

BACKGROUND

- Cervical intraepithelial neoplasia (CIN) is a common gynecological condition often associated with persistent human papillomavirus (HPV) infections, characterized by abnormal cell growth on the cervix.
- The primary treatment for CIN involves the Loop Electrosurgical Excision Procedure (LEEP), which effectively removes lesions in most cases. However, some patients do not achieve HPV negativity even after lesion removal.
- Research has indicated that LEEP can alter the cervicovaginal microenvironment, impacting patient outcomes. While there have been efforts to characterize the cervicovaginal bacterial communities associated with HPV, the causal relationships related to HPV clearance failures remain underexplored.
- The intratissue microbiota has gained recognition as a crucial component of the tissue microenvironment, particularly concerning cancers arising from mucosal areas
- The cervicovaginal microenvironment includes host-derived and microbially produced metabolites that may influence HPV status.

OBJECTIVE

- This study aims to compare the microbiota of HPV-cleared versus non-cleared CIN patients post-LEEP and to identify specific microbiota and metabolites associated with HPV clearance.

METHODS

- A total of 80 participants, aged 20–50 and diagnosed with either cervical intraepithelial neoplasia I (CIN I; n = 30, persisting for over 18 months) or CIN II/III (n = 50), were enrolled.
- At 6 months post-treatment, 43 participants completed postoperative follow-up, including 11 with CIN I and 32 with CIN II/III.
- 5R 16S rRNA gene sequencing analysis of cervicovaginal secretion and cervical tissue microbiomes
- Non-targeted and targeted metabolomic assessments of the cervicovaginal metabolome

