African Collaborative Center for Microbiome and Genomics Research (ACCME)

Clement Adebamowo
Institute of Human Virology, Abuja, Nigeria

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African Collaborative Center for Genomics and Microbiome Research

- Led by Institute of Human Virology, Nigeria
- Collaborating African Institutions:
  - Center for Infectious Diseases Research, Zambia
  - National Hospital, Abuja, Nigeria
  - University of Abuja Teaching Hospital, Gwagwalada, Nigeria
  - University of Ibadan, Nigeria
  - African University of Science and Technology, Abuja, Nigeria

- Collaborating International Partner Institutions:
  - Queen Mary’s School of Medicine and Dentistry, London
  - University of New Mexico, Albuquerque, NM
  - Institute of Genome Sciences, University of Maryland, Baltimore, MD
  - Cancer Epidemiology Division, NIH/NCI, Bethesda, MD
  - Center for Genomics Research and Global Health, NIH/NHGRI, Bethesda, MD
  - Greenebaum Cancer Center, University of Maryland School of Medicine, MD
  - Institute of Human Virology, University of Maryland, Baltimore, MD
HPV Associated Cancers

291 million women worldwide (Point prevalence 10.1%) are estimated to have human papillomavirus infection of the cervix at a given point in time.
HPV Carcinogenesis

• There are more than 100 types of HPV infecting humans

• Almost all African women are infected by hrHPV at some point in their lives

• Persistent hrHPV is a necessary but not sufficient cause of cervical cancer
  • HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-45, HPV-52, HPV-58, HPV-68 and HPV-69 are recognized as carcinogenic – 71\% of global ICC are believed to be due to types 16 and 18
  • Recently classified as carcinogenic HPV-39, HPV-51, HPV-56, HPV-59

• Smoking, diet, sexual health and behavior are independent risk factors

• There is significant regional variation within Africa in cervical cancer incidence
Epidemiology of HPV Infection

- Persistent infection with hrHPV infection occurs in only 4 – 12%

- There is little data on prevalence of persistence and its determinants in African populations

- hrHPV infection leads to progressive changes in the cervical epithelium that can be detected before frank malignancy

- Estimated absolute risk for CIN3+
  - 47.4% (95% CI = 34.9% to 57.5%)
  - > 12 years of follow up after 2 positive qualitative type specific hrHPV

- Estimated absolute risk for CIN3+ or worse 3.0% (95% CI = 2.5% to 3.5%)
  - following a qualitative hrHPV test
Pattern of HPV infections in Nigerian women

• We studied 278 women enrolled in cervical cancer screening programs at 2 sites in Abuja, Nigeria

• Among HIV+ women, HPV35 (8.7%) and HPV56 (7.4%) were the most prevalent hrHPV types, while HPV52 and HPV68 (2.8%, each) were the most prevalent among HIV- women

• HIV infection was associated with increased risk of any HPV, hrHPV and multiple HPV infections.
High risk HPV in HIV+ and HIV- women

• The multivariate prevalence ratio for any hrHPV and multiple hrHPV were 4.18 (95% CI 2.05 – 8.49, p-value <0.0001) and 6.6 (95% CI 1.49 – 29.64, p-value 0.01) respectively, comparing HIV+ to HIV- women

• Oncogenic HPV types 35, 52, 56 and 68 may be more important risk factors for cervical pre-cancer and cancer among women in Africa. Current HPV vaccines do not cover the prevalent hrHPV types in this population and degree of cross reactivity of the vaccine needs to be ascertained.
Vaginal microbiome and prevalent high risk HPV infection in Nigerian women

In the same women, we characterized the vaginal bacterial composition and abundance (community state type, CST) by deep sequencing of barcoded 16S rRNA gene fragments (V4) and compared this by prevalent hrHPV status using logistic regression, weighted UniFrac distances, and LDA (Linear Discriminant Analysis) effect size (LEfSe) algorithm.
Vaginal microbiome and prevalent high risk HPV (hrHPV) infection in Nigerian women

Our findings

- CST IVB, (Characterized by low relative abundance of *Lactobacillus* spp.) most prevalent CST present in 139 (50%)
- CST III (often dominated by *L. iners*) in 109 (39.2%)
- CST I (often dominated by *L. crispatus*) in 22 (7.9%)
- CST – CST VI (dominated by *Proteobacteria*) in 8 (2.9%) participants – previously undescribed.
- Association between CST IVB and hrHPV in HIV negative participants (adjusted OR =5.63, 95%CI 1.19–26.7, p=0.03).
- LEfSeE analysis revealed an association between prevalent hrHPV infection and increased microbial diversity, with a decreased abundance of *L. iners* and increased abundance of anaerobes particularly of the genera *Bacterioidetes* and *Fusobacteria* in HIV- women.
Genomic risk of prevalent hrHPV infection in Nigerian women

• Significant associations with 2 SNPs - rs2305809 on Ribosomal Protein gene S19 (RPS19) and rs2342700 on Thymidylate Synthase gene (TYMS) - and prevalent hrHPV infection.

