brings tremendous mental distress, causing pain, low self-esteem, anxiety, and even impacting affective relations and career[5]. Moreover, VVC has been reported to cause adverse conditions such as cervicitis [6], pelvic inflammatory disease[7], genital tract malignancy[8], infertility[9], fetal intrauterine infection[10], HPV[11], and preterm birth[12].

A variety of microorganisms are present in the vagina of healthy women, which form a mutually regulated, coordinated, and dynamically balanced vaginal microenvironment. The balance of the vaginal microenvironment plays an important role in maintaining the self-cleaning function of the vagina and the health of the host. *Lactobacillus* accounts for the majority of the normal vaginal microbiota and helps defend against pathogens by lowering the vaginal pH to maintain an acidic environment[13]. Furthermore, cytokines and chemokines are regulated to maintain an anti-inflammatory vaginal environment[14], competing for adhesion sites to form a mechanical barrier[15], and secreting bio-surface-active substances to regulate the host immune response[16, 17]. However, dysbiosis of the vaginal microbiota occurs due to a variety of endogenous or exogenous factors that cause an imbalance in the vaginal internal environment. The main pathogens causing vaginal inflammatory diseases include *Gardnerella vaginalis*, *Candida albicans*, and *Trichomonas vaginalis*, which are associated with bacterial vaginitis, vulvovaginal candidiasis, and trichomoniasis, respectively.

Candida albicans is a serious opportunistic fungal pathogen[18] and is the most common infectious yeast strain in women with VVC, showing a prevalence of 90%[19]. It can colonize the mucosal surfaces of the genitourinary and gastrointestinal tracts, as well as the asymptomatic oral cavity and skin of healthy people[2]. Changes in microbial community composition and systemic or local immunosuppression (e.g., pregnancy, diabetes, allergies, broad-spectrum antibiotics, oral steroids, psychosocial stress, estrogen, and sexual activity) may result in increased load and virulence of *C. albicans*, which may exceed the tolerance threshold of epithelial cells, leading to the production and release of pro-inflammatory factors and inducing an intense inflammatory response[20]. However, *C. albicans* overload is not the only cause of VVC, as it can invade host cells by inducing endocytosis and active penetration[21]. Endocytosis is a passive process mediated by epithelial cells in which *C. albicans* transforms from a yeast phase to a more adherent mycelial phase, leading to loss of epithelial integrity and barrier function through the interaction of the fungal invasin Als3 and host E- or N-cadherin on the surface of the mycelium[22]. Meanwhile, *C. albicans* secreted related enzymes (SAPs)

can promote tissue invasion by activating epithelial calpain1, mediating active fungal penetration[23]. In addition, mycelial-phase *C. albicans* produces a peptide toxin "candida lysin" that activates the p38/cFos and ERK/MKP1 signaling pathways by recruiting innate immune cells such as neutrophils, macrophages, and innate type 17 cells, thereby leading to cytokine and chemokine secretion[24]. Furthermore, candida lysin promotes *Candida* invasion by affecting mitochondria and altering immunomodulatory signaling in vaginal epithelial cells [25].

VVC is typically treated with short-term topical and single-dose oral antimycotic agents to restore normal vaginal flora[26]. Nevertheless, due to the high level of resistance to commonly used antifungal drugs, antibiotic efficacy is greatly reduced and a high recurrence rate is observed despite standardized and multiple courses of antifungal therapy[27]. Although VVC has been studied for more than ten years[28], little is known about its pathogenesis. Therefore, exploring the pathogenesis of VVC and identifying new targets for alternative antibiotic therapy has great significance.

Metabolomics is an important part of modern omics research. Potential biomarkers are identified by studying the upstream biological processes, their metabolites, and metabolic pathways[29]. With the development of metabolomics, a growing number of metabolites have been discovered and studied as biomarkers, providing new insights for disease diagnosis, pathogenesis, and drug intervention. Furthermore, the metabolic profile of the cervicovaginal microenvironment can effectively be used to distinguish between HPV infection, cervical dysplasia, and ICC[30]. Several metabolites are significantly associated with the clinical signs and symptoms of bacterial vaginosis (BV), which has obvious metabolic signatures across multiple metabolic pathways[31]. Moreover, the composition of bacterial communities is affected during genital infection due to the changes in vaginal metabolome composition; significantly elevated metabolites such as TMA-NO_x (TMAO), taurine, and methanol have been observed in VVC vaginal discharge[32]. Nonetheless, the potential role of vaginal bioactive metabolites in the development of VVC has not been reported.

In the present study, vaginal discharge profiling was performed with non-targeted metabolomics methods, such as liquid chromatography-mass spectrometry (LC-MS), to explore the difference in the metabolite expression profiles between VVC patients and normal controls (CTL). In addition to analyzing the global characteristics between VVC and CTL, active substances affecting *C. albicans* infection could also be identified, providing insights into the mechanism of VVC infection and the corresponding defense mechanism of the body. The findings highlight a new perspective on the treatment of *C. albicans* vaginal infection.