RESULTS

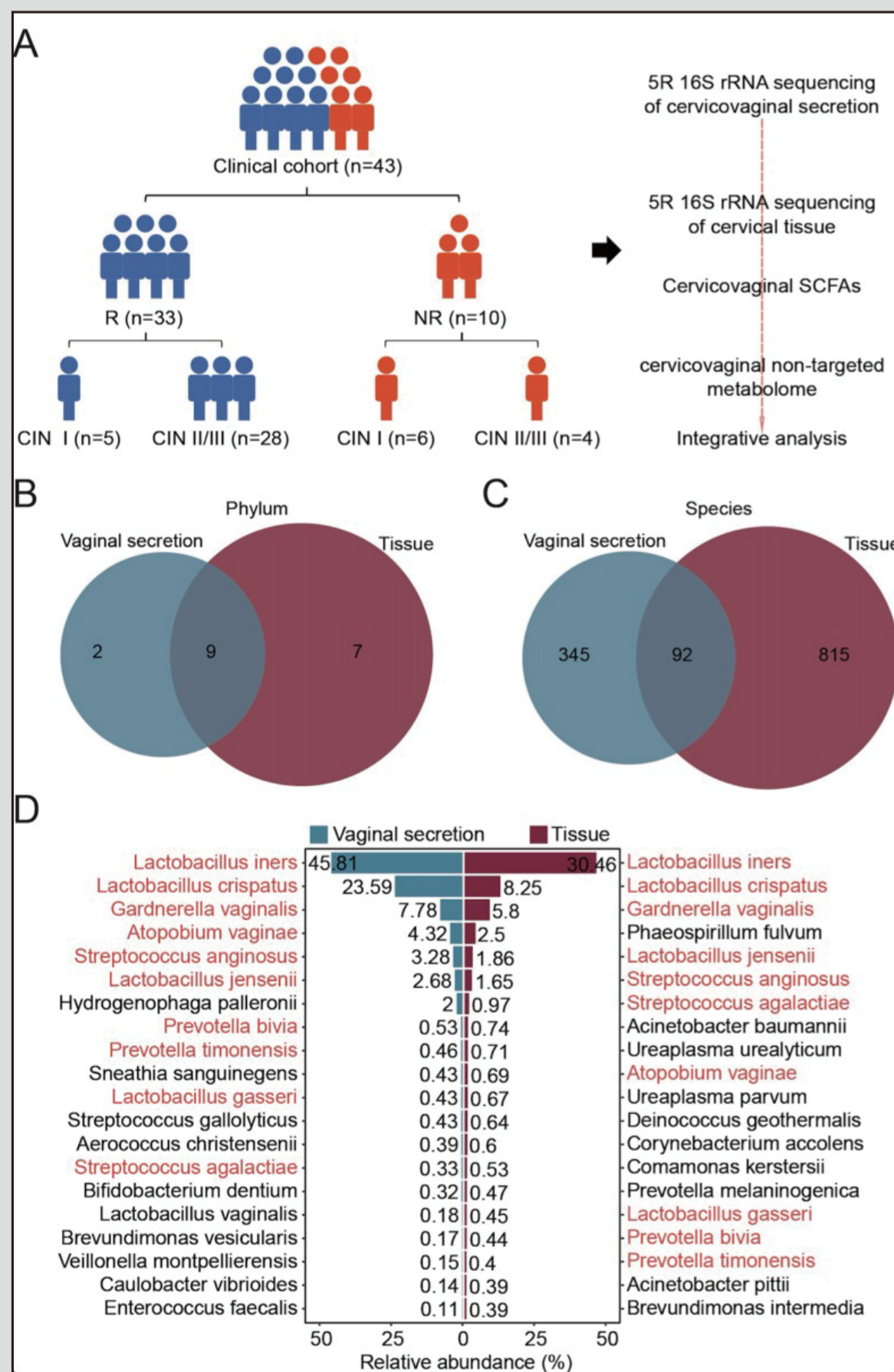


Figure1 Comparison the results of the 5R 16S rRNA gene sequencing from cervicovaginal secretions and cervical tissue samples.

(A) Study workflow illustrating that participants underwent sequencing of cervicovaginal secretion and cervical tissue 5R 16S rRNA, along with targeted metabolomic analysis of SCFAs and non-targeted metabolomics, followed by bioinformatic integration of microbiome and metabolome data. (B–C) Venn diagrams indicating the distribution of shared and unique microbiota at the phylum level (B) and species level (C). (D) A butterfly diagram showcasing the top 20 bacterial species from cervicovaginal secretion and cervical tissue samples, with shared species highlighted in red.

Abbreviations: R, HPV-cleared; NR, HPV non-cleared;

