Association between Cervical Microbiota, HPV and the risk for cervical cancer in Hispanics

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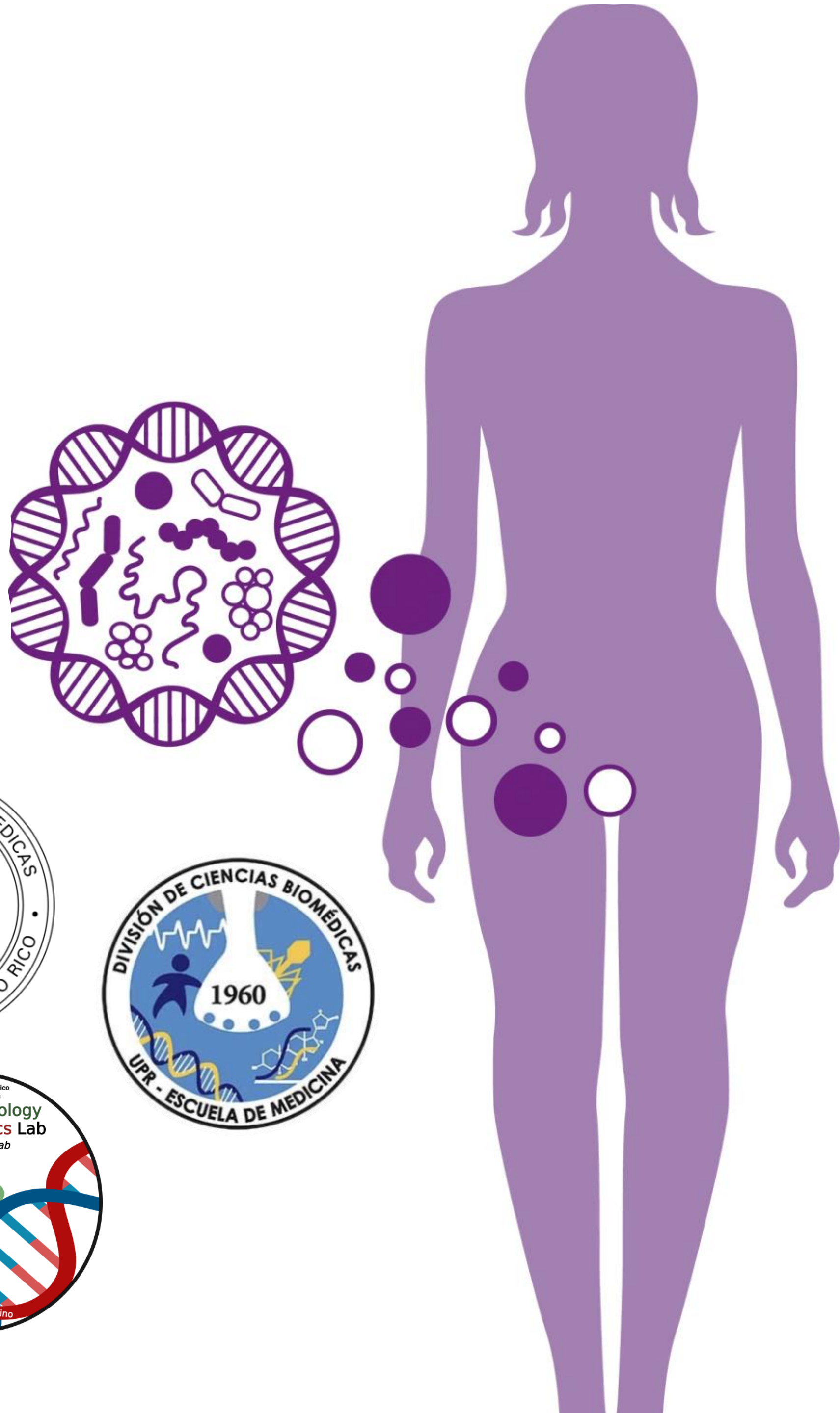

FLASCO 2023 Session: The Emerging Role of the Microbiome in Cancer Outcomes

Conflict of Interest Statement

No conflicts of interest to declare

UPR Microbiomes Lab

UPR-MSC Dept Microbiology & Medical Zoology

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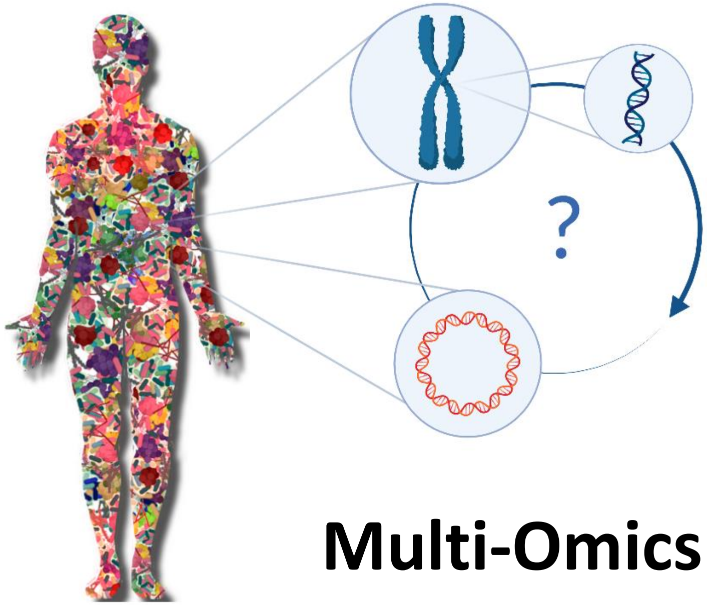
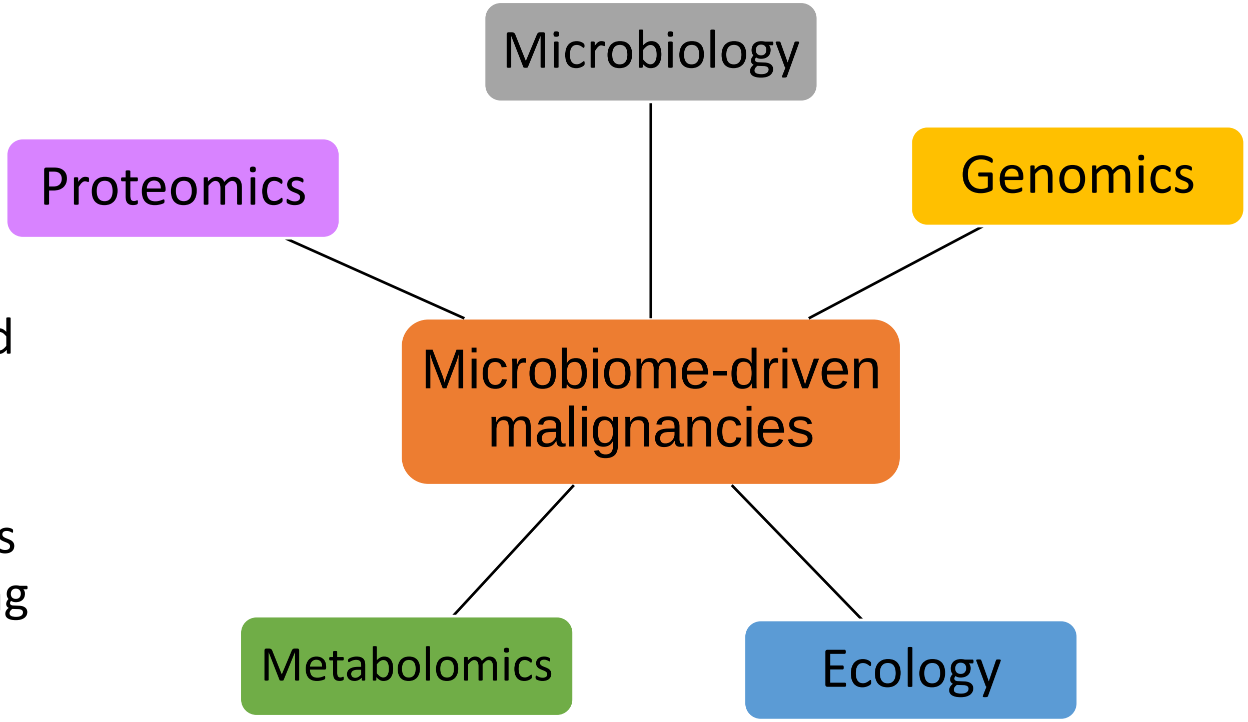


University of Puerto Rico
School of Medicine
Microbial Ecology
and Genomics Lab
Microbiome Lab
PI: Dr. Filipa Godoy Vitorino

- We study **microbiomes**, including dynamics and functions of microbial communities in host-associated ecosystems (mostly in humans/Women health, and animals)
- We aim at understanding the **coevolution** of microbes with their hosts, the symbiotic mechanisms underlying microbial **resilience** as well as the processes that lead to **dysbiosis** and disease phenotypes.

