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# The inferred modulation of correlated vaginal microbiota and metabolome by cervical differentially expressed genes across distinct CIN grades

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## Abstract

**Background** In vitro studies have demonstrated the modulation of vaginal microbiota (VM) by cervical peptides which levels varied with the status of HPV infection and cervical intraepithelial neoplasia (CIN) grades. However, there is a deficiency in population-based studies investigating the modulation of VM compositions and metabolome by cervical differentially expressed genes (DEGs) across different grades of CIN.

**Methods** This study included 43 HPV-positive women, classified into low-grade (CIN1, $n=23$) and high-grade (CIN2+, $n=20$) groups. Vaginal swabs were collected for both microbiota and metabolome analysis. Cervical exfoliated cells were collected for RNA-Seq analysis.

**Results** We identified 258 differentially expressed genes (DEGs), among which 176 CIN1-enriched genes were linked to immune responses, cell chemotaxis, negative regulation of cell migration, and B cell differentiation, activation, and proliferation. Eighty-two genes upregulated in CIN2+cohorts were associated with epidermis development and keratinization. Then, we identified 5,686 paired correlations between DEGs, VM, and metabolome, with 2,320 involving *Lactobacillus*. Further analysis revealed *Lactobacillus* as the primary determinant of metabolic profiles, followed by *Gardnerella*, *Faecalibacterium*, *Aerococcus* and *Streptococcus*, such as the notable positive correlation between *Lactobacillus* with D-lactic acid and DL-indole-3-lactic acid. Applying mediation analysis, we found that *Lactobacillus* mediated the association of 14 CIN1-enriched DEGs, such as COL4A2, CCBE1 and SPON1, with the production of 57 metabolites, including D-lactic acid, oleic acid and various amino acids. Additional analysis indicated significant mediation effects of 79 metabolites on the association of DEGs with the growth of *Lactobacillus*, *Gardnerella*, *Fannyhessea* and *Aerococcus*.

**Conclusions** Our findings provide valuable population-based evidence for the inferred modulation of correlated VM and metabolome by cervical DEGs across different CIN stages.

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**Keywords** Human papillomavirus, Cervical intraepithelial neoplasia, Vaginal microbiota, Vaginal metabolome, Host gene expression

## Introduction

The interaction between human papillomavirus (HPV), host and vaginal microbiota (VM) impacts the risk of persistent HPV infection and progression of cervical intraepithelial neoplasia (CIN) [1]. There is accumulated population-based evidence for HPV-modulated or VM-orchestrated host responses that are linked to the risk of persistent HPV infection as well as CIN progression [2–6]. Nevertheless, the opposite regulation axis that means the modulation of host products on VM remains to be understood.

A recent study found notably decreased cervical levels of several innate peptides among women with high-grade CIN and even cervical cancer, compared to low-grade CIN and HPV-negative women [7]. Then they demonstrated that some of those downregulated host innate peptides were utilized as nutrient resources to promote the growth of vaginal *Lactobacillus* species [7]. Zhu M, et al. demonstrated the selective positive impact of fatty acids on the promoted growth of *Lactobacillus crispatus* as well as on the inhibition of *Lactobacillus iners* [8]. In addition, our prospective study indicated more significant dynamic shifts of the vaginal metabolome in response to therapeutic removal of high-grade CIN, compared to VM compositions [9]. Those findings suggested the modulation of VM compositions and metabolism by host-derived products that varied across distinct CIN grades [7, 10–12].

However, there is a deficiency of population-based studies investigating the modulation of VM components and metabolism by cervical differentially expressed genes (DEGs) across distinct stages of CIN. In this study, we conducted cervical transcriptome, VM and metabolome analysis for a cohort of HPV-positive Chinese women diagnosed with either low-grade or high-grade CIN. Our primary objectives were to elucidate the inferred modulation of VM and associated metabolome by cervical DEGs across distinct grades of CIN.

## Materials and methods

### Study design, sample collection, and clinical diagnosis

Following strict inclusion criteria, as previously detailed in our research [13], this study included 43 HPV-positive females with distinct CIN grades, who provided qualified samples for cervical gene expression, VM and metabolome analysis. The inclusion was conducted via interviews within the Obstetrics and Gynecology Department at Peking University Shenzhen Hospital in China. Those criteria required that participants must be over the age of 18, non-menopausal, and devoid of history pertaining to cervical ablation, resection surgery, hysterectomy, pelvic radiotherapy, recent sexual intercourse (three days before sampling), vaginal douching or medication use (one week before sampling), antibiotic exposure (one month before sampling), hormone replacement therapy or GnRH-a administration (three months before sampling), no autoimmune diseases as well as HIV infection. Moreover, vaginal smears were screened and confirmed negative for $H_2O_2$, leukocyte esterase, neuraminidase, b-glucuronidase, and acetylaminoglucosidase, to exclude common genital infections. All participants provided written consent after being thoroughly informed.

Vaginal swabs were collected at least three days post-menstruation. During colposcopy, a skilled physician collected cervical exfoliated cells via Pap brushes and vaginal smears from the posterior fornix via swabs. A qualified technician immediately conducted RNA extraction from Pap brush samples (Qiagen RNeasy Mini Kit, QIAGEN, German). All vaginal swabs were preserved in sterile 2 ml tubes on ice and promptly transferred to −80 °C storage within 30 min.