RESULTS

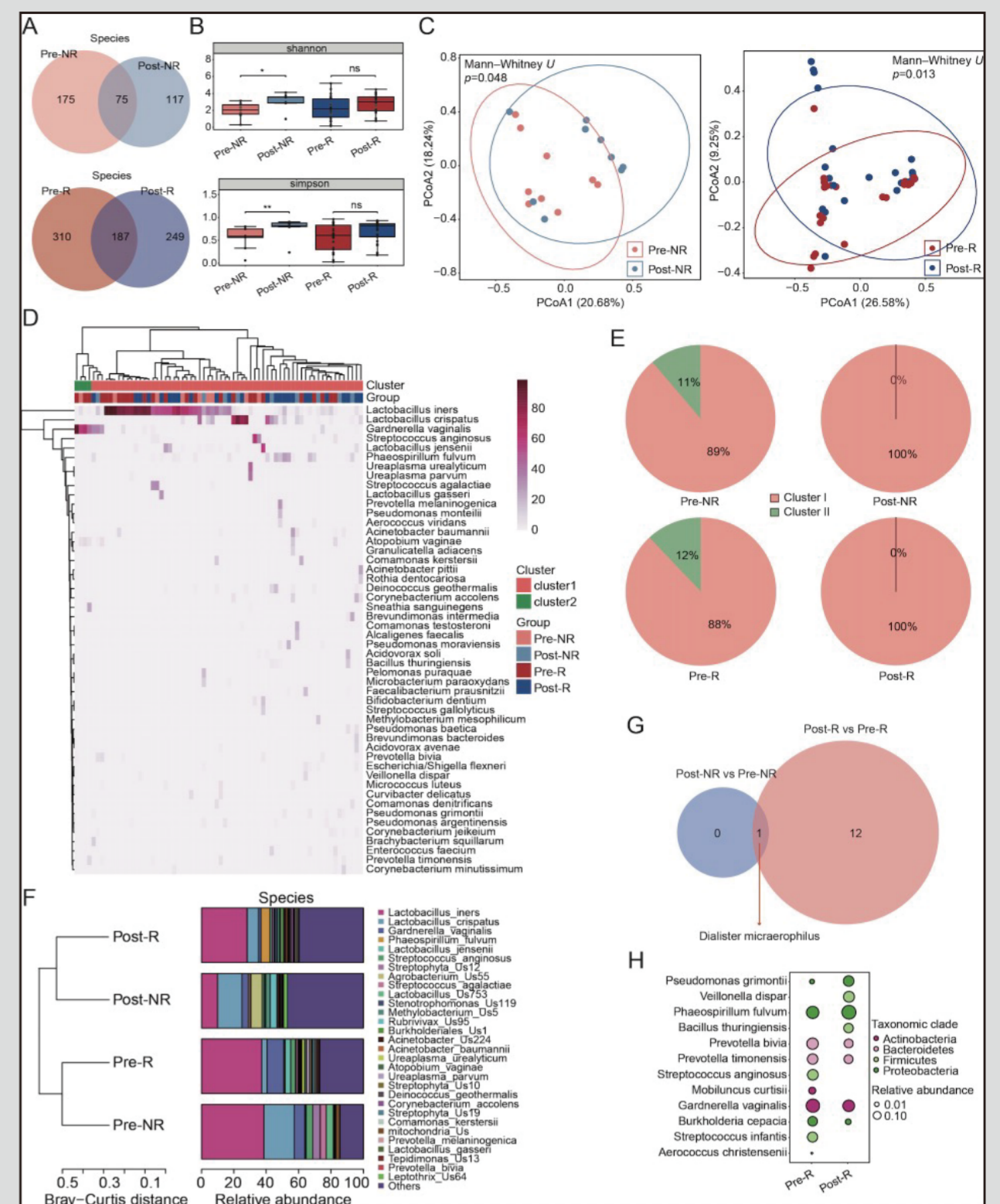


Figure2 Analysis of bacterial diversity and composition of cervical tissue pre- and post-LEEP surgery.

(A) Venn diagrams comparing bacterial species before and following LEEP, specifically for pre-NR vs post-NR (top) and pre-R vs post-R (bottom). (B) Assessment of alpha diversity via Shannon (top) and Simpson (bottom) indices between the sample pairs, using Mann-Whitney U test for statistical analysis. (C) Beta diversity determined through PCoA based on Bray-Curtis distances between pre-NR and post-NR (left), as well as between pre-R and post-R (right), with significance evaluated by ANOSIM. (D) Heatmap illustrating the top 50 bacterial species across 68 samples. (E) Visualization of bacterial clusters within the assessed groups. (F) Hierarchical clustering on the average proportions of the 30 most abundant species among the four groups based on Bray-Curtis distances. (G) A Venn diagram representing unique DABs for the R group (Mann-Whitney U test, $p < 0.05$). (H) A bubble diagram illustrating eleven DABs identified within the R group.