Multi-Omics Approach



Microbiology

Proteomics

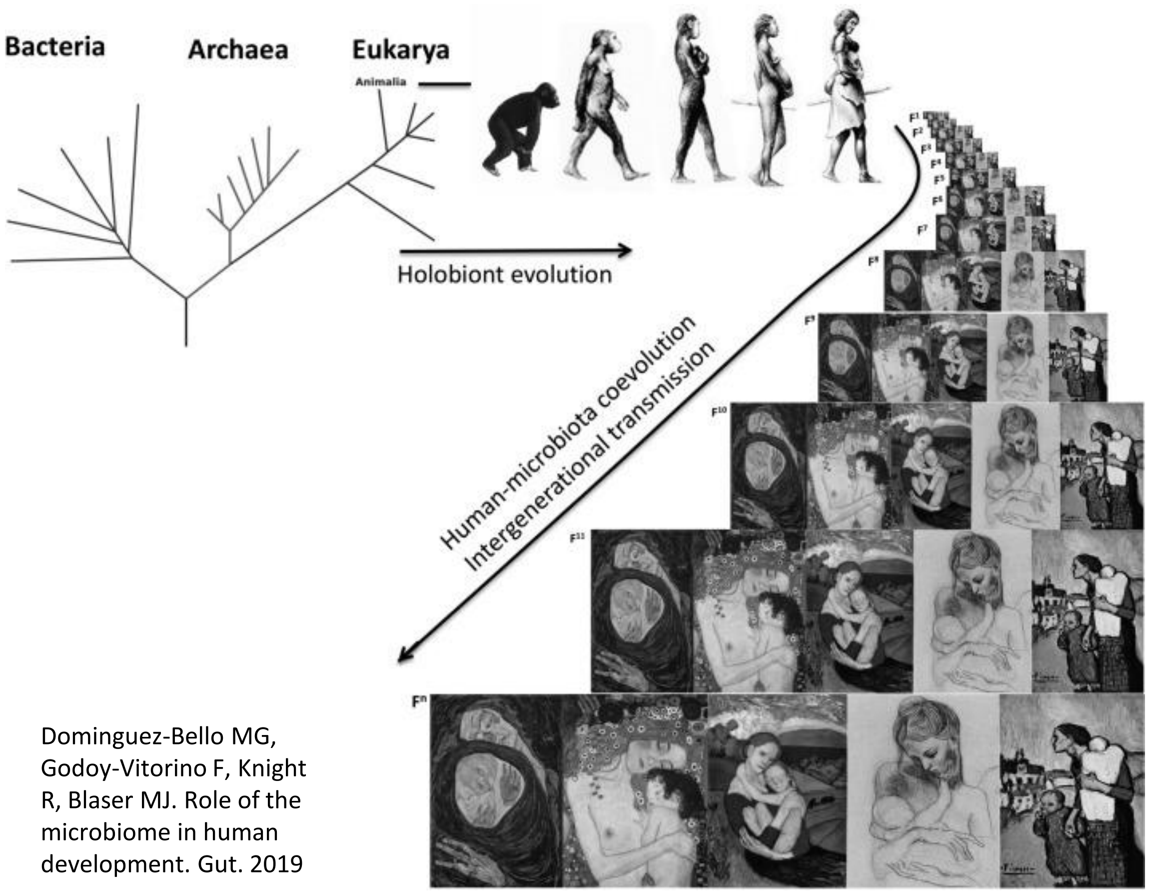
Genomics

Microbiome-driven malignancies

Metabolomics

Ecology

Evolution of the holobiont and vertical transmission through human generations



Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ. Role of the microbiome in human development. Gut. 2019

Leaders | Modern medicine

Microbes maketh man

People are not just people. They are an awful lot of microbes, too

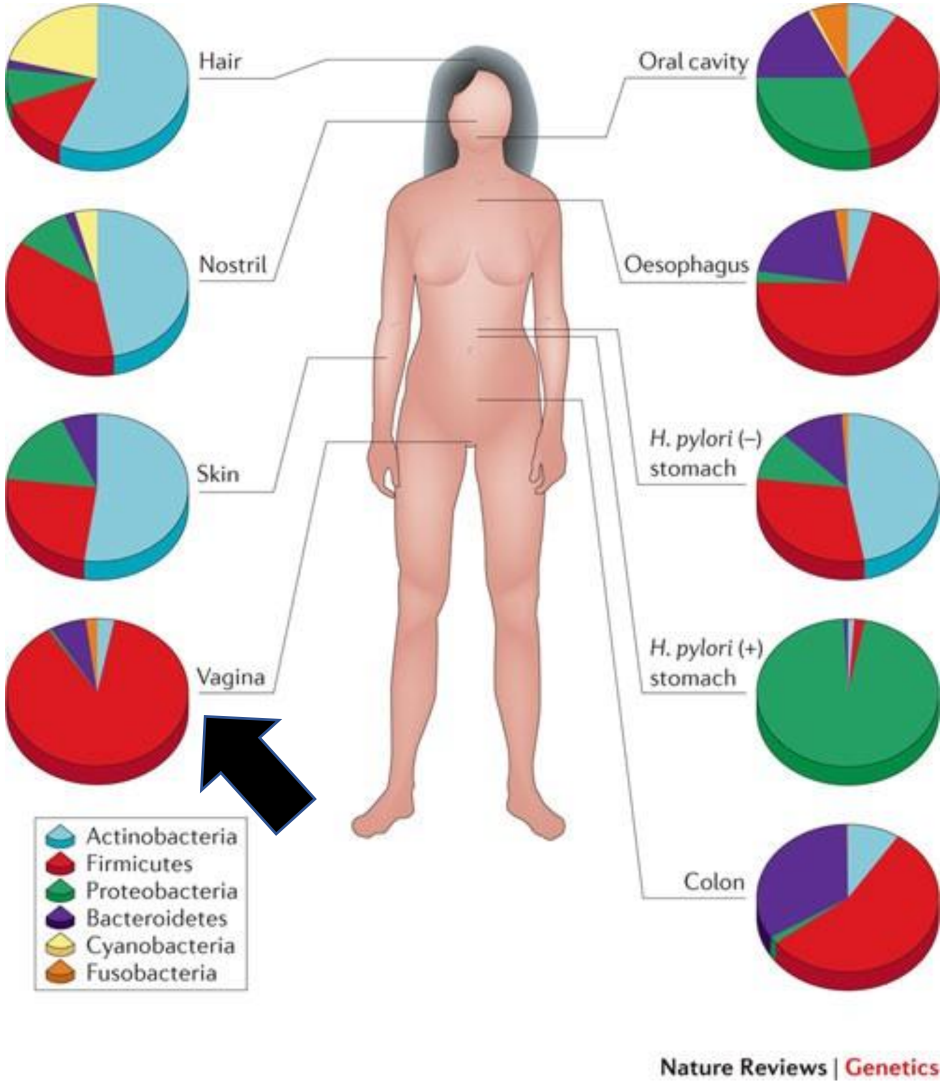
Jon Berkeley/SPL

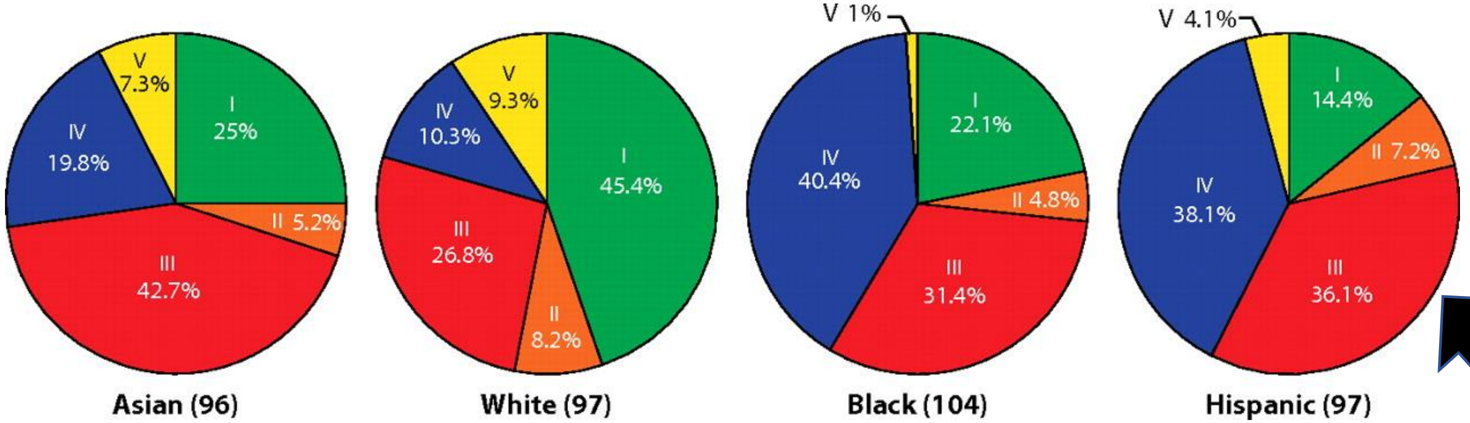
https://www.economist.com/leaders/2012/08/18/microbes-maketh-man

- **Evolution** of all complex life forms has occurred **in associations with bacteria**, the first forms of life on earth.
- The human body carries **representatives of all branches of the tree of life** (Animalia-Homo sapiens, protozoa, fungi, archaea and bacteria conforming the microbiota).
- The **microbiota has been transferred throughout generations of humans**, with the matrilineal line transferring the primordial birth microbiota.
- The **vertical human transmission** has led to conservation of a **phylogenetic signal in human microbiota** communities.

Human body niches:
Is the vagina a low diversity organ?