Vaginal swabs were utilized for HPV testing via Roche Cobas®4800 HPV testing system, which targeted specific detection of HPV16/18, along with 12 additional high-risk HPV genotypes, including HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. We used reagents (CINtec® Histology, Roche, USA) for p16 immunohistochemical staining. CIN diagnosis was performed by two qualified pathologists, who relied on p16 immunohistochemical staining as an ancillary method to differentiate between CIN1 and CIN2+lesions.. Cytologically diagnosed CIN2 samples that demonstrated p16 positivity were categorized as CIN2+, whereas those lacking p16 positivity were classified as CIN1 [14, 15].

Under the assistance of a research assistant, the clinician documented vital metadata for each participant, including smoking, sexual history, contraceptive use, pregnancy, abortion histories, HPV genotypes, and the history of HPV vaccination.

### Sequencing and data processing of cervical gene expression, VM and metabolome

Microbial DNA of vaginal swabs was extracted using the Dneasy PowerSoil Pro Kit from Qiagen (Germany), ensuring the highest quality of DNA. The Agilent 5400

Fragment Analyzer system was applied to determine the concentration and purity of extracted DNA, based on automatic capillary electrophoresis system (Agilent Technologies, Inc., Santa Clara, USA). Then hypervariable V4-V5 regions of 16S rRNA gene were amplified (primers: 515-FR (GTGCCAGCMG CCGCGGTAA) and 926-RR (CCGTCAATTCMTTTRAGTTT). Subsequently, the Illumina NovaSeq platform (Illumina, San Diego, CA, USA) was used to generate reads of 250 bp for V4-V5 regions. Then comprehensive VM profiles for each vaginal swab was generated via the QIIME2 pipeline [16].

For untargeted metabolome analysis, we utilized high-resolution liquid chromatography coupled with mass spectrometry (LC–MS) on Orbitrap Exploris™ 480 (Thermo Fisher Scientific platform, Ottawa, USA), to generate the raw intensity data. Then the raw data were converted to the mzXML format. Subsequently, the Progenesis QI software (version 2.2) was applied to extract ion features, excluding those were absent in more than half of the quality control samples or exceeding 80% absence among test samples, or had a relative standard deviation greater than 30%. We then performed comprehensive searches against the Human Metabolome Database (HMDB, version 5.0) and Kyoto Encyclopedia of Genes and Genomes (KEGG, version 96.0) to annotate filtered metabolites. The resulting metabolite abundance matrix served as the foundation for subsequent analysis.

Total RNA was extracted from cervical exfoliated cells using the Qiagen RNeasy Mini Kit and sequenced on the Illumina NovaSeq platform. The library preparation was performed using Optimal Dual-mode mRNA Library Prep Kit (BGI-Shenzhen, China). Then the generated DNA nanoball were loaded into the patterned nanoarray and paired reads of 150 bp were generated via sequencing platform. Raw sequencing reads were filtered via Cutadapt (v.2.5) [17] to remove low-quality and adapter sequences. Then filtered reads were aligned to the human genome (GRCh38) using Hisat2 (v.2.1.0) [18]. Expression levels of each gene were quantified and normalized using RSEM (v.1.3.3) [19] and DESeq2 (v.1.20) [20]. Then the DESeq2 (v.1.20) software was applied to analyze the differences in host gene expression between CIN1 and CIN2 + cohorts [20]. Functional enrichment of DEGs was conducted based on the DAVID Knowledgebase (v2023q4) [21].

### Bioinformatics and statistics

The designation of VM clusters was assigned based on the presence of a dominant bacterial genus or species, constituting at least 50% of the microbial community. For VM community state type (CST) that lacked a single predominant genus or species, the cluster was uniquely classified as MixedLD.

PERMANOVA was applied to assess the contribution of VM CST to metabolic profiles, based on the vegan package in R software. The synergy between VM and metabolome was assessed via O2PLS analysis, implemented in the OmicsPLS package of the R software. All continuous variables were standardized to conform to a standard normal distribution ($N \sim (0, 1)$) by employing an empirical normal quantile transformation. By applying the data of all microbial samples, the Spearman rho coefficient was used to evaluate the correlation between DEGs, VM and metabolome. Given small sample size in this study, the statistic significance was determined by FDR < 0.1, representing less stringent standard than FDR < 0.05, to discover DEGs with high fold change of expression levels as well as more potential correlations between DEGs and VM/metabolome.

Mediation effects were examined using the mediate function in the R mediation package (4.5.0) [22], and age included as the covariate. The mediation analysis included fitting two linear models for two regulation axis:

Axis: DEGs-Genus(mediator)-Metabolite

$$\text{Genus}_i = \alpha_1 + \beta_1 \text{Metabolite}_i + \delta_1^T X_i + \varepsilon_{i1}$$

$$\text{Gene}_i = \alpha_2 + \beta_2 \text{Metabolite}_i + \gamma_i \text{Genus}_i + \delta_2^T X_i + \varepsilon_{i2}$$

Axis: DEGs-Metabolite(mediator)-Genus

$$\text{Metabolite}_i = \alpha_1 + \beta_1 \text{Genus}_i + \delta_1^T X_i + \varepsilon_{i1}$$

$$\text{Gene}_i = \alpha_2 + \beta_2 \text{Genus}_i + \gamma_i \text{Metabolite}_i + \delta_2^T X_i + \varepsilon_{i2}$$

where $\text{Genus}_i$, $\text{Metabolite}_i$ and $\text{Gene}_i$ denotes the relative abundance of each genus, metabolite and DEG, respectively. Xi represents a vector of covariates. After fitting these two modules, the product of two coefficients $\beta_1\gamma_1$ was interpreted as an estimate of average causal mediated effect and the coefficient $\beta_2$ was interpreted as an estimate of the average direct effect. Significant mediation effects were determined based on an FDR < 0.1 for the proportion of mediation, with the lowest value of the 95% confidence interval (CI) exceeding zero.