Materials and Methods

Study subjects and sample collection

This study included subjects aged 20 to 45 years who individuals with VVC and normal controls. Clinical samples from female patients attending the Department of Gynecology of Tongren Hospital were randomly collected, and 14 VVC-diagnosed patients and 15 normal controls were recruited after vaginal discharge testing. All individuals were informed and provided written informed consent, and the study was approved by the Ethics Committee of Tongren Hospital, Shanghai Jiao Tong University. The participants were screened, women who were pregnant, postmenopausal, menstruating, taking antibiotics and antifungals during the first three months of the study, using vaginal rinse during the first three weeks of the study, having sexual intercourse or using vaginal lubricants within 48 hours prior to sample collection were excluded from the study.

Samples of vaginal discharge from 29 subjects were collected on sterile cotton swabs, two samples per person, half of which were made into smears for Gram stain and sent to the Department of Clinical Laboratory, Shanghai Tongren Hospital for examination. The other half collected cotton swabs from the vaginal discharge of the study participants were added with 0.3mL solvent (methanol) containing internal standard (4μg/mL, L-2-chloro-phenylalanine), and were clarified by centrifugation (13000rpm for 10 min at 4°C). Frozen 200μL of supernatant at -80°C for subsequent global metabolomics identification and quantification analysis.

Detection of Metabolic Profiling by LC-MS

LC-MS analysis uses Ultra-High Performance Liquid chromatography (Ultimate 3000, USA) combined with the Thermo-Orbitrap Elite mass spectrometer. Metabolic profiling was performed in electrospray ionization (ESI) positive and ESI negative ion mode using an ACQUITY UPLC I-Class system (Waters Corporation, USA) coupled with an AB SCIEX Triple TOF 5600 System (AB SCIEX, USA). The binary gradient elution systems consisted of water containing 0.1% formic acid (v/v, A) and acetonitrile containing 0.1% formic acid (v/v, B). Separation was achieved using the following conditions: 5% B for 0-2min; 5-95% B for 2-13min; and afinal 95% B for 13-15min; post time was 5min. The chromatographic conditions were as follows: injection volume was 3 μ l; column temperature was 25 °C; flowrate was 0.4ml/ min; post time was 5min. Mass spectrometry uses positive ion mode combined with negative ion mode with the following parameters: Positive mode: Heater Temp 300 °C, Sheath Gas Flow rate, 45arb, Aux Gas Flow Rate , 15 arb, Sweep Gas Flow Rate, 1arb, spray voltage, 3.0KV, Capillary Temp, 350 °C, S-Lens RF Level, 30%. Scan ranges: 200-1500. Negative mode: Heater Temp 300 °C, Sheath Gas Flow rate, 45arb, Aux Gas Flow Rate, 15arb, Sweep Gas Flow Rate, 1arb, spray voltage, 2.5KV, Capillary Temp, 350 °C, S-Lens RF Level, 60%. Scan ranges: 200-1500.

Statistical Analysis

The compound Finder Software (Thermo Science, USA) was used for compound component extraction and data preprocessing in samples, including analysis such as baseline filtering, peak identification and integration, retention time correction, peak alignment, and mass fragment attribution. The edited data matrix was imported into Simca-P software (version 11.0) for multivariate statistical analysis after Centralization and Pareto scaling. The dissimilarities in the metabolome dataset among the groups were analyzed using principal component analysis (PCA) and partial least squares discrimination analysis (PLS-DA). The VVC and Control groups were looked for differentially expressed metabolites according to the variable importance in projection (VIP) value (>1) of the PLS-DA model, statistical significance was observed at P value < 0.05 . Receiver Operating Characteristics (ROC) analysis was used to identify the metabolites that distinguished the patients in the VVC group from control group and the strength of the discriminators were measured with the Area Under the Curve (AUC) values. AUC values above 0.8 were considered as good and above 0.9 were considered as excellent discriminators. Furthermore, we used the human metabolome database (HMDB, <https://hmdb.ca>) and our internal standard metabolite library to identify and analyze the metabolites. Additionally, the Kyoto Encyclopedia of Genes and Genomes (KEGG) online database and the ingenuity pathway analysis (IPA) server were applied to understand networks of the metabolic pathways between differential metabolites.

Molecular Docking

The 3D structure in SDF format was acquired from PubChem data according to the CAS number (60-33-3) of the small molecule metabolite linoleic acid, and the structure was imported into ChemBio3D Ultra 14.0 for energy minimization. The Minimum RMS Gradient was set to 0.001, and the small molecule metabolite was saved in mol2 format. The protein long-chain acyl-CoA synthetase 1 (ACSL1) that interacts with the small molecule metabolite linoleic acid was identified from the database STITCH (<http://stitch.embl.de/>). Downloaded the protein structure of ACSL1 from the Uniprot database, and the protein structure was processed to remove protein crystal water, original ligand, etc on PyMOL2.3.0 platform. The optimized small molecules and protein structures were imported into AutodockTools-1.5.6 software for hydrogenation, charge calculation, charge distribution, and saved as "pdbqt" format as ligands for molecular docking. POCASA 1.1 was used to predict protein binding sites, and the interaction mode analysis of molecular docking results were completed by PyMOL2.3.0 software.