- The female reproductive tract is known to be the **<u>least diverse microbial ecosystem of the human body .</u>**
- It has an epithelial barrier under estrogen influence.
- *Lactobacillus* are characteristically dominant of “normal” healthy vagina.
- **<u>Changes in communities due to ethnicity, lifestyle and infections!</u>**

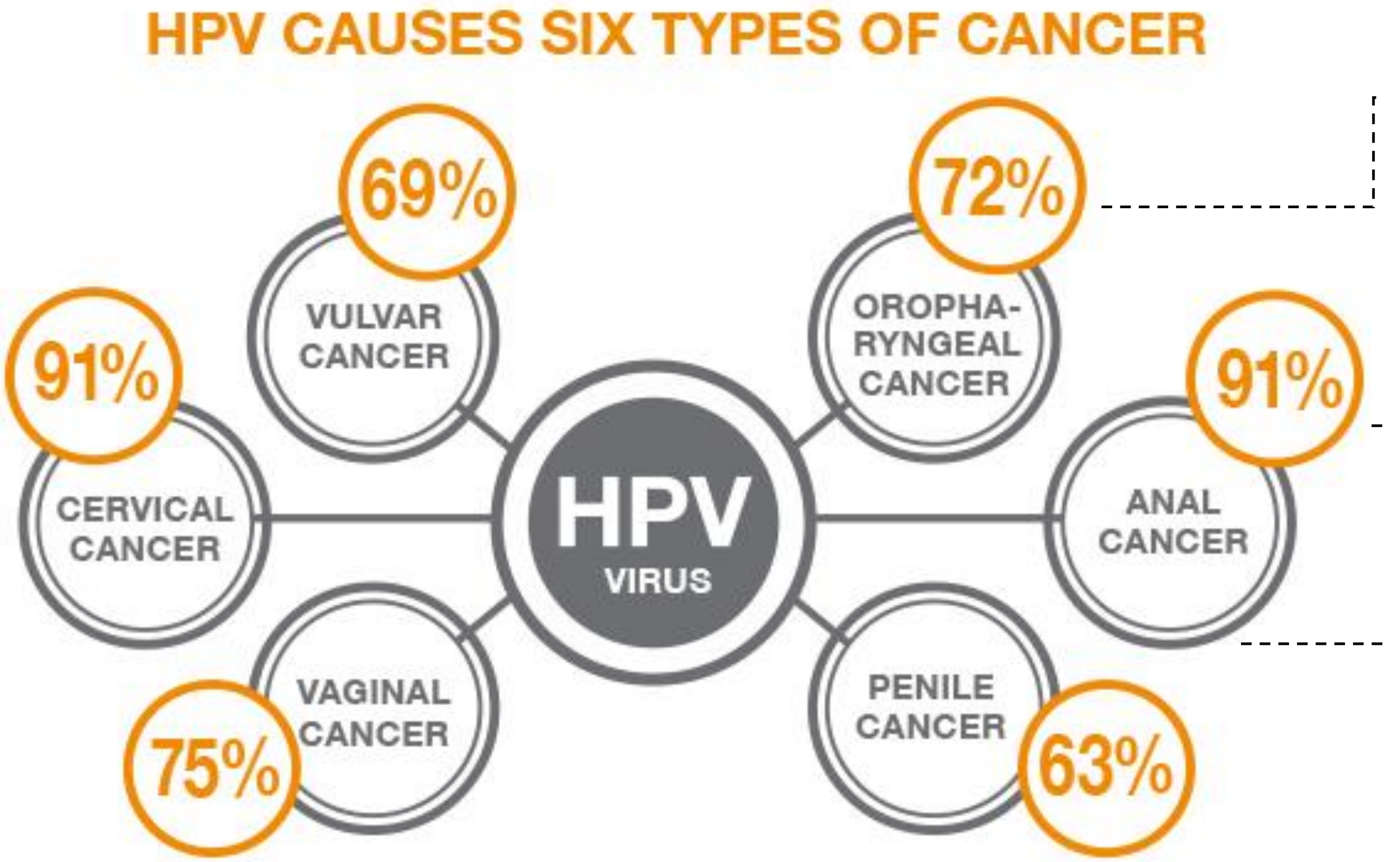




Ravel et al. The vaginal microbiome of reproductive age women. PNAS. 2011.

Cho, I., Blaser, M. The human microbiome: at the interface of health and disease. *Nat Rev Genet* **13**, 260–270 (2012)

Human Papillomavirus-Induced Carcinogenesis on the rise in PR



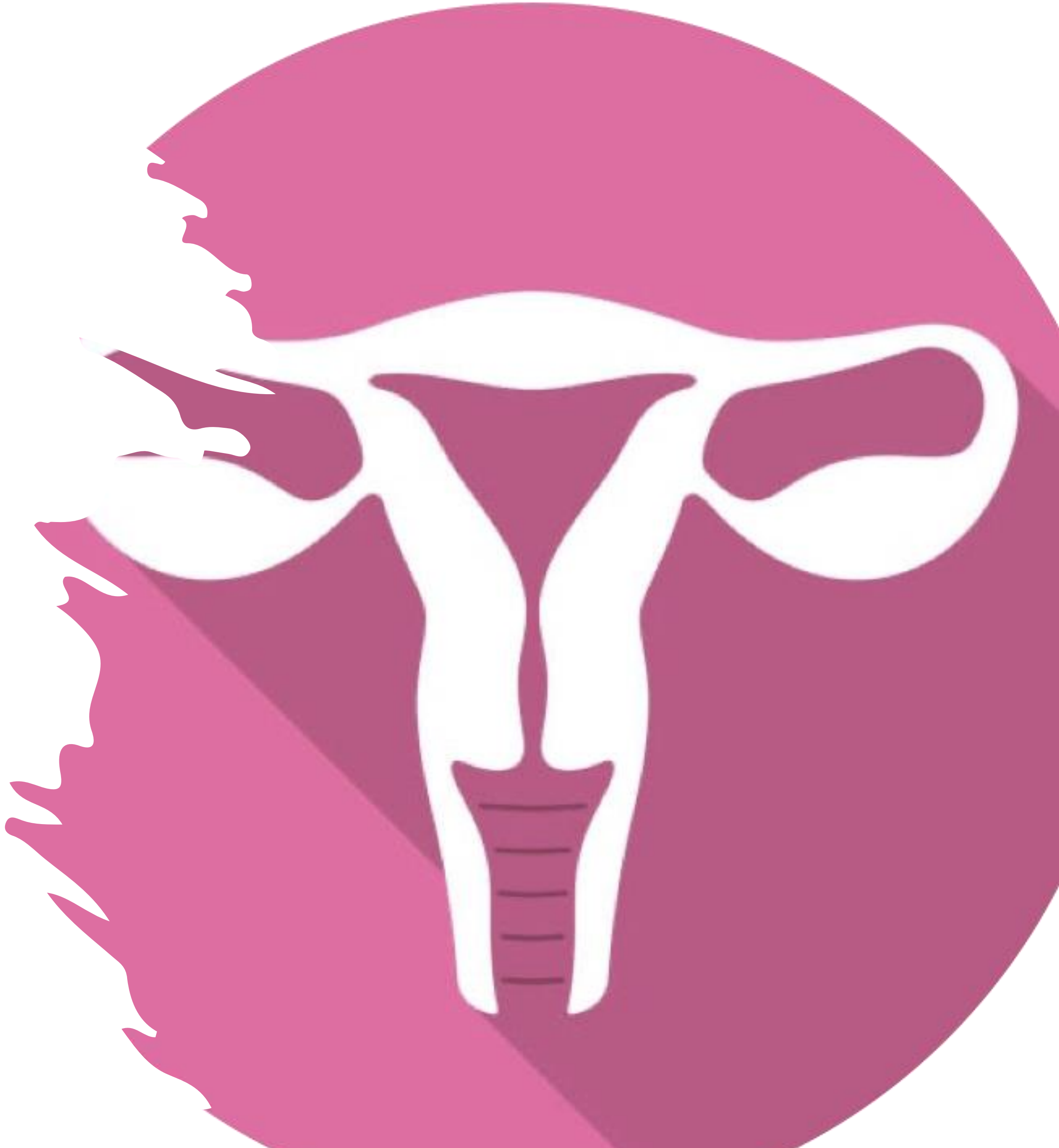
Human papillomaviruses (HPV) are the most common STI worldwide, causing oral, anal and cervical carcinomas

Nearly all cervical cancer is due to HPV; with two types, HPV16 and HPV18, accounting for 70% of cases.

99% of cervical cancers have HPV DNA within the tumor (CDC).

Human Papillomavirus-Induced Carcinogenesis on the rise in PR

- **Despite advances in HPV vaccination**, cervical cancer remains a significant global problem, being **second most common cancer in women in Latin America and the Caribbean**. (PAHO)
- From **2001 to 2017, cervical cancer cases were on the rise in Puerto Rico**, increasing the incidence per 100,000 person-years from 9.2 to 13. Ortiz,AP JAMA Oncology 2021.
- **Factors promoting hr-HPV persistence and those leading to cervical cancer are not completely understood**.



At least half of sexually active people will have HPV at some point in their lives, but few women will get cervical cancer.

Microbiome might be the missing link.....

Hypothesis

- We hypothesize that the cervical microenvironment, including the immune response and the microbiome influence the course of HPV infections and cervical disease.

Experimental Work

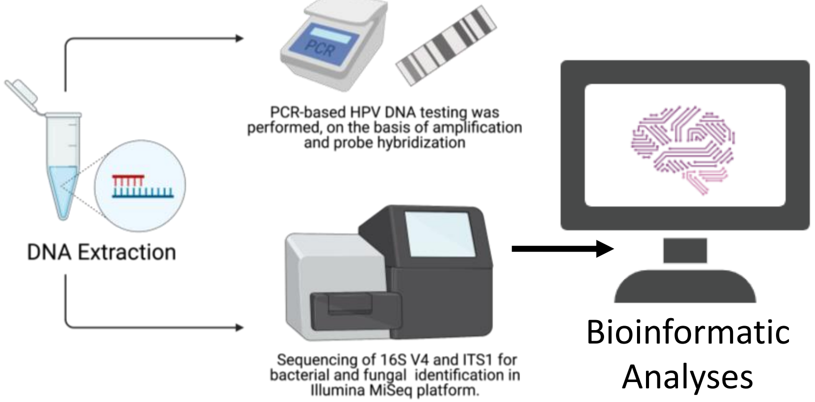
Josefina Romaguera, MD
Clinician

Eduardo Tosado, MSc
PhD. candidate

Recruitment
>500 (21-60 Years old)

Clinical questionnaire
(Demographics, BMI, Sexual information etc.)
Clinician acquires samples