RESULTS

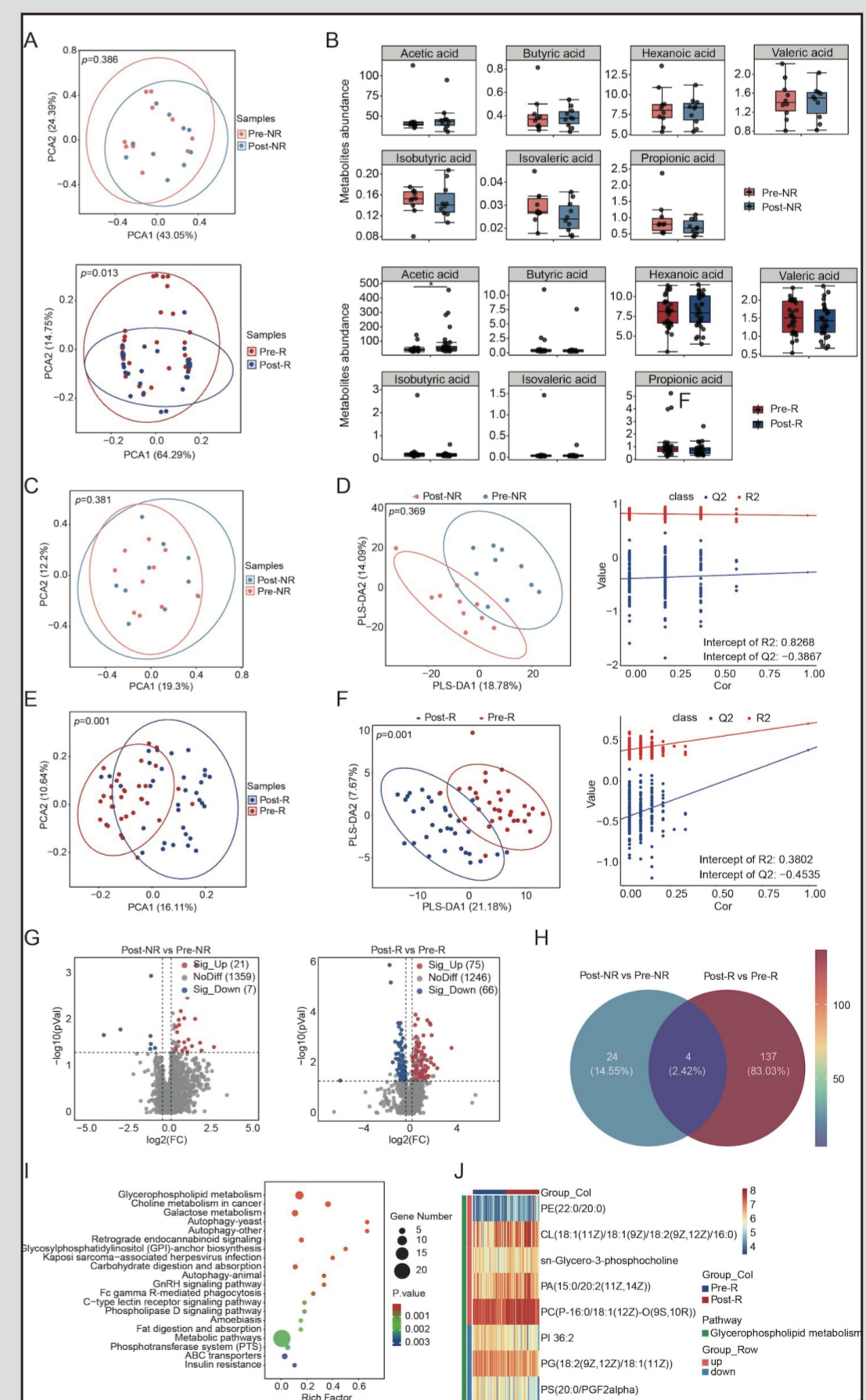


Figure3 Metabolic profiling of the cervicovaginal metabolome pre- and post-LEEP surgery

(A) PCA plots based on Bray-Curtis distances for SCFAs comparing pre-NR vs post-NR (top) and pre-R vs post-R (bottom), with significance derived from ANOSIM. (B) Box plots depicting SCFA levels for comparing pre-NR and post-NR (top), as well as pre-R and post-R (bottom) before and after LEEP. (C and E) PCA plots for non-targeted metabolomics relating to pre-NR vs post-NR (C) and pre-R vs post-R (E), assessed for significance via ANOSIM. (D and F) PLS-DA was utilized for comparisons between pre-NR and post-NR (D), alongside the pre-R and post-R (F), with model validity confirmed through 200 permutations. (G) Volcano plots display metabolic alterations between pre-NR vs post-NR (left) and pre-R vs post-R (right), with notable metabolites indicated by red (up) and blue (down) dots based on VIP score and Mann-Whitney U test thresholds. (H) A Venn diagram showing unique differential metabolites specific to the R group. (I) A bubble chart detailing the 20 enriched KEGG pathways associated with the different metabolites shown in (H). (J) Heatmap illustrating significantly altered metabolites related to glycerophospholipid metabolism.

CONCLUSIONS

- The presence of beneficial Lactobacillus species and the production of SCFAs, particularly acetic acid, were linked to successful HPV clearance;
- Certain anaerobic bacteria were more prevalent in patients with persistent infections;
- We identified distinct microbial communities and metabolic alterations associated with HPV status, highlighting specific bacterial species and metabolites that may influence the likelihood of HPV clearance.