Results

General and Morphological Characteristics of Study Participants

The demographic information and clinical characteristics of the 29 patients are summarized in Table 1. In total, 15 patients were included in the normal group (CTL), and 14 were diagnosed with vulvovaginal candidiasis (VVC) due to *C. albicans*. There were no significant differences in age between the groups. The

median body mass index (BMI) across the groups was around 23, with no significant difference between the two groups. Moreover, both the Nugent score and AV score of the subjects were 0-1/0-2, showing no significant difference between the CTL and VVC groups. The LBG classification was also similar between the two groups. However, women in the VVC group exhibited symptom and sign scores [?]7 (Table 2); controls scored only 0-1 on this criterion. All subjects suffering from VVC had a disruption of the vaginal microbiome, and the cleanliness of vaginal discharge was III-IVdeg.

Table 1. Characteristics of Study Participants

	CTL(n=15)	VVC(n=14)
Enrolment criteria	Asymptomatic and negative microbiological tests	Vaginal symptoms and detection of C. .
Age, yr, median (range)	29 (20, 46)	40 (27-45)
BMI, kg/m2, median (range)	23.6 (21.5, 25.2)	23.0 (20.6, 26.3)
Vaginal microbiome	Balance	Imbalance
Leucorrhea cleanliness	I-II°	III-IV°
Nugent score	0-1	0-2
LBG classification	I	I-IIa
AV score	0-1	0-2
VVC symptom and sign score	0-1	[?]7

Table 2. VVC symptom and sign scoring criteria

	0	1	2	3
Itching	-	Occasional	Frequent	Persistent
Pain	-	Mild	Moderate	Severe
Hyperemia, Edema	-	Mild	Moderate	Severe
Scratches, Rhagadia, Erosion	-	-	-	+
Amount of discharge	-	Slightly more than normal	Large amount, no spillage	Large amount, spillage

Microscopic observation of the morphological features of Gram stain of vaginal discharge in healthy people and VVC patients captured 2 typical smears. Lactobacillus genus morphology showed gram-positive rods (Fig. 1A), while Candida spore morphology demonstrated gram-positive oval cells (Fig. 1B); the blastospores had sprouts and hyphae extended from the germ tubes. The observed morphological features of Lactobacillus and Candida species were consistent with those described in a previous study[33].

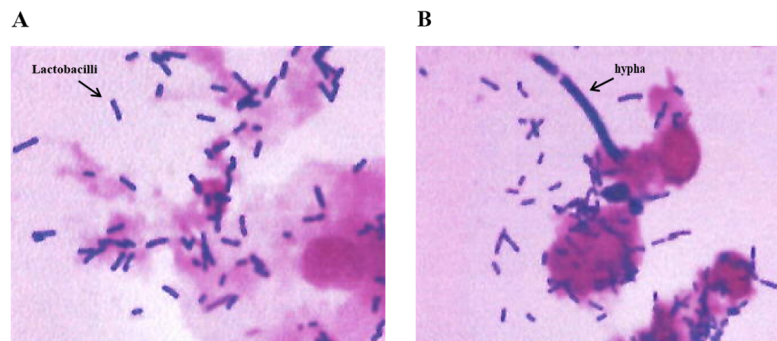


Figure 1. Microscopic visualization of vaginal gram-stain smears of 2 typical subjects. (A) vaginal smears in healthy women: a large number of Lactobacilli and normal epithelial cells ($\times 400$). (B) vaginal smears in patients with VVC: presence of Candida spores, blastospores and hyphae ($\times 400$).

Differences in Metabolic Profiles of CTL and VVC

In order to identify the differences in the metabolic profile of the CTL and VVC groups, the vaginal metabolites were analyzed in healthy individuals and VVC patients. The established PCA model revealed clusters in the VVC group that were significantly alienated from the CTL group on both PC1 and PC2 in positive and negative modes (Fig. 2A). A supervised PLS-DA model was obtained with two principal predictive components, which indicated a clear dispersion between the two groups (Fig. 2B). Furthermore, a permutation test of the PLS-DA model was implemented. The R^2 and Q^2 intercept values were (0.0, 0.639) and (0.0,

-0.751) in positive mode between CTL and VVC groups, while the R2 and Q2 intercept values were (0.0, 0.758) and (0.0, -0.554) in negative mode (Fig. 2C). The above results suggested that the metabolism of the CTL group was significantly different from that of the VVC group.