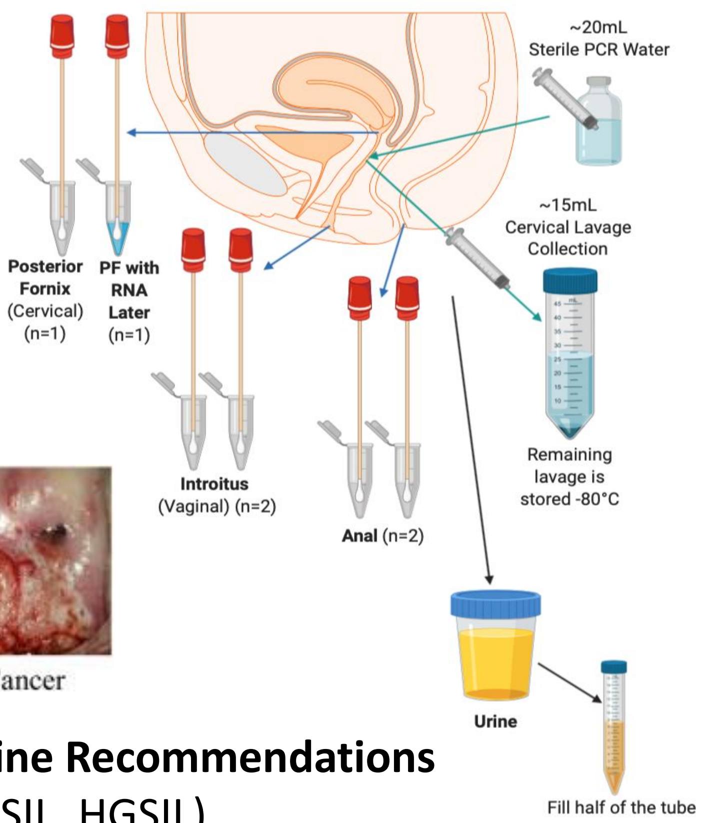
IRB Protocol ref. 1050114 / IBC # 94620

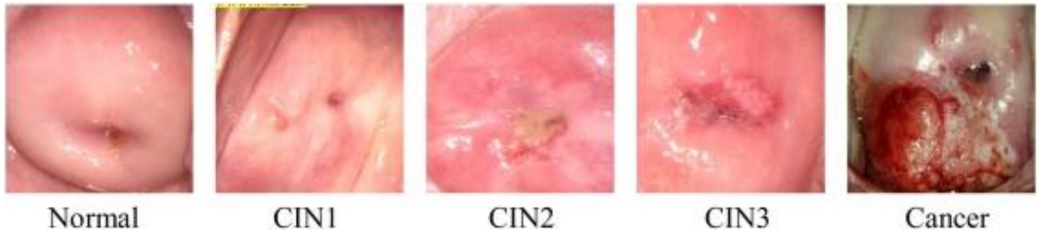


Over 600 Participants Recruited and Ongoing

- Microbiome 16S rRNA: n=500
- Microbiome ITS2 rRNA: n=200
- Urine Metabolomics: n=250
- Cervical Lavage Metabolomics: n=200
- Cytokine Profile: n=120
- Shotgun Sequencing: n=80
- Quantitative Proteomics: n=50

Sampling Process





Terminology Used in ASCCP Consensus Guideline Recommendations

Cytology screening = PAP test (NSIL, ASCUS, LGSIL, HGSIL)

Pathology = Colposcopy Biopsy (NILM / CIN's 1-3)

DNA Extraction
- gDNA extractions with QIAGEN Powersoil kit
- Stringent QC for genomic analyses (gDNA, Amplicons)

HPV Typification
- Reverse hybridization with the HPV SPF10-LiPA25 kit (DDL, Netherlands); PCR -65bp fragment amplification from HPV L1 gen (DNA ELISA Kit HPV SF10, version 1) 2- followed by Reversed Hybridization Assay (RHA kit SF10-LIPA25, version 1)

Bacterial Biota (16S) (cervix, vagina, anus)
- Amplification of 16S rDNA V3-V4 region (~275bp)
- Illumina miseq (amplicons)

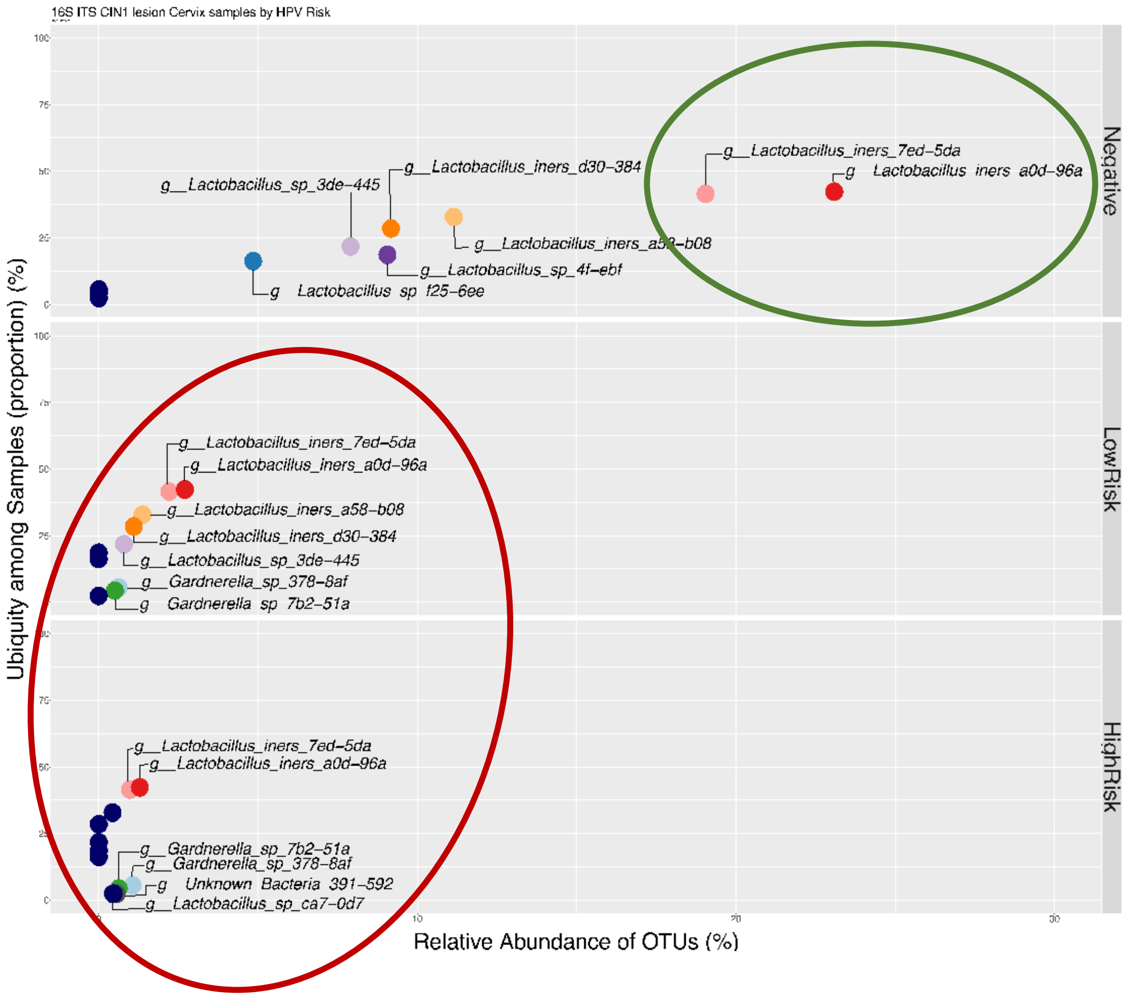
Fungal Biota (ITS-2) (cervix)
- Amplification of ITS 2 (~300bp)
- Illumina miseq (amplicons)

Shotgun Metagenomics
- Good quality gDNA (150-200ng)
- Shotgun Illumina NextSeq

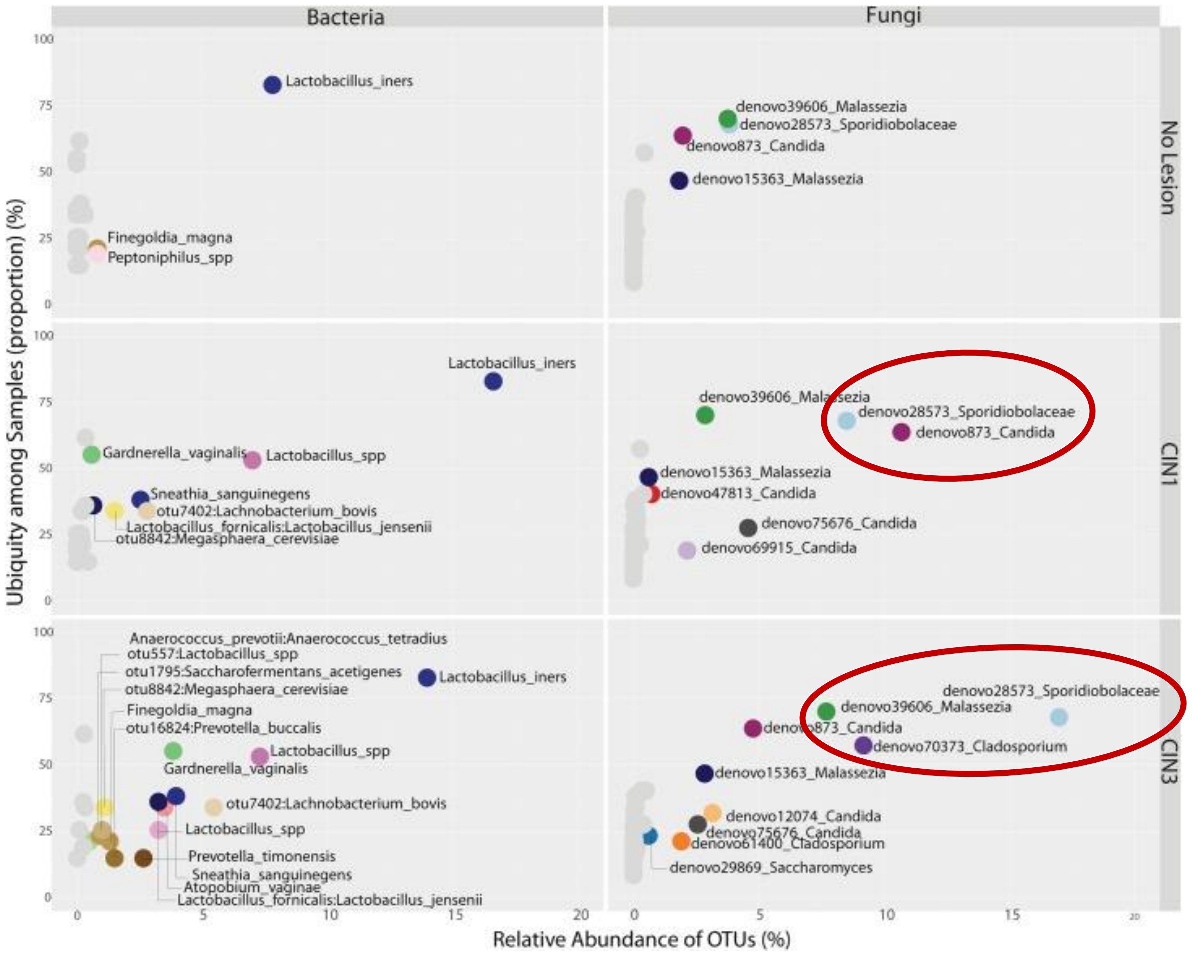
Cytokine Profiling from Cervical Lavages
- Luminex Multiplex Assay