## Results

### Study population

This study recruited 43 HPV-positive women, who provided qualified vaginal swabs and pap-brush samples

(Fig. 1A, Table 1). Of these, 43 pap-brush samples were qualified for cervical transcriptome analysis. Additionally, 86 vaginal swabs were applied for subsequent analysis, with 43 samples qualifying for VM examination and 43 for vaginal metabolome assessment.

For included HPV-positive women, there were no significant variations in the average age between CIN1 and CIN2 + cohorts (Table 1). Most women in both groups tested positive for HPV16/18-excluded high-risk HPV genotypes, with 21.7% and 25.0% of women in the CIN1 and CIN2 + cohort exhibiting HPV16 positivity. However, the proportion of HPV18-positive women was higher in the CIN1 group compared to the CIN2 + group (Table 1). Among the 43 women included, 3, 9, and 10 women had received the 2-valent, 4-valent, and 9-valent HPV vaccination respectively (Table 1). Furthermore, there were no significant differences observed in other phenotypes, such as smoking, between CIN1 and CIN2 + cohorts (Table 1).

### Cervical DEGs between CIN1 and CIN2 + cohort

For collected cervical exfoliated cells, we generated expression information for 21,082 genes. Our analysis unveiled distinct expression patterns between CIN1 and CIN2 + cohorts, demonstrating that 176 host genes were upregulated and 82 genes were downregulated in the CIN1 cohort (Fig. 1B-C, Supplementary Table 1). Functional enrichment analysis of upregulated genes within the CIN1 cohort predominantly linked them to immune responses, cell chemotaxis, negative regulation of cell migration, as well as B cell differentiation, activation, and proliferation (Fig. 1D, Supplementary Table 1–2). Notably, several genes exhibiting ≥ twofold change in the CIN1 cohort were implicated in innate or adaptive immune responses, including CCR6, CXCR3, CXCR5, CD200, CD79A, CD79B, CD19, CDH6, CADM1, DEFB103A, and DEFB103B (Fig. 1C, Supplementary Table 1). Additionally, CCBE1, IL36RN, SPON1, SERPINA5, and COL4A2 were among the other genes with ≥ twofold