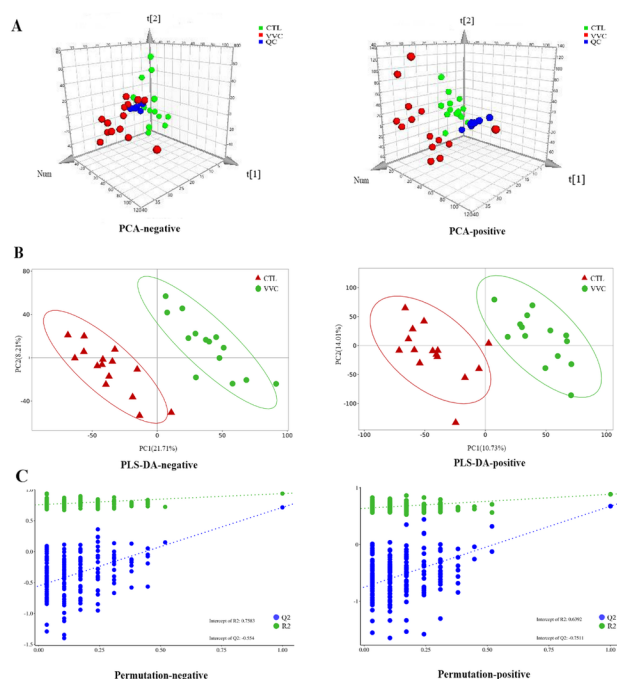


Figure 2. Differential metabolic profiles of VVC vs CTL groups. (A) Principal component analysis (PCA) suggested that VVC has unique metabolic characteristics. (B) Statistical validation of permutation analysis (200 times) of PLS-DA models for VVC and CTL. (C) The permutation tests were performed based on LC-MS data from the VVC group and the CTL group. The intercept values of the regression line and the Y-axis were R2 and Q2, respectively.

Enrichment Analysis on Differential Metabolites

metabolites between VVC and CTL groups were shown with heat maps, and increased and decreased metabolites were indicated in red and blue, respectively. (B) The volcano plot represented all metabolites of the VVC vs CTL groups. (C) Approximate classification of these differential metabolites.

Pathway Analysis of Differential Metabolites

Enrichment analysis by KEGG pathway revealed that the differential metabolites may be involved in α -linolenic acid and linoleic acid metabolism, purine metabolism, homocysteine degradation, phosphatidylethanolamine biosynthesis, and so on (Fig. 4A). Similarly, the bubble chart of metabolic pathway analysis demonstrated that the differential metabolites were concerned with linoleic acid metabolism, phenylalanine metabolism, taurine, and hypotaurine metabolism, tyrosine and tryptophan biosynthesis (Fig. 4B).

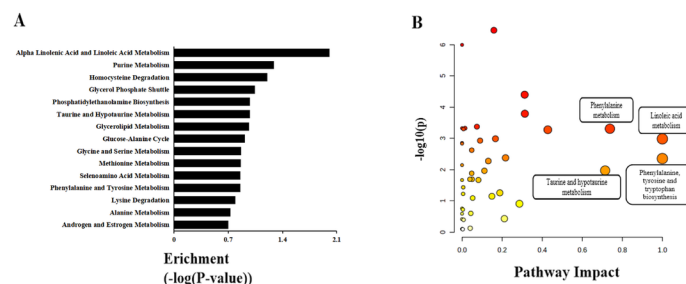


Figure 4. (A) KEGG enrichment analysis. (B) Metabolic pathway clustering.

Identification of Differential Metabolites

Changes in metabolite abundance among groups were reflected in relative differences between metabolic species. The metabolites with the most significant changes are listed in Table 3. Metabolites that positively correlated with VVC were mainly derived from sugar alcohols, amino acids, and unsaturated fatty acids, including L-(-)-arabitol, D-(+)-arabitol, L-tyrosine, epsilon-(gamma-glutamyl)-lysine, L-methionine sulfoxide, L-(+)-alanine, arachidonic acid, linoleic acid, and docosahexaenoic acid. The relative abundance of these metabolites was significantly higher in the VVC group compared to the CTL group (Fig5. A-C). Most metabolites that were downregulated in the VVC group were organic acids and fatty acyls such as ethyl 3-oxohexanoate, arachidic acid, and phloionolic acid (Fig5. D-E).