Urine and Cervical Lavage GC-MS Untargeted Metabolomics
- Sample prep for metabolite extraction
- Derivatization
- GC-MS

Changes in the ubiquity and prevalence of certain taxa associate with HPV and lesion status



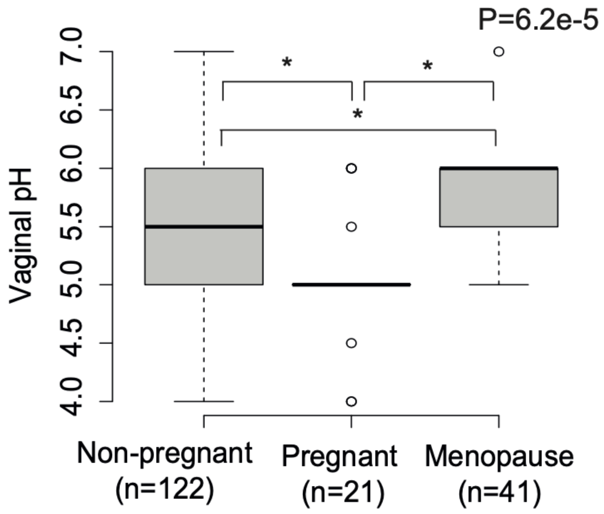
Reduction of *Lactobacillus* with HPV

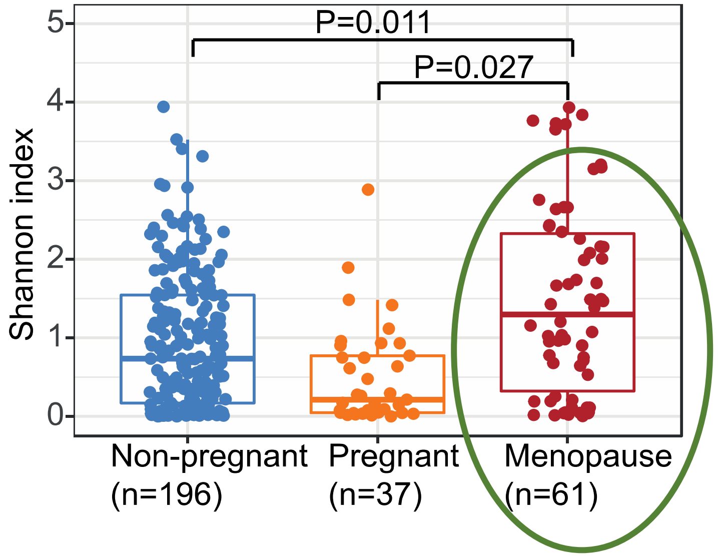


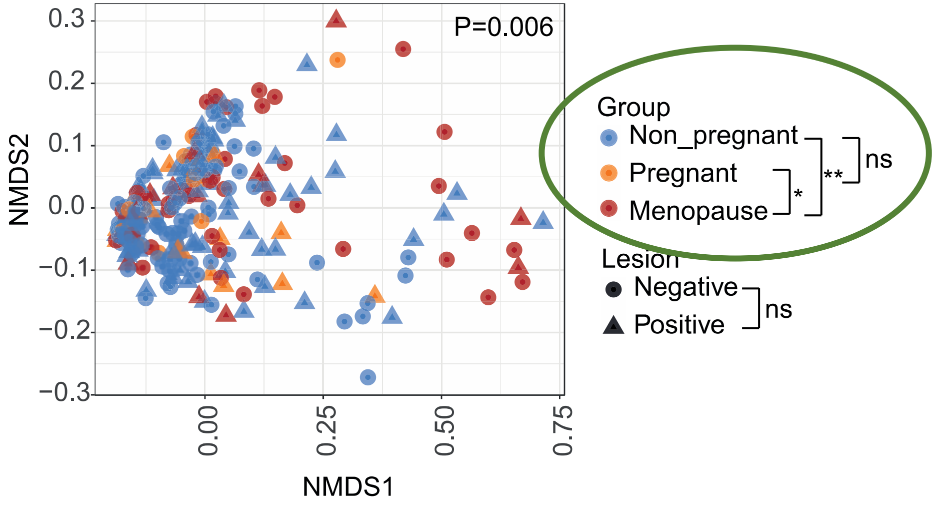
Increase in *Candida* and *Sporidiobollaceae* and *Malassezia* with cervical lesions.

Godoy-Vitorino et al Frontiers in Microbiology, PMID: 30405584

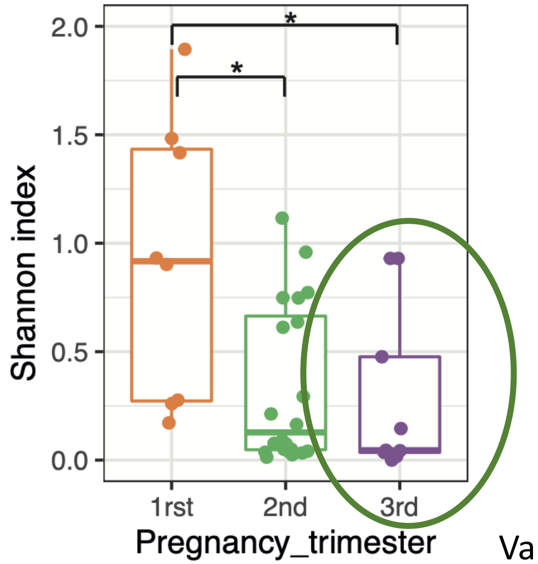
Vaginal microbiota comparison among non-pregnant, pregnant and menopause women reveal a highly volatile community