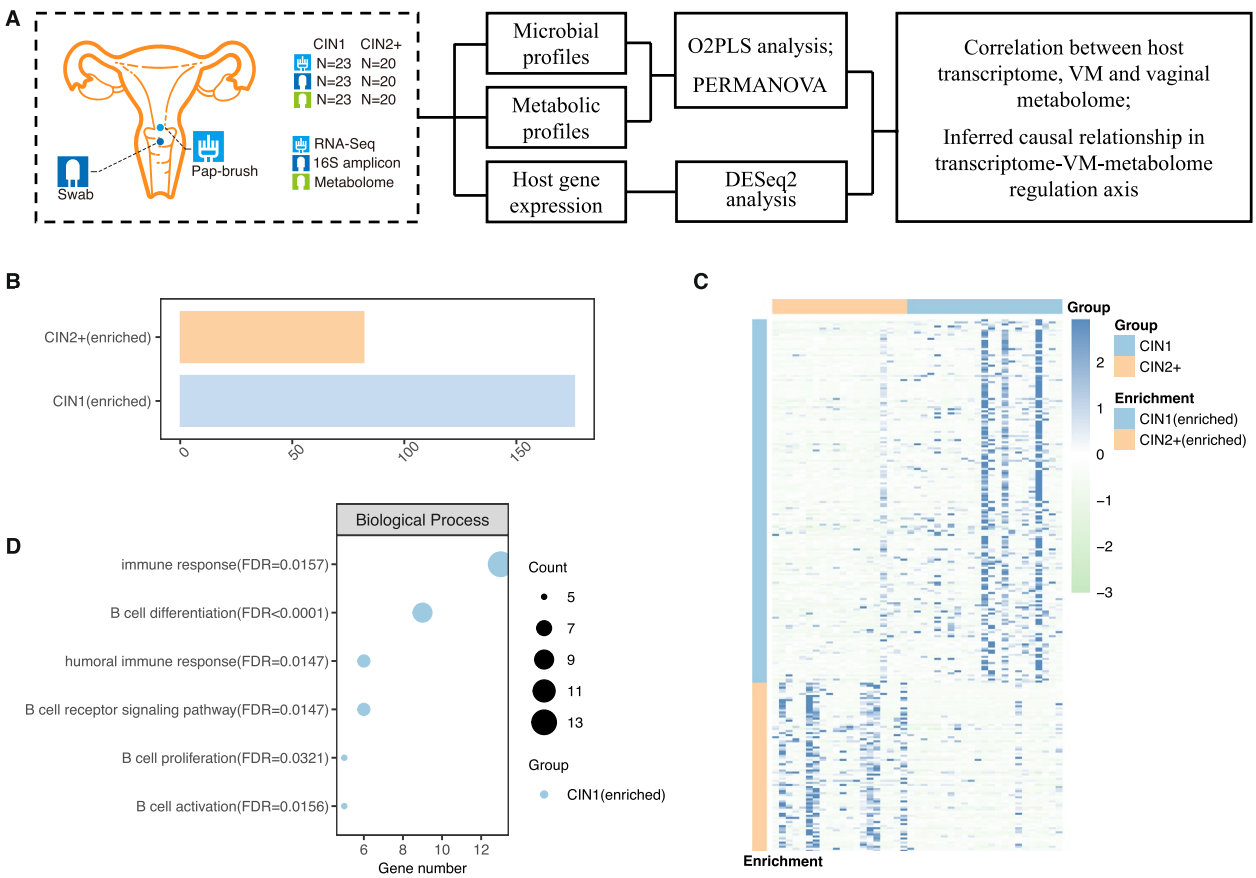


**Fig. 1** Study design and DEGs between CIN1 and CIN2 + cohort. **A** Study pipeline. **B** This plot displays 258 genes with significant variation (FDR < 0.1) between CIN1 and CIN2 + cohorts, emphasizing the magnitude and statistical significance of expression changes. **C** The heatmap depicts the normalized expression levels of 258 DEGs (FDR < 0.1), alongside functional enrichments associated with these genes, offering a comprehensive view of their biological relevance. **D** Functional enrichment of DEGs upregulated in CIN1 cohort

**Table 1** Information of included 43 HPV-positive women

| CIN grade | CIN1 (*N* = 23) | CIN2 + (*N* = 20) | *p*-value (Fisher's exact test) |
|---|---|---|---|
| **Age (mean: min–max)** | 33.7(27–48) | 30.8(23–46) | Wilcoxon rank-sum test($p > 0.05$) |
| **HPV infection** | | | |
| HPV16 + | 21.7%(5/23) | 25.0%(5/20) | 1 |
| HPV18 + | 13.0%(3/23) | 0.0%(0/20) | 0.2359 |
| Other 12 HPV genotypes + | 87.0%(20/23) | 95.0%(19/20) | 0.6105 |
| **Vaccination** | | | |
| 2-valent | 4.3%(1/23) | 10.0%(2/20) | 0.5900 |
| 4-valent | 26.1%(6/23) | 15.0%(3/20) | 0.4674 |
| 9-valent | 13.0%(3/23) | 35.0%(7/20) | 0.1480 |
| **Smoking** | 0.0%(0/23) | 5.0%(1/20) | 0.4651 |
| **No. of sex partner (mean: min–max)** | 2.6(1–16) | 2.8(1–7) | Wilcoxon rank-sum test($p > 0.05$) |
| **Age with first sex activity (mean: min–max)** | 22.0(18–28) | 21.8(16–28) | Wilcoxon rank-sum test($p > 0.05$) |
| **Condom contraception** | 87.0%(20/23) | 50.0%(13/20) | 0.1480 |
| **Gestation** | 65.2%(15/23) | 45.0%(9/20) | 0.2276 |
| **Abortion** | 34.8%(8/23) | 35.0%(7/20) | 1 |
| **CST(genus level): LD** | 82.6%(19/23) | 70.0%(14/20) | 0.4728 |
| **CST(species level) of LD samples** | | | |
| *Lactobacillus crispatus*-dominant (LCD) | 26.32%(5/19) | 21.43%(3/14) | 1 |
| *Lactobacillus iners*-dominant (LID) | 42.10%(8/19) | 50.00%(7/14) | 0.7325 |
| *Lactobacillus gasseri*-dominant (LGD) | 5.26%(1/19) | 0.0%(0/14) | 1 |
| CSTs dominated by more than two *Lactobacillus* species (MixedLD) | 26.32%(5/19) | 28.57%(4/14) | 1 |

*CST* Community state type, *LD Lactobacillus*-dominant

change that were enriched in the CIN1 cohort (Fig. 1C, Supplementary Table 1).

Conversely, genes enriched in CIN2 + cohorts were primarily associated with epidermis development, keratinocyte differentiation, keratinization, and epidermal cell differentiation (Supplementary Table 1–2). Specifically, genes with high overall expression levels in this cohort included FABP5, TP63, KRT24, UPK3BL1, PHGDH, KCNS1, LGALS7B, and CALML5 (Fig. 1C, Supplementary Table 1).

### The correlation between VM and vaginal metabolome

We identified 29 genus and 45 species, based on 137 identified operational taxonomic units (OTUs). In combination with identified 1,372 vaginal metabolites, permutational multivariate analysis of variance (PERMANOVA) indicated notable differences between *Lactobacillus*-dominated (LD) and non-LD (NLD) samples as well as insignificant differences among species-level LD microbial samples (Fig. 2A). Thus, we analyzed the correlation between VM and vaginal metabolome at the genus level.

The two-way orthogonal partial least squares (O2PLS) analysis uncovered a remarkable synergistic interplay between the genus-level VM components and metabolome profiles (Fig. 2B). Further analysis emphasized the pivotal role of vaginal *Lactobacillus* as the primary determinant of metabolic profiles, followed by *Fannyhessea*, *Gardenerella*, *Aerococcus* and *Streptococcus* (Fig. 2B). These five genera represented > 99% abundance in most of included microbial samples. Therefore, we analyzed the correlation between those five genera and vaginal metabolites in subsequent analysis, suggesting 1,549 paired correlations (FDR < 0.1) (Fig. 2C, Supplementary Table 3). Vaginal *Lactobacillus* had statistically significant positive correlations with 234 metabolites, mainly including organic acids and derivatives such as DL-indole-3-lactic acid, D-lactic acid and various amino acids, as well as lipids and lipid-like molecules, organoheterocylic compounds and benzenoids (Fig. 2C, Supplementary Table 3). One hundred and forty-four metabolites were in negative correlations with *Lactobacillus* level, most of which were classified as lipids and lipid-like molecules as