Table 3. Significantly different metabolites ($P < 0.05$)

Class	Name	VIP	P-value	Fold change (VVC/CTL)
Sugar alcohols	L-(-)-Arabitol	1.37058	0.008358575	39.46677859
Sugar alcohols	D-(+)-arabitol	1.67212	0.025116803	39.94592315
Amino acids	L-Tyrosine	2.51556	0.00029274	7.853724438
Amino acids	L-phenylalanine	1.49033	0.048175252	2.040453298
Amino acids	L-Methionine sulfoxide	2.34713	0.000908617	3.674473575
Amino acids	L-(+)-Alanine	1.54826	0.002538006	3.379222055
Unsaturated Fatty Acids	Arachidonic acid	2.11427	1.98578E-05	7.014585629
Unsaturated Fatty Acids	Linoleic acid	1.43611	0.004693232	10.0937047
Unsaturated Fatty Acids	Docosahexaenoic acid	1.67075	0.000832678	5.235192182
Organic acids	Ethyl 3-oxohexanoate	1.65782	0.000676157	0.386337726
Fatty Acyls	Arachidic acid	1.31731	0.011309088	0.241618361
Fatty Acyls	Phloionolic acid	1.3601	0.007586884	0.387641675

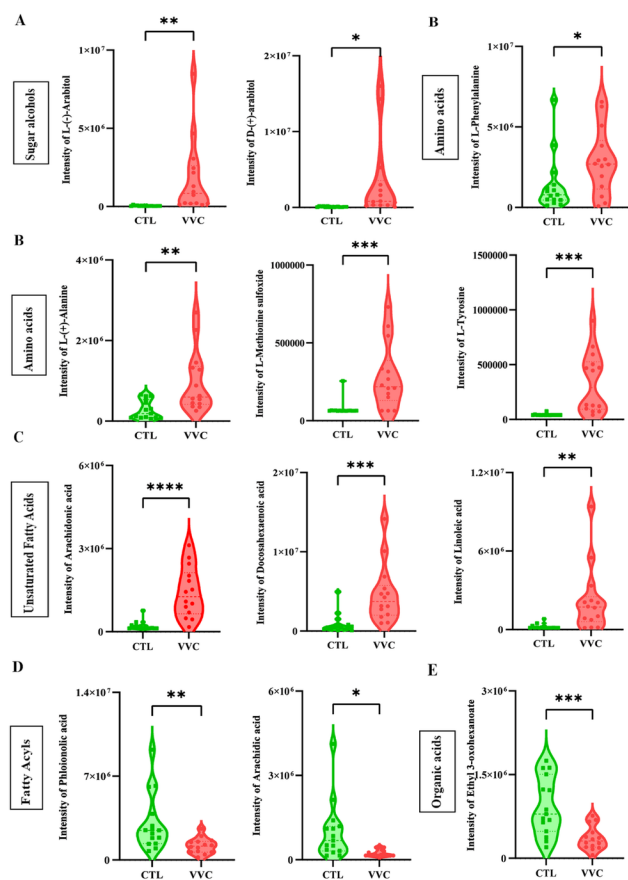


Figure 5. The intensity of significantly differential expressed metabolites between CTL and VVC groups. (A-C) The sugar alcohols, amino acids, and unsaturated fatty acids content in the VVC group were significantly increased. (D-E) The CTL group contained higher levels of organic acids and fatty acyls. (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$)

Diagnostic and Predictive Performance of Differential Metabolites

A receiver operating characteristics (ROC) analysis of vaginal discharge samples from the CTL and VVC groups was performed to explore the potential effect of *C. albicans* infection on the vaginal metabolome. In addition, the AUC values revealed the diagnostic potential of metabolites as biomarkers specific to distinguishing healthy individuals from patients with VVC. When comparing the samples from the CTL group and the VVC group, 11 metabolites showed discriminative ability (AUC values > 0.8). Among these, TBHQ,

3-Methoxy-4-hydroxyphenylglycol glucuronide, and deoxycholic acid had AUC values > 0.85 , and the AUC value of lecanoric acid > 0.9 , which was considered an excellent discriminator (Fig. 6A). This indicated significant consumption of these metabolites in the VVC group. Subsequently, the features of VVC were investigated using ROC analysis. The results showed that 25 differential metabolites had AUC values of > 0.8 , and 14 metabolites had AUC values > 0.9 (Fig. 6B). Among the metabolites that are discriminant factors of VVC, L-(-)-arabitol, L-tyrosine, D-(+)-arabitol, psychosine had AUC values > 0.97 , and were identified as more accurate discriminating factors and provided better predictive estimates than any other metabolite detected. Overall, ROC analysis showed that VVC induced significant changes in the metabolic profile of the vaginal microenvironment, and these metabolites could distinguish between normal individuals and VVC patients, which were potential markers for predicting VVC.