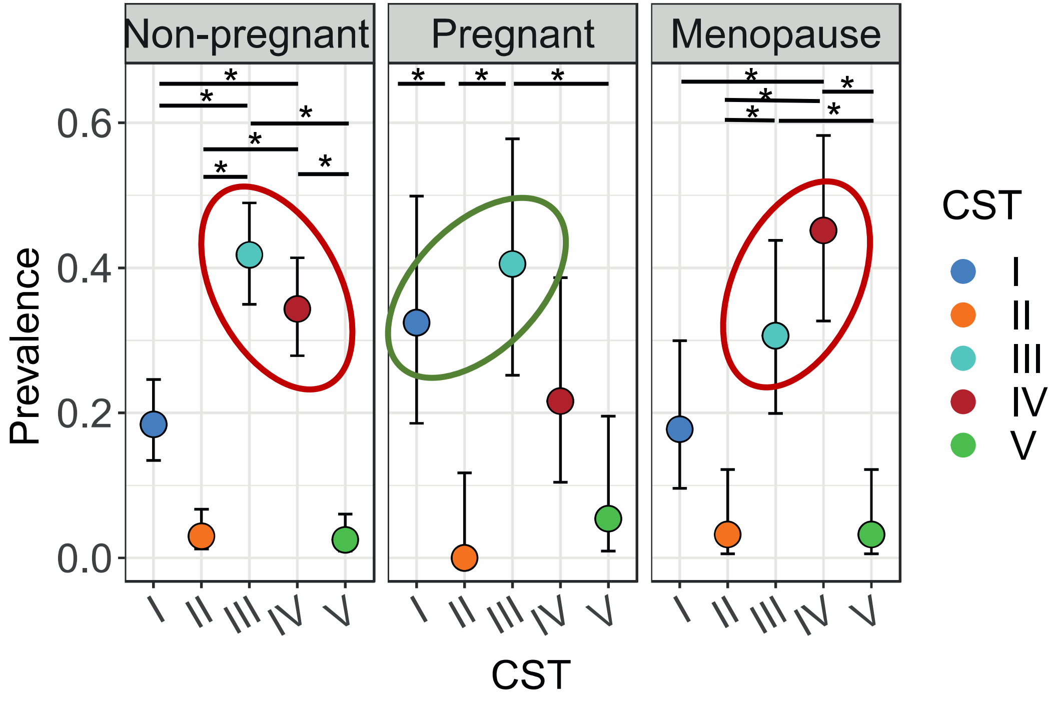


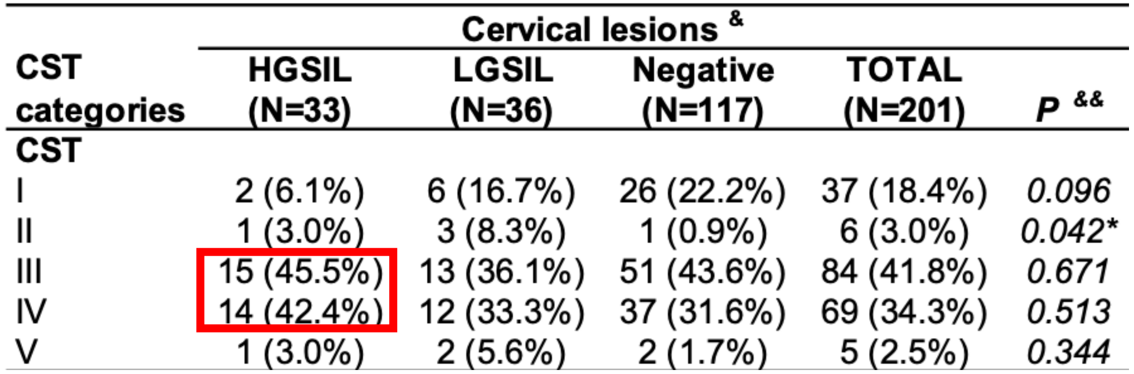
- Vaginal microbiota diversity significantly related to increase in pH.
- Low *Lactobacillus* induces high cervicovaginal pH leading to an environment more susceptible to infections.
- Women in menopause have highly diverse microbiomes and different community composition from pregnant and non-pregnant women.
- Pregnancy reduces diversity specially in the third trimester.



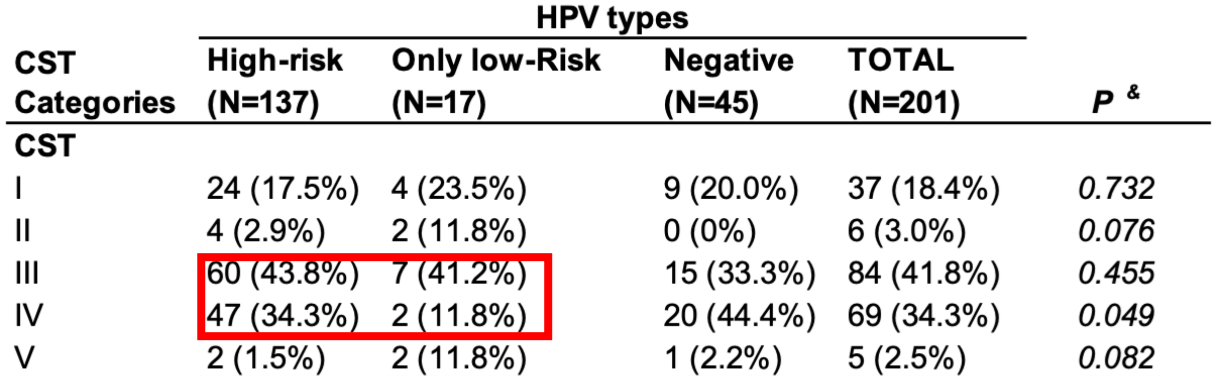
Vargas-Robles, submitted

CST architecture among groups of women





CST categories	Cervical lesions & HGSIL (N=33)	LGSIL (N=36)	Negative (N=117)	TOTAL (N=201)	P &&
CST					
I	2 (6.1%)	6 (16.7%)	26 (22.2%)	37 (18.4%)	*0.096*
II	1 (3.0%)	3 (8.3%)	1 (0.9%)	6 (3.0%)	*0.042**
III	15 (45.5%)	13 (36.1%)	51 (43.6%)	84 (41.8%)	*0.671*
IV	14 (42.4%)	12 (33.3%)	37 (31.6%)	69 (34.3%)	*0.513*
V	1 (3.0%)	2 (5.6%)	2 (1.7%)	5 (2.5%)	*0.344*



CST Categories	HPV types High-risk (N=137)	Only low-Risk (N=17)	Negative (N=45)	TOTAL (N=201)	P &
CST					
I	24 (17.5%)	4 (23.5%)	9 (20.0%)	37 (18.4%)	*0.732*
II	4 (2.9%)	2 (11.8%)	0 (0%)	6 (3.0%)	*0.076*
III	60 (43.8%)	7 (41.2%)	15 (33.3%)	84 (41.8%)	*0.455*
IV	47 (34.3%)	2 (11.8%)	20 (44.4%)	69 (34.3%)	*0.049*
V	2 (1.5%)	2 (11.8%)	1 (2.2%)	5 (2.5%)	*0.082*

- **CST-III was more prevalent in non-pregnant women,** while in contrast **CST-IV predominated in menopausal women.**
- **Pregnant women** mainly have *Lactobacillus* dominant **CST-I and CST-III**
- **CSTIII and IV** are dominant across women groups and mostly **associated with cervical lesion and HR HPV**.

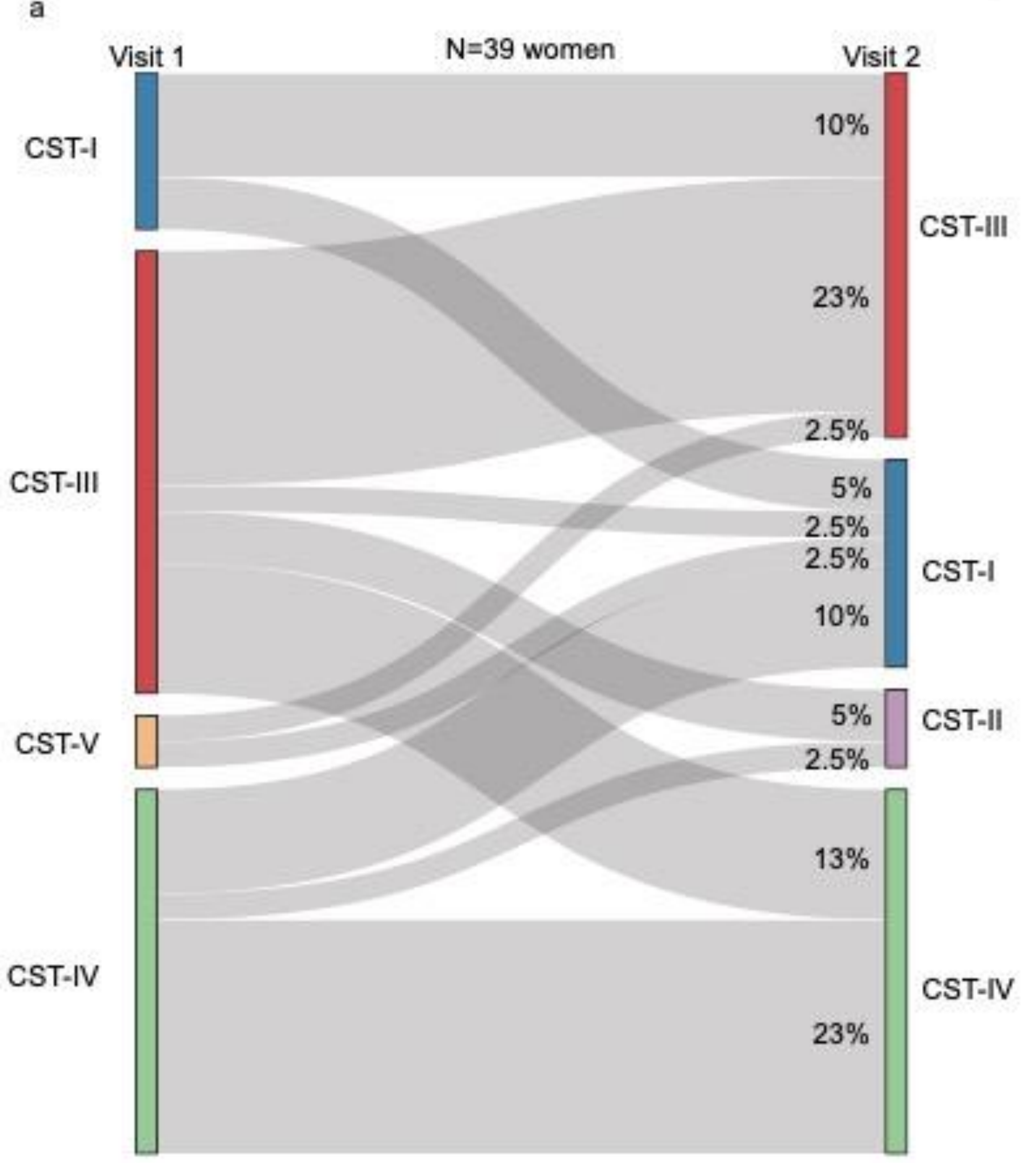
CST I communities are **dominated by *L. crispatus***
CST II communities are **dominated by *L. gasseri***
CST III communities are **dominated by *L. iners***
CST IV **low levels of *Lactobacillus***