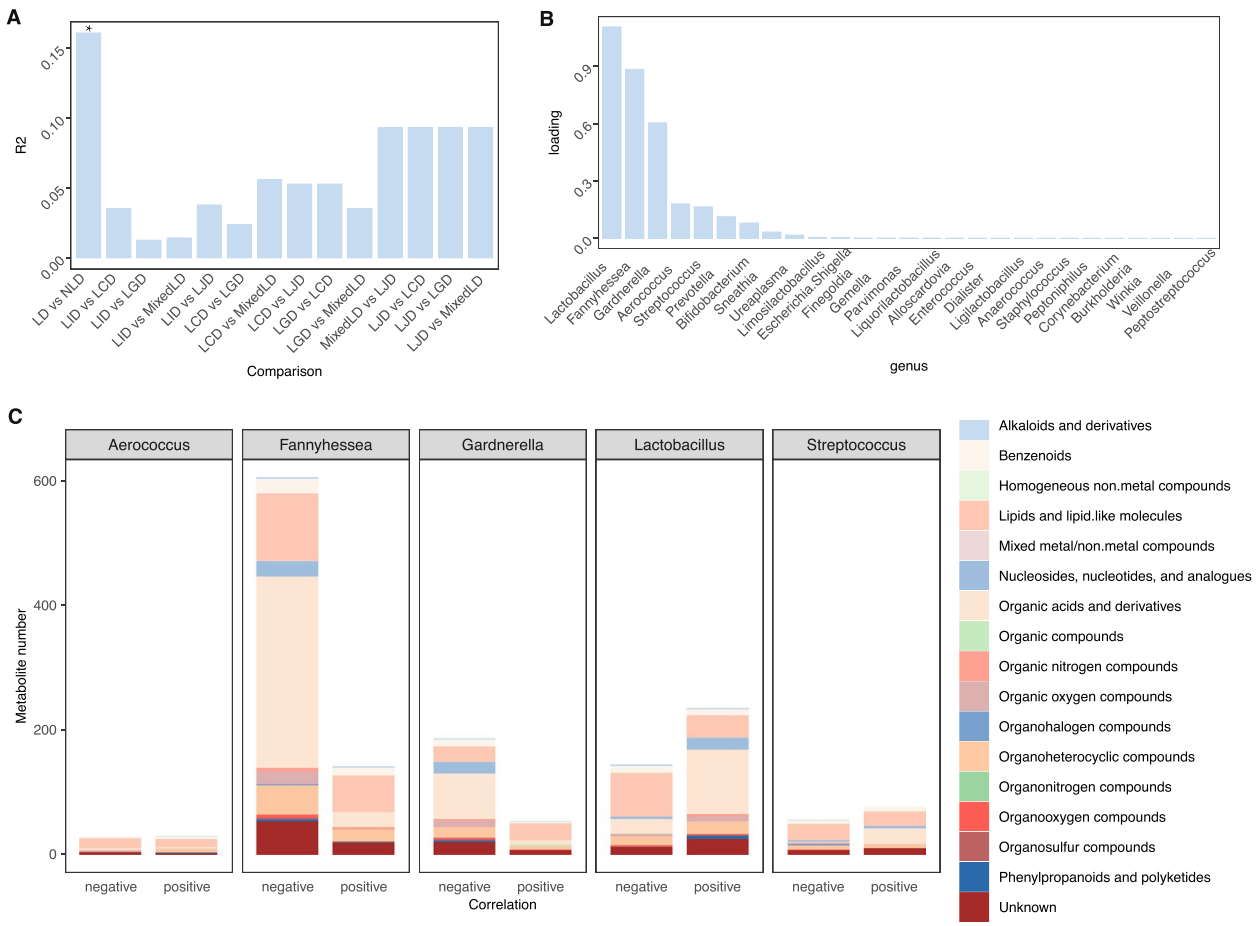


**Fig. 2** Correlations between VM and metabolome. **A** Explained variance(R2) for variations of metabolic profiles. * represents FDR < 0.1. **B** The O2PLS analysis indicates the loading value of genera in the correlation with metabolic profiles. **C** Being assessed by spearman rho coefficient, there are notable correlations (FDR < 0.1) between five genera with metabolites

well as organic acids and derivatives, such as capric acid, 11-oxohexadecanoic acid, and oleic acid. *Fannyhessea*, *Gardnerella*, *Aerococcus,* and *Streptococcus* had notable correlations with 745, 238, 57, and 131 metabolites, respectively (Fig. 2C). Further analysis showed that several metabolites that positively correlated with *Lactobacillus* had negative correlations with *Fannyhessea* and *Gardnerella* while had positive correlations with *Aerococcus*, such as L-3-Phenyllactic acid, DL-3-(4-Hydroxyphenyl)lactic acid, DL-indole-3-lactic acid, and D-lactic acid (Fig. 2C, Supplementary Table 3).