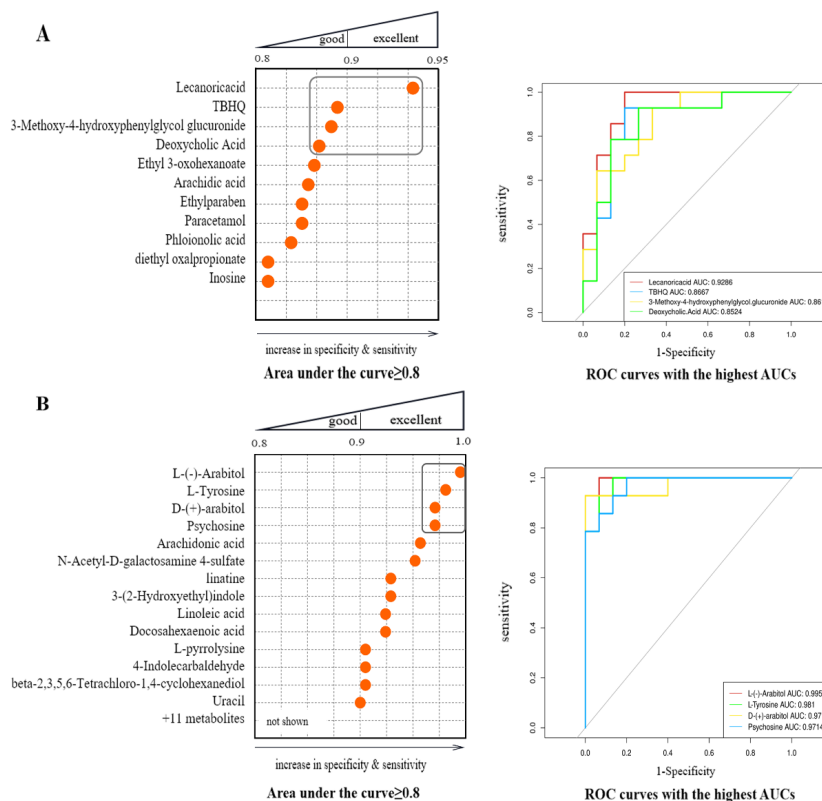


Figure 6. Diagnostic and predictive performance of differential metabolites between VVC and CTL. (A) Metabolites that discriminated CTL from VVC. (B) Metabolites that discriminated VVC from CTL.

Bioinformatics

The differential metabolites were imported into the IPA software, revealing several relevant biological pathways, such as MAPK, NF- κ B, Dectin-1/Syk, and IL-17 signaling pathways (Fig. 7). Moreover, molecular docking experiments indicated that the binding energy of the small molecule metabolite linoleic acid to ACSL1 was -7.2kcal/mol, suggesting that ACSL1 may be the target protein of linoleic acid (Fig. 8A). Linoleic acid formed hydrogen bonds with MET-325 and PHE-326 of ACSL1, with hydrogen bond lengths of 2.8Å and 2.1Å, respectively (Fig. 8B-C).

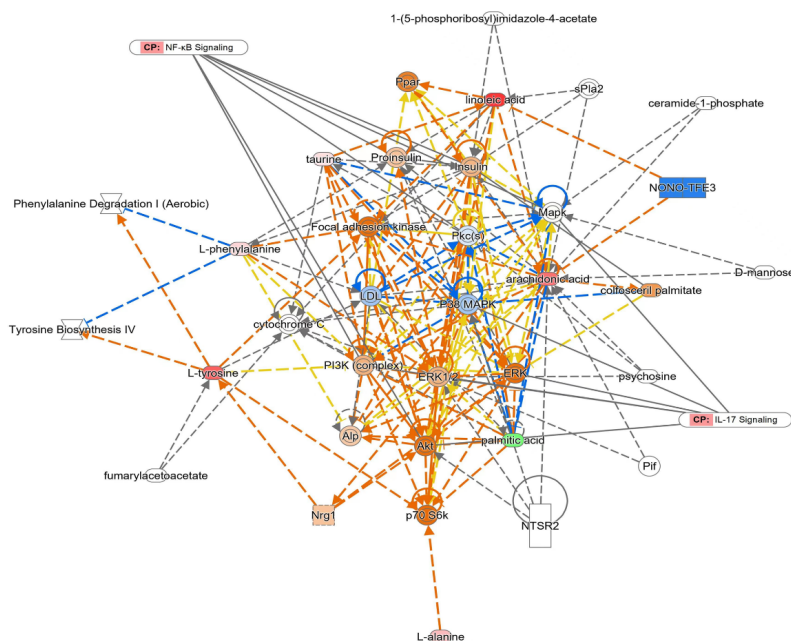


Figure 7. Biological networks, pathways, and functions of metabolites analysis by Ingenuity Pathway Analysis (IPA). Red and green represent metabolites that are significantly upregulated and down-regulated, respectively. CP represents signaling pathways associated with differential metabolites. Direct relationships are represented by solid lines and indirect relationships are by dashed lines.