Vargas-Robles, submitted

Longitudinal analyses reveals resilience of CST-III and IV

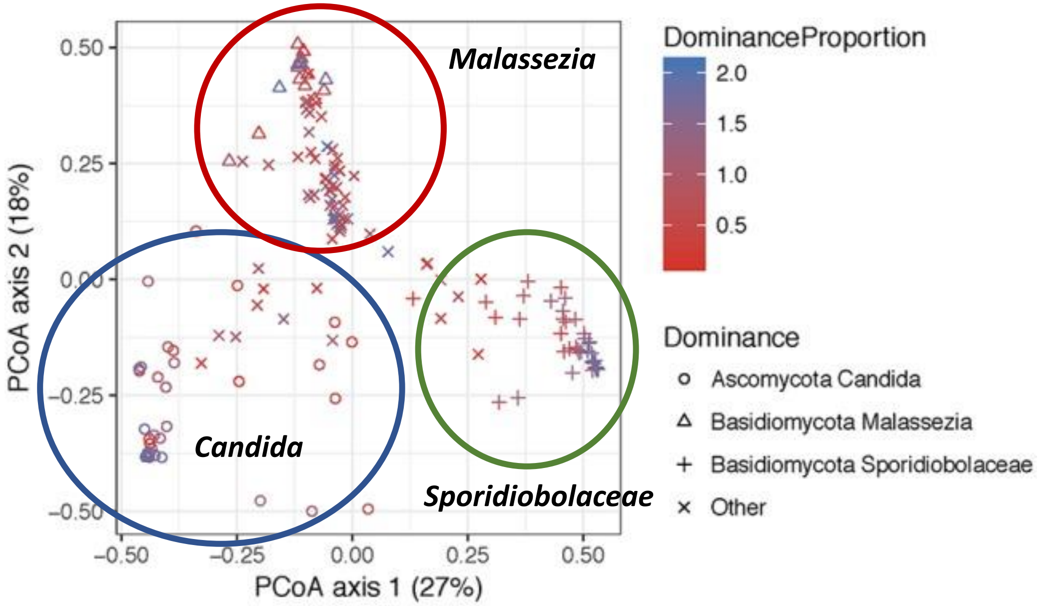
- Only 5% women maintain stable CST-I communities
- CST-III remains constant or evolves mostly to CST-IV
- Only 10% of CST-IV improves to CST-I

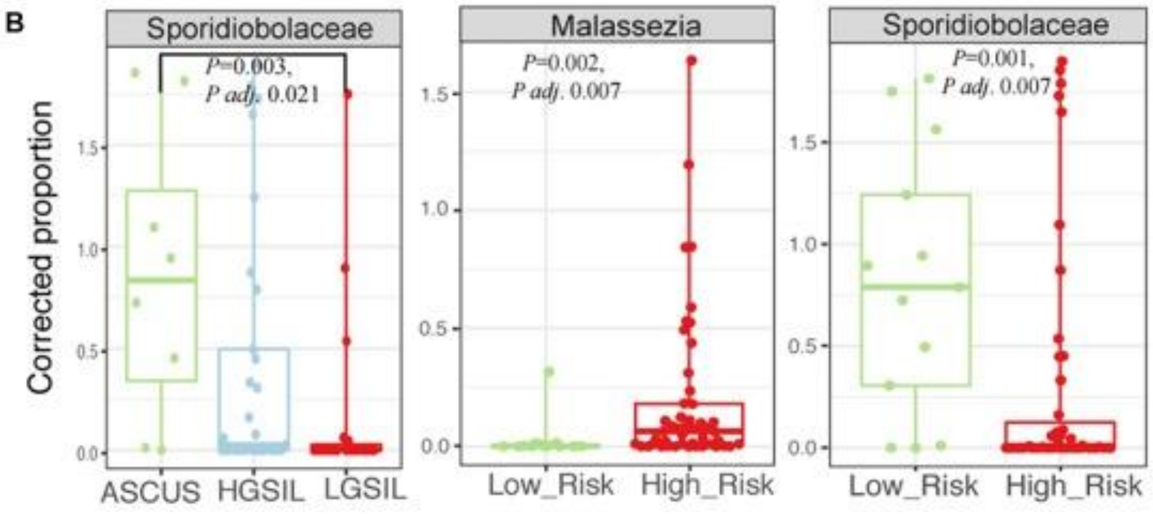
Approximately half of women (51.3%, 20/39) kept the same CST between visits

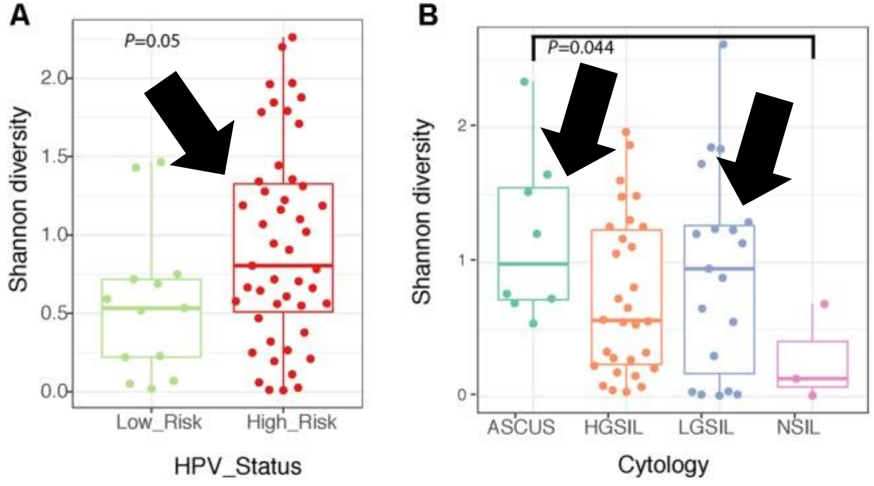


Vargas-Robles, submitted

What about fungi? – Increase in diversity with high grade lesions and hrHPV



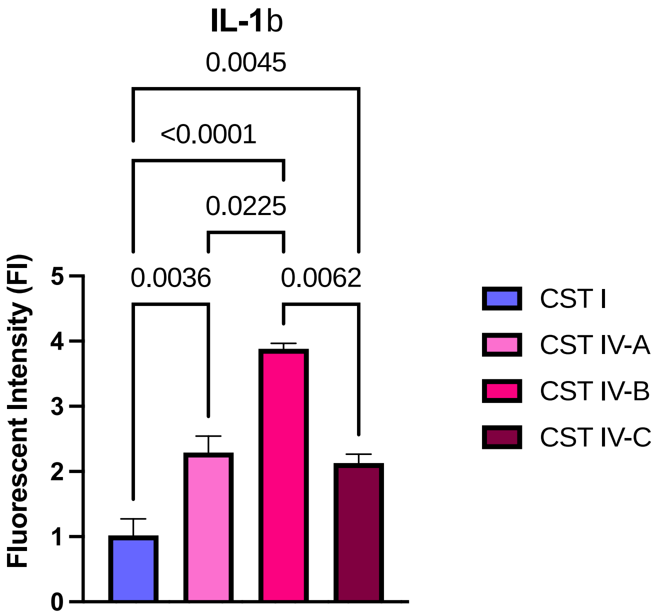


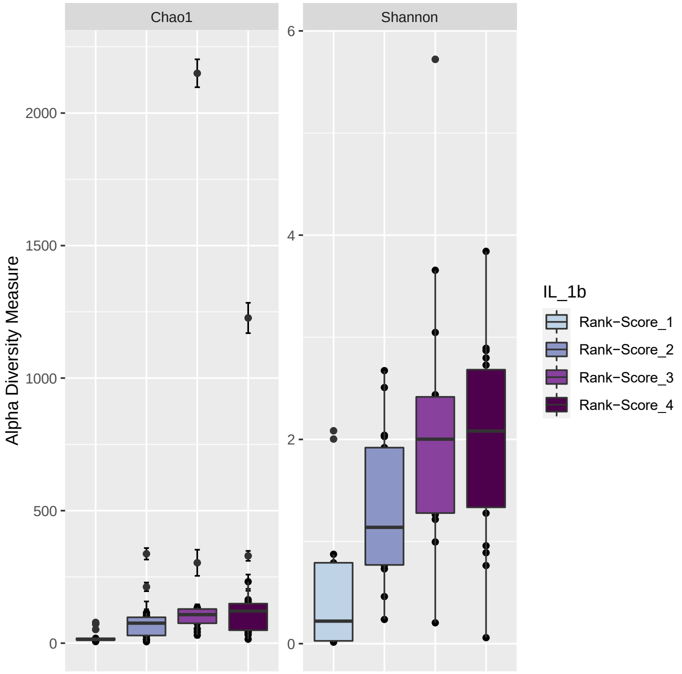


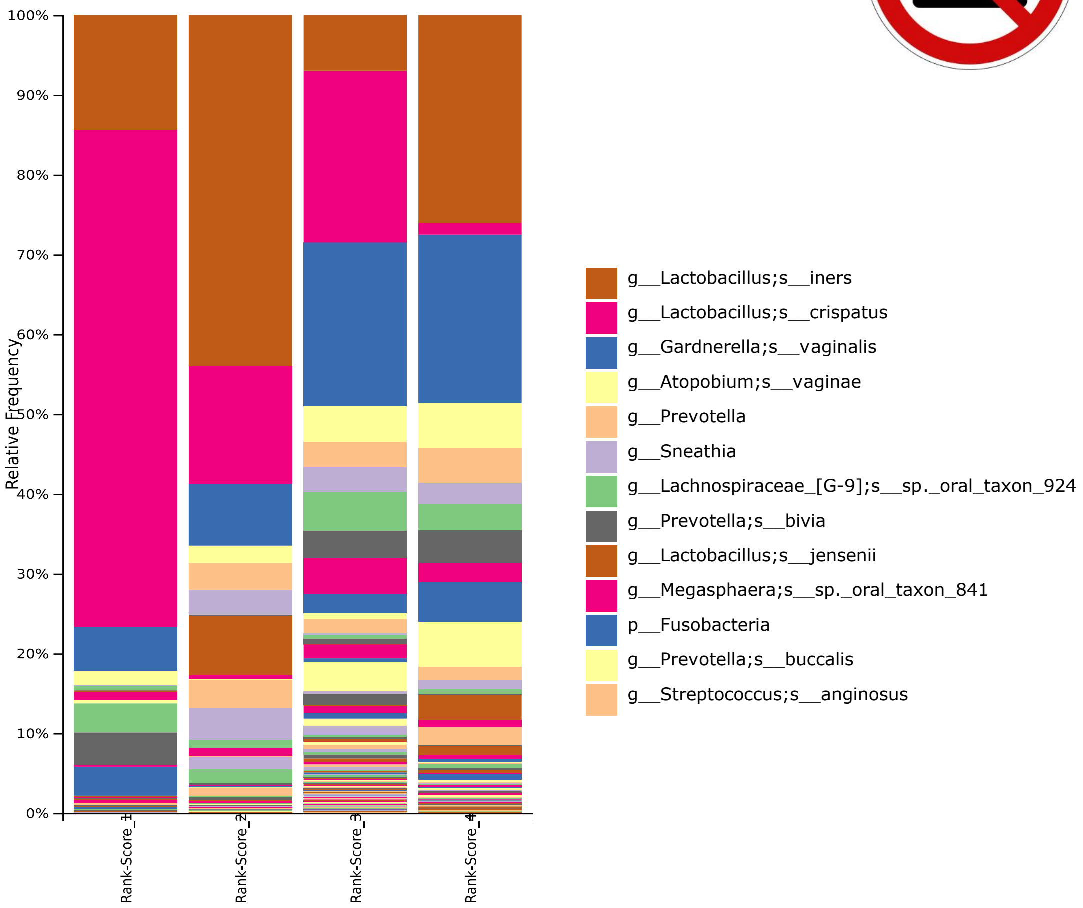
- *Candida*, *Sporidiobolaceae* and *Malassezia* dominates cervical fungal communities.
- Higher fungal diversity is associated with high-risk HPV infections; ASCUS and LGSIL.
- *Sporidiobolaceae* is more dominant in ASCUS and low-risk infections, while Malassezia is more dominant in high-risk infections across vagina, cervix and anus.