### The paired correlations between cervical DEGs, VM and vaginal metabolome

We found 5,686 paired correlations involving the above-mentioned five genera, 843 vaginal metabolites, and 132 DEGs (FDR < 0.1) (Fig. 3, Supplementary Table 4). Among these, vaginal *Lactobacillus* was involved in 2,320 paired correlations, concerning 369 metabolites and 28 DEGs (Fig. 3, Supplementary Table 4). For instance, we identified negative FMO2:*Lactobacillus*, and FMO2:D-lactic acid, as well as positive *Lactobacillus*:D-lactic acid paired correlations (Fig. 3, Supplementary Table 4). We also found paired correlations between vaginal *Lactobacillus*, CIN1-enriched gene CCBE1, and metabolite dextromethorphan as well capric acid (Fig. 3), and the paired correlation between *Lactobacillus*, CIN1-enriched gene COL4A2, and metabolite racemoramide (Fig. 3).

A total of 3,366 paired correlations were identified between 117 DEGs, 727 metabolites and *Fannyhessea*, *Gardnerella*, *Aerococcus,* and *Streptococcus* (FDR < 0.1) (Fig. 3, Supplementary Table 4). Specifically, there existed a paired correlation between *Gardnerella*, gene CIN1-enriched gene SPRR2F and several metabolites including inosine, N8-acetylspermidine, piperoic acid as well as FAHFA 18:0; FAHFA(16:0/2:0) (Supplementary Table 4). Other paired correlations included the associations between *Aerococcus*, several DEGs(SIGLEC6, IKZF3 AFF3), and N-undecylbenzenesulfonic acid, LysoPS 18:2; LysoPS 18:2 as well as LysoPS 18:1; LysoPS 18:1 (Supplementary Table 4).

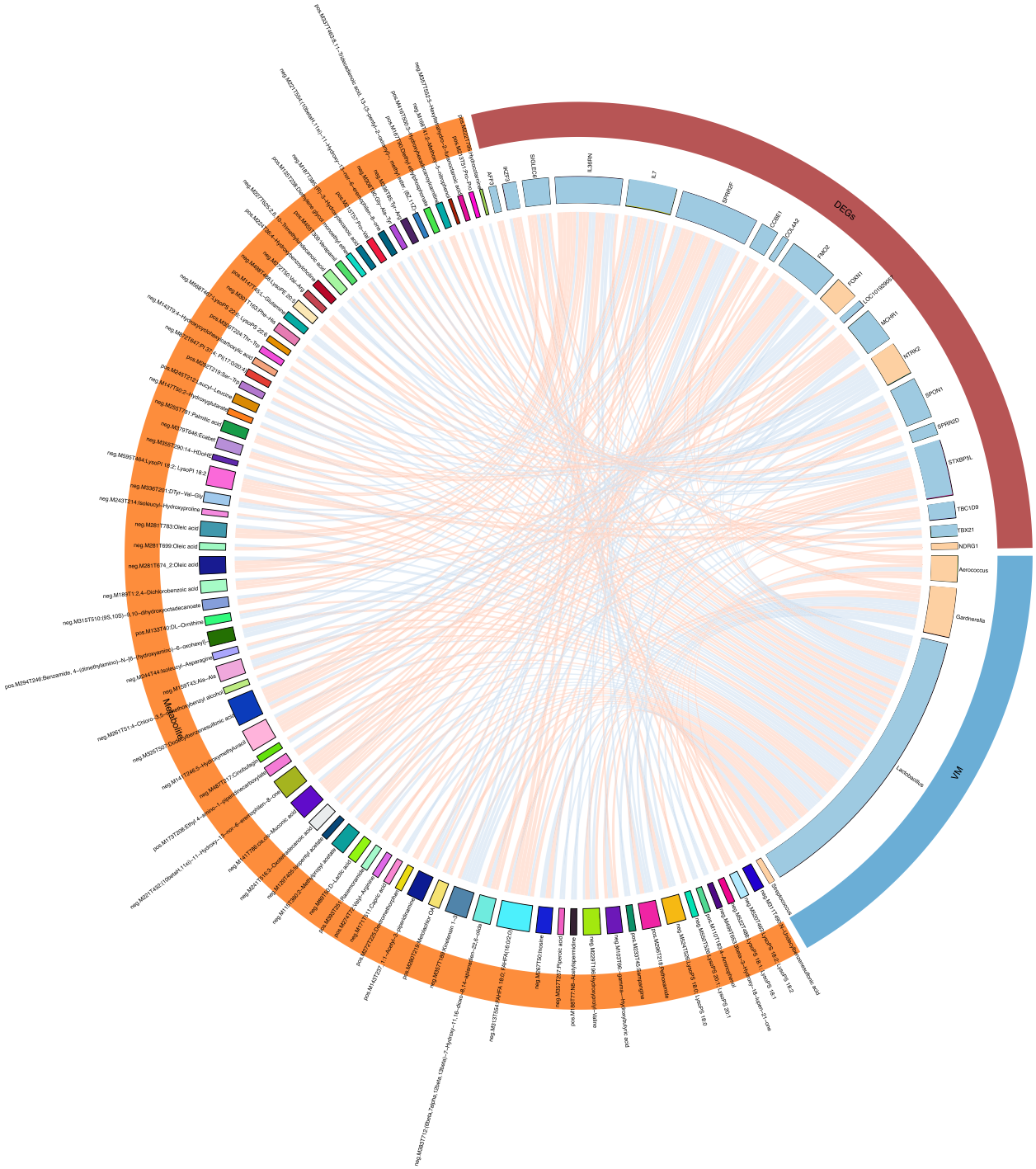


**Fig. 3** Paired correlations between DEGs, VM and metabolome and DEGs (FDR < 0.1). Red and blue lines signify positive and negative correlations, respectively