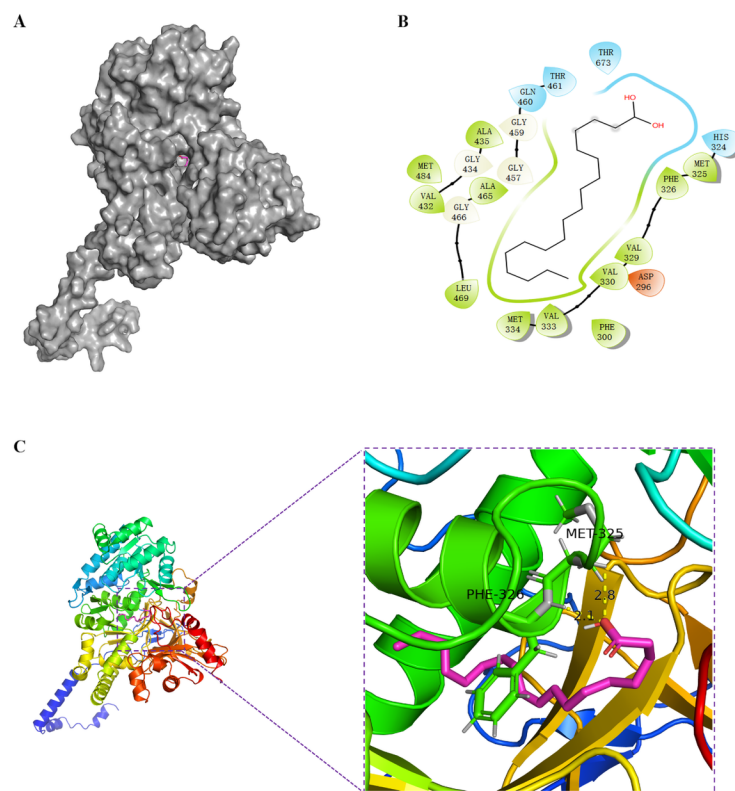


Figure 8. Study on the molecular docking of LA with ACSL1. (A) The 3D structure of docked molecule binding to ACSL1. (B) Amino acid residues MET-325 and PHE-326 at the binding site of LA to ACSL1. (C) A close observation of the binding site of LA to ACSL1.

Discussion

VVC is a mucocutaneous mycosis caused by *Candida* that has a significant impact on women's quality of life

and leads to increased healthcare costs due to high recurrence rates and increased antifungal resistance[34]. *C. albicans* is the most common opportunistic pathogen in VVC and is present in the respiratory, gastrointestinal, and genitourinary tract of more than 30 percent of healthy individuals during their lifetime[2]. Usually, yeast-phase *C. albicans* is tolerated by the vaginal epithelium. However, in VVC, *C. albicans* exhibits an aggressive form of hyphae, producing various extracellular enzymes, such as secretory aspartate protease (SAP), phospholipase, and hemolysin, which invade tissues through adhesion, penetration, hyphae invasion, and endothelial colonization of vaginal epithelial cells[35].

An increasing number of metabolites have been discovered and defined with the development of metabolomics research. Metabolomics studies can investigate a variety of biological processes and phenotypes by means of metabolites[36], such as amino acid and lipid metabolites[37]. Metabolomics has been extensively studied in inflammation-related diseases such as hyperuricemia[38], inflammatory bowel disease[39], rheumatoid arthritis[40], sepsis[41], liver failure[42], and endometriosis[43]. Research evaluating cervical and vaginal lavage fluids in patients with inflammatory vaginal disease has found associations between the composition of vaginal microflora and metabolite profiles[32]. Furthermore, a lipidomics study of VVC and cellolytic vaginopathy (CV) have shown significant differences in lipid composition. Lipids play an essential role in maintaining the homeostasis of the vaginal microenvironment[44]. However, few studies have comprehensively studied the metabolomics profile of VVC.

In the present study, untargeted metabolomic analysis was performed to further explore the potential biological functions of these metabolites and their role in VVC genesis following *C. albicans* infection, and to determine the differential metabolic profiles between the VVC and CTL groups. The metabolic profiles of patients with VVC were significantly different from those without. In total, 211 differential metabolites were identified, including 128 upregulated metabolites and 83 downregulated metabolites in VVC patients compared with healthy individuals.

Among these differential metabolites, significant increases in sugar alcohols, amino acids, unsaturated fatty acids, and pyrimidines were observed in the VVC group. Conversely, higher levels of organic acids and fatty acyl groups were observed in the CTL group. An analysis of the diagnostic capability of well-characterized biomarkers (ROC) showed that L-(-)-arabitol, L-tyrosine, D-(+)-arabitol, and psychosine could effectively distinguish VVC from healthy people (AUC>0.97). Additionally, more than ten metabolites (AUC>0.8), including linoleic acid, arachidonic acid, L-pyrrolysine, and uracil, were found to have good discrimination capacity, which are potential biomarkers to distinguish VVC from CTL patients. Tyrosine was thought to be neurotoxic and capable of causing neurological disorders such as encephalitis[45]. Arabitol in serum[46] and urine[47] has been used as a biomarker to rapidly diagnose invasive candidiasis and can be used to inform antifungal therapy and prognosis.

Amino acid metabolism plays an important role in the activation of immune cells and the production of antibodies, and excess amino acids may be detrimental to the immune system[48]. In this study, amino acid metabolism was one of the major metabolic pathways, including phenylalanine, tyrosine, and tryptophan biosynthesis, phenylalanine metabolism, and taurine metabolism. Similarly, one research reported that the same metabolic pathways were enriched in sepsis[41], indicating that amino acid metabolism was inextricably linked to the occurrence of inflammation. Moreover, phenylalanine metabolism promotes the neutrophil-evasive state[49], and excess L-phenylalanine was found to affect antibody production by inhibiting protein synthesis[50]. Similar metabolic changes were observed in our study, and excess L-phenylalanine may contribute to the development of VVC.