Godoy-Vitorino et al Frontiers in Microbiology, PMID: 30405584

Correlation between the bacterial biota and host immune response







Dr. Loyda Mendez
UAGM

Eduardo Tosado, MSc
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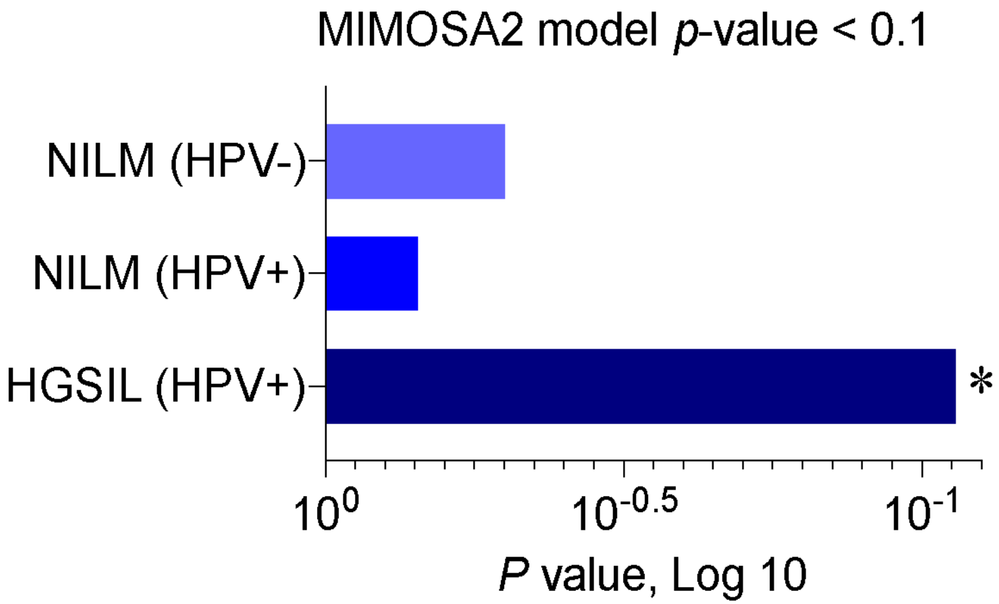
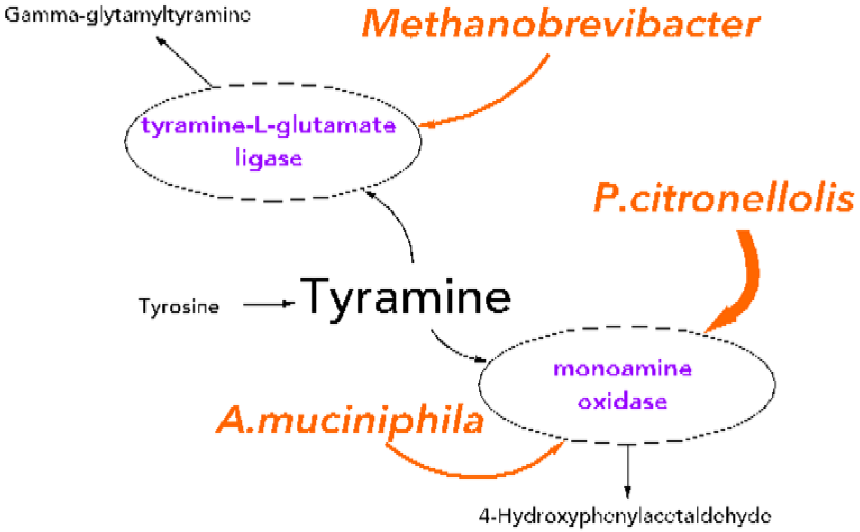
- IL-1β was **significantly more abundant in participants with a CST-IV** microbial profile and **associated with higher microbial diversity and a decrease of *Lactobacillus***.
  - **CST-IV →** associated with a loss in *Lactobacillus* and higher proportions of the genus *Atopobium, Prevotella, Sneathia and Gardnerella.*
- The rise in IL-1β can be attributed to cellular mechanisms such as tumorigenesis or angiogenesis.

Tosado-Rodríguez, submitted

GC-MS of cervical lavages show that HPV infections trigger enhanced Tyramine consumption in the cervix





- **HPV triggers consumption of Tyramine** – the genotoxicity of this biogenic amine linked to cancer development.
- Biogenic amine activity associated to species of ***Prevotella* and *Methanobrevibacter*** *in the cervix of HGSIL HPV+.*

Dr. Nataliya Chorna
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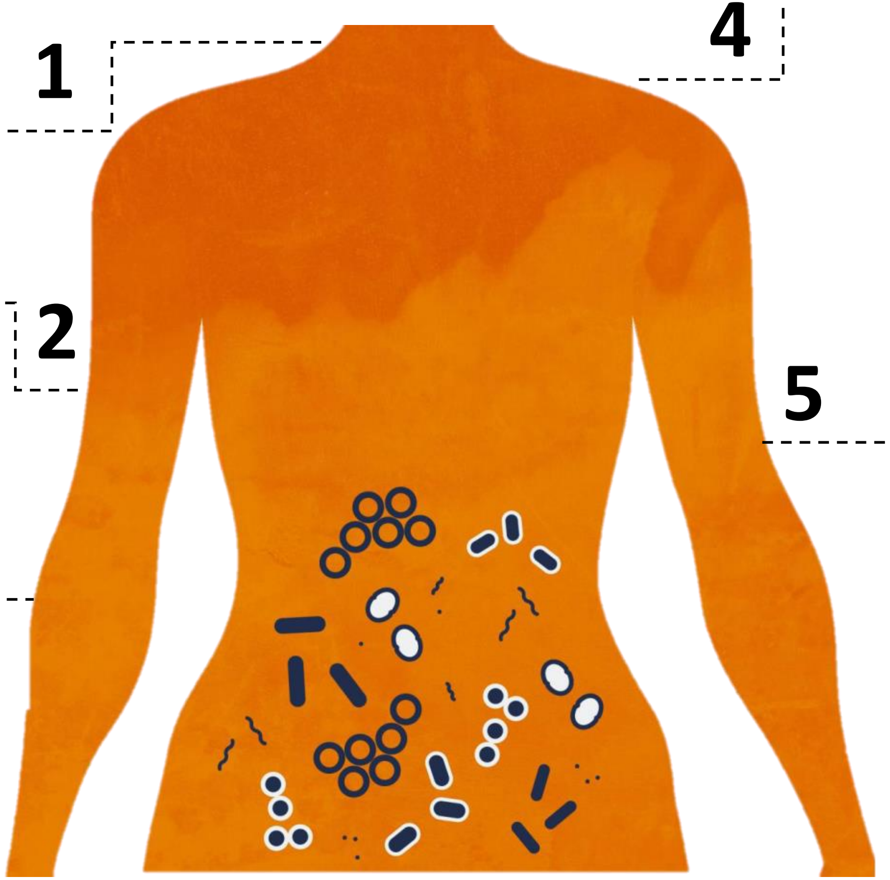
Takeaways



1

The cervical microbiota of Caribbean women is characteristically diverse, with a decrease in Lactobacillus promoting inflammatory processes, likely associated with disease progression.

2

Pregnancy improves vaginal homeostasis and *Lactobacillus* dominance (specially 3rd trimester).

3

Gardnerella, *Atopobium*, *Prevotella* and *Sneathia* are the dysbiotic bacterial markers in cervical epithelial changes.

4

Participants with high-grade disease revealed higher levels of all four pro-inflammatory cytokines (IL-1β, TNFα, IFNγ, IL-6).

5

Metabolomics reveals important role of amino acid metabolism with biogenic amine production in women with high-risk HPV.

The “rules” for community structure (interkingdom) in the cervix need to be understood to allow the development of new therapies.

New R01-like funding allowing for unique in-depth metagenomics on a longitudinal cohort (ongoing)

Acknowledgements

UPR MICROBIAL ECOLOGY TEAM

- NIMHD-RCMI U54 MD00760036-8538
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PRINBRE
IDeA Network of Biomedical Research Excellence

CCR RCMI HD
Center for
Collaborative Research
in Health Disparities

UC San Diego

Puerto Rico
Science, Technology
& Research Trust

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Questions?

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