### The inferred modulation of correlated VM components and metabolites by cervical DEGs across different grades of CIN

Mediation analysis indicated the modulation of 19 DEGs on 72 metabolites, being mediated by *Lactobacillus*, *Gardnerella*, *Streptococcus* and *Aerococcus* (Fig. 4A). Specifically, *Lactobacillus* mediated the modulation of 14 CIN1-enriched DEGs, such as COL4A2, CCBE1, FMO2 and SPON1, on the production of 57 metabolites, including D-lactic acid, DL-ornithine, oleic acid, L-glutamine

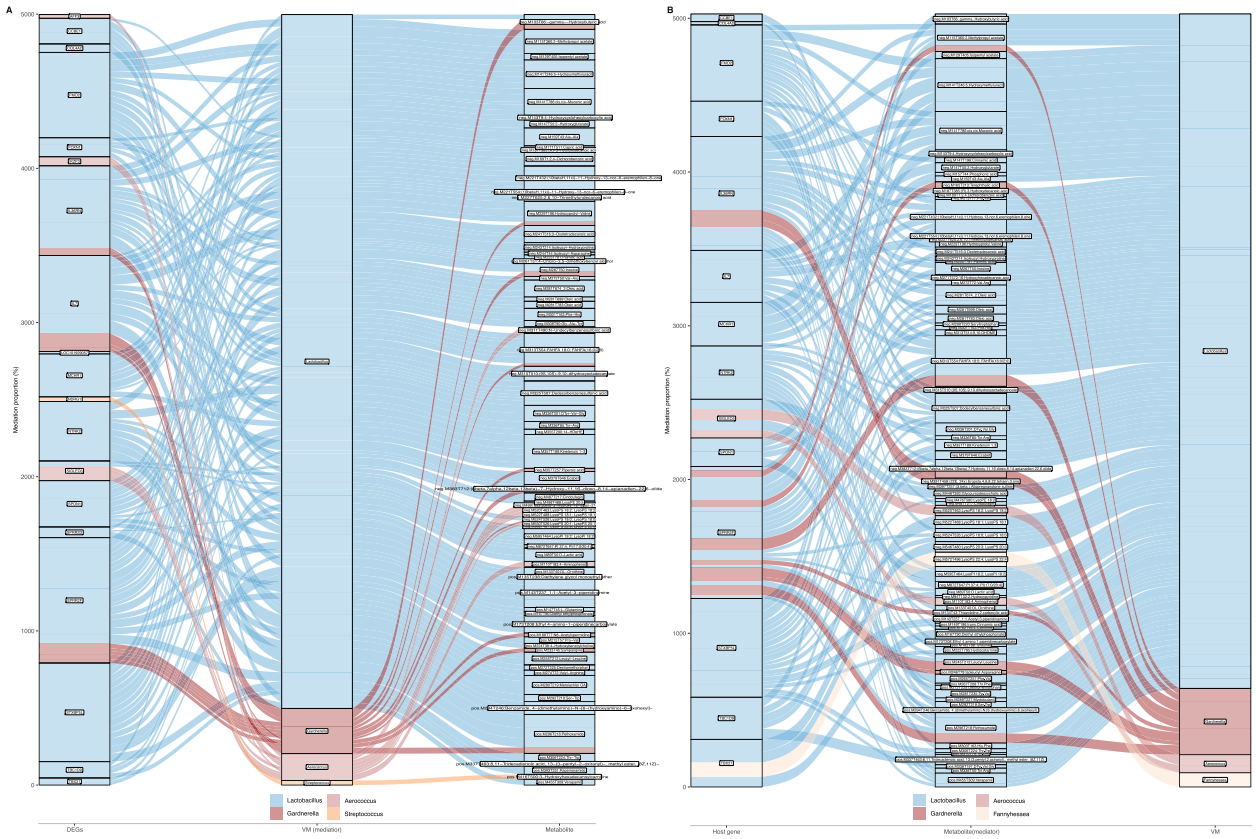


**Fig. 4** Being assessed by mediation effects, the inferred modulation of DEGs on correlated VM and metabolome. **A** This analysis explores the mediation effects of VM in the influence of DEGs on metabolites. **B** This analysis explores the mediation effects of metabolites in the influence of DEGs on VM

and several amino acids (Fig. 4A). *Lactobacillus* also mediated the association of two CIN1-depleted DEGs, NTRK2 and FOXN1, with 11 metabolites, such as dodecylbenzenesulfonic acid, leucyl-leucine and 1-acetyl-3-piperidinamine (Fig. 4A). *Gardnerella*, *Streptococcus* and *Aerococcus* seemed to mediate the modulation of 6 CIN1-enriched and 1 CIN1-depleted DEGs on 17 metabolites, including N-undecylbenzenesulfonic acid, LysoPS 18:2, 4-aminophenol, sampangine, piperoic acid, inosine and 3-hydroxyhexadecanoylcarnitine (Fig. 4A).