Furthermore, linoleic acid metabolism also played a key role, as it showed the most obvious and broadest variation in the VVC group. Linoleic acid and arachidonic acid in the VVC group both increased significantly. Linoleic acid (LA; ω -6,18:2) and arachidonic acid (AA; ω -6,20:4) are essential fatty acids for the human body and are two of the most abundant polyunsaturated fatty acids (PUFAs). AA can be obtained directly from the diet or can be synthesized from LA. One study found that a larger amount of AA was synthesized from LA than from dietary sources[51]. Therefore, the significant elevation of LA caught our attention. Although LA was associated with a reduced risk of cardiovascular disease[52], studies have shown that excess LA can

promote the occurrence of inflammatory events[53, 54]. Similarly, metabolites of LA mediated inflammation through oxidative stress, and oxidized linoleic acid (LA) metabolites (OXLAMs) induced mitochondrial dysfunction, apoptosis, and activation of NLRP3 inflammasome by means of oxidative stress, promoting the development of nonalcoholic steatohepatitis (NASH)[55]. LA-derived hydroxyoctadecenoic acids (HODEs) have been found to contribute to the progression of atherosclerosis as inflammatory regulators[56]. Additionally, the LA metabolites epoxyoctadecenoic acids (EpOMEs) and dihydroxyoctadecenoic acids (DiHOMEs) activate NF- κ B and AP-1 transcription factors to induce an inflammatory reaction[57]. Therefore, some lipid metabolites of VVC may be related to inflammation, which was consistent with previous findings[44]. LA, found in the VVC group, may act as a pro-inflammatory factor in the response of the vaginal mucosa to *C. albicans*.

IPA network analysis was performed to further explore the potential role of metabolites in VVC, revealing that the changes in differential metabolites were mainly related to MAPK signaling, NF- κ B signaling, Dectin-1/Syk signaling, and IL-17 signaling pathways. The study found that *C. albicans* significantly activated the epidermal growth factor receptor (EGFR) in human vaginal epithelial cells, activating the inflammatory response through several pathways, including mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B)[58, 59]. Among them, *Candida* mainly initiated the mucosal immunity of the vaginal epithelium through the MAPK signaling pathway and activated MAPK phosphatase 1 (MKP1) and c-Fos by activating MAPK-associated proteins (p38 and ERK1/2)[60, 61]. The activation of MKP1 marked the transition of *Candida* from the colonizing yeast phase to the invasive toxin-producing hyphal phase.

Further molecular docking experiments showed that LA could bind to ACSL1, suggesting that ACSL1 may be the target protein of LA. As a member of the family of long-chain acyl-CoA synthetase genes, ACSL1 acted on long-chain fatty acids (FAs), shuttling FAs into mitochondria in heart and adipose tissue for β -oxidation and lipid synthesis[62, 63]. Moreover, the potential role of ACSL1 in sepsis has been reported[64]. One study confirmed that inhibition of ACSL1 activity attenuated phosphorylation of p38 MAPK, ERK1/2, and NF- κ B, suggesting that ACSL1 was an upstream regulator of MAPK and NF- κ B signaling pathways to mediate inflammation[65]. LA may promote the occurrence of VVC by binding to ACSL1 to regulate the MAPK and NF- κ B signaling pathways. Therefore, LA has the potential to become a new method for the clinical treatment of VVC, and further research should explore its function and mechanism. This study had certain limitations. In the future, we will further validate cell and animal samples and refine in vivo and in vitro experiments to confirm our findings.

Conclusion

This study revealed differences in the metabolic profiles of vaginal secretions in patients with VVC due to *C. albicans* infection and in healthy people. These differentially expressed metabolites may be involved in the VVC process. The analysis results showed that significantly elevated LA levels in VVC could bind to ACSL1, suggesting that LA may exert its biological function through the MAPK and NF- κ B signaling pathways. Our results provide new insights into the diagnosis and treatment of VVC, and further research is needed to verify the mechanism of LA in VVC.

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Institutional review board statement

The Ethics Committee of the Tongren Hospital, Shanghai Jiao Tong University School of Medicine (Protocol No. JG-2021-01-008), approved this study. The patients/participants provided their written informed consent to participate in this study.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no competing interests.

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Author contributions

Conceptualization, R.Z. and J.Q.; Data curation and Methodology, X.C. and J.C.; Investigation, J.W. and G.W.; Supervision, R.Z.; Writing—original draft, X.C. and J.W.; Writing—review and editing, G.W. and R.Z.; Funding acquisition, J.C. and J.Q.; Project administration, J.Q. They have also given their final approval of this study.