Further analysis indicated notable mediation effects of 79 metabolites on the modulation of 14 DEGs on the growth of *Lactobacillus*, *Gardnerella*, *Fannyhessea* and *Aerococcus* (Fig. 4B). Seventy-two metabolites had the potential to mediate the modulation of 14 DEGs on the growth of vaginal *Lactobacillus*, generating 125 of 137 mediation axis (Fig. 4B). Among those 125 mediation axis, dodecylbenzenesulfomic acid and L-carnitine mediated the positive correlation between CIN1-enriched DEG CCBE1 as well as COL4A2 and *Lactobacillus*, respectively (Fig. 4B). CIN1-enriched gene FMO2 seemed to impact *Lactobacillus* levels via the mediation of several metabolites, such as 2-methylpropyl acetate, 3-oxotetradecanoic acid, cis,cis-muconic acid, oleic acid, palmitic acid and cinnamic acid (Fig. 4B). LysoPS 18:2, LysoPS 12:4, 4-aminophenol, thiazolidine-2-carboxylic acid, leucyl-leucine and 2-methylpropyl acetate mediated the expression of SIGLEC6, IL36RN, SPRR2F on the levels of *Aerococcus*, *Fannyhesesea* and *Gardnerella* (Fig. 4B).

## Discussion

Partially consistent with prior studies indicating distinct expression of cervical peptides and cytokines [7, 11, 12], we identified DEGs in cervical exfoliated cells. Our analysis indicated that genes enriched in the CIN1 cohort are primarily associated with immune responses and negative regulation of cell migration, echoing previous reports that implicated altered cell migration and compromised immunity in HPV-infected high-grade CIN and cervical cancer [23, 24]. Specifically, the CIN1-enriched COL4A2 gene, which exhibited a high fold change, encodes type IV collagen, a major structural component of basement membranes that plays a pivotal role in maintaining

membrane integrity [25]. This partially aligns with prior studies indicating elevated levels of type IV collagen in the cervix of individuals with lower CIN grade [26–30]. More intriguingly, amiloride has been shown to suppress the migratory and invasive capabilities of HeLa cells by inhibiting the degradation of type IV collagen through the downregulation of type IV collagenase expression [31]. Additionally, canstatin, which is the C-terminal fragment of type IV collagen, has been identified as a potent inhibitor of tumor growth [26, 32, 33]. Those findings underscore the multifaceted role of COL4A2 and its products in modulating cancer progression. Conversely, the CIN1-depleted FOXD1 gene has been implicated in cancer cell proliferation, cancer progression, as well as poor prognosis [34–37].

Existing evidence has demonstrated the modulation of differentially produced gene products across distinct stages of HPV infection and CIN progression on the growth of *Lactobacillus* via the regulation of host peptides [7]. Furthermore, Zhu et al. found that vaginal *Lactobacillus* was selectively affected by specific metabolites [8], which may differ across distinct states of HPV infection and CIN grades [9, 12, 38, 39]. Partially consistent with these findings, we observed mediation effects in the potential modulation of DEGs on VM and the metabolome. For instance, CIN1-enriched COL4A2 expression modulated the levels of D-lactic acid and L-glutamine via the mediation of *Lactobacillus* and regulated the growth of *Lactobacillus* via the mediation of L-carnitine. Although the precise mechanisms underlying these mediation effects remain elusive, our findings suggest intricate modulations of cervical gene expression on VM and vaginal metabolome.

Further analysis identified a positive correlation between *Lactobacillus* and D-lactic acid as well as DL-indole-3-lactic acid, which play crucial roles in vaginal health and the inhibition of tumor growth [3, 40–45]. Consistent with previous research, it has been demonstrated that D-lactic acid derived from *Lactobacillus* benefits lower genital health [3, 40, 41]. Although reports on the role of vaginal *Lactobacillus*-derived indole-3-lactic acid in the risk of CIN progression are scarce, accumulated evidence indicates that it protects against tumor growth and inflammation [42–45]. As expected, *Fannyhessea*, *Gardnerella*, *Aerococcus,* and *Streptococcus* exhibited negative correlations with these metabolites, partially aligning with the demonstrated inhibition of these genera by *Lactobacillus* [41, 46–48].

The limitations of the current study must be acknowledged. Firstly, the modest sample size present constraints in ensuring definitive conclusions. However, this limitation is partially mitigated by excluding participants with prevalent vaginal infections and menopause, which are known to profoundly impact VM and associated metabolome [49]. Secondly, 16S rDNA amplicon sequencing limited our capacity to comprehensively understand the functional communities of VM [41, 50]. Then we were unable to delve deeply into intra-group heterogeneity and inter-group variations among vaginal *Lactobacillus* strains and their functional landscapes. Thus, we should employ more exhaustive sequencing technologies, such as metagenome and metatranscriptome, to bridge these gaps. Lastly, although our analysis suggested modulation of host gene expression on VM and the metabolome, mediated by each other, mechanisms underlying these connections and causality should be further explored.

In conclusion, our analysis suggested the modulation of VM and associated metabolome via cervical DEGs across distinct stages of CIN. These findings will provide significant population-based evidence for the orchestration of cervical gene expression on VM and metabolome during CIN progression.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03922-8.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.

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**Clinical trial number**
Not applicable.

**Authors' contributions**
D.W, W.R. and D.H. conceived the study. D.H., L.C., H.J. and G.C. recruited attenders and collected samples. W.D. and X.R. performed sample storage and associated experimental analysis. D.W., L.Y., J.X. and X.R. performed data analysis and drafted the manuscript. All authors reviewed the manuscript.

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**Data availability**
The raw data of VM and metabolome have been deposited in CNGB Sequence Archive (CNSA) under Project No. CNP0005859. Sequencing data of host transcriptome analysis have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022) with accession code HRA008087.

## Declarations

**Ethics approval and consent to participate**
This study was approved by the Ethics Committee of Peking University Shenzhen Hospital (registration number: 2021–006). All participants were

fully informed and then provided signed consents. We declare that our study adhered to the Declaration of Helsinki.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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