• Genes/regions in the immune genes 2’,5’ oligoadenylate synthetase gene 3 (OAS3), sulfatase 1 (SULF1), epidermal dysplasia verruciformis (EV)-associated EVER1 and EVER2 genes, transmembrane channel-like 6 and 8 (TMC6, TMC8), peroxiredoxin 3 (PRDX3), interleukin 2 receptorα (IL2RA), telomere maintenance 2 (TELO2), and complement component 1, r subcomponent-like (C1RL) were not associated.

• RPS19 gene codes for a ribosomal protein expressed by hematopoietic and non-hematopoietic tissues while TYMS gene product is used in the DNA synthesis and repair pathway.
Epigenetics of HPV infection

A

DNA cytosine

5'---G-C---3'
3'---C-G---5'

DNA 5-methyl cytosine

5'---G-C---3'
3'---C-G---5'

DNMT3a, 3b de novo methylation

Demethylation

B

1. Bisulfite treatment

$\text{mC} \xrightarrow{} \text{mC}$

C $\xrightarrow{}$ U

2. PCR amplification

$\text{mC} \xrightarrow{} \text{C}$

U $\xrightarrow{}$ T

Original sequence: GGA$\text{mC}$/CGCTAGACTGCTA
After bisulfite: GGA$\text{mC}$/GUGTAGACTGCTA
After PCR: GGAG$\text{mC}$/GTAGATTGTTA

3. The degree of methylation is analyzed as a C/T SNP, $\%C = C/(C+T)$
Epigenetics of HPV
Epigenetics of HPV 16 L1.3 and HPV 18 L2

HPV16 L1.3

HPV18 L2
Epigenetic changes in somatic cervical cells

hrHPV positive: LMX1

HPV Negative: LMX1
Epigenetic changes in somatic cervical cells

hrHPV positive: EPB41L3

HPV negative: EPB41L3
Summary of results of Cervical somatic cells epigenetics

**LMX1**

- Average methylation %
  - hrHPV
  - Negs
  - lrHPV

**EPB41L3**

- Average methylation %
  - hrHPV
  - Neg
  - lrHPV
Reproducibility LMX1 - Corr. = 0.525; Corr = 0.694
Current ACCME research –
Epidemiology and carcinogenesis of hrHPV

• The pilot work done till date provides a foundation for the current research in ACCME

• Aims:
  – Study the epidemiology and molecular genetic patterns of persistent hrHPV infection African women
    • Prevalence of persistent hrHPV infection
    • Multiple hrHPV infections
    • Differential duration of persistence of specific hrHPV types in individuals with multiple hrHPV and their association with CIN2+
    • Evaluate the epidemiological risk factors for persistent hrHPV infections
Gaps in knowledge about the role of the vaginal microenvironment

• Evaluate the epidemiological determinants and role of epigenetic changes in hrHPV and cervical cells as biomarkers of persistent hrHPV infection and CIN2+.
  • Determine the pattern of epigenetic changes in hrHPV and cervical cells, and its association with persistence of infection and CIN2+
  • Analyze the epidemiological determinants and secular trend of epigenetic changes in hrHPV and cervical cells in each category of hrHPV status and CIN2+

• Evaluate the role of cervical cytokines and that of the classes and stability of the vaginal microbiota and their association with persistent hrHPV infection and CIN2+
  – Examine germline risk factors for
    • persistent hrHPV infection
    • patterns of cervical cytokines secretions
    • pattern and stability of vaginal microbiome
    • CIN2+ in African women
1. Baseline visit:
   15,000 HIV(-), women aged 18 years and above screened, consented and enrolled
   Questionnaire; vaginal pH; Sampling for HPV types and blood for germline DNA

2. Follow up visits at 6, 12, 18 and 24 months visits
   Follow-up questionnaire; Sampling for HPV types

3. At end of enrollment of cohort in 24 months
   3a. 1,500 HIV(-) women with prevalent HPV identified
   3b. 13,500 women available as controls for different studies

4a. For Project 1 (prevalence of hrHPV) – select 800 cases with persistent hrHPV infection from 3a
4b. For Project 2 (cervical cytokines) – Randomly select 320 cases with persistent hrHPV from 4a.
4c. For Project 2 (vaginal microbiome) – randomly select 75 cases with persistent hrHPV and cervical cytokines results from 4b.
4d. For Project 3 (GWAS) the germline DNA of the 800 cases with persistent hrHPV infection in 4a are used

5a. For Project 1 (prevalence of hrHPV) – select 800 controls without persistent hrHPV infection from 3b.
5b. For Project 2 (cervical cytokine study) – Randomly select 320 controls without persistent hrHPV from 3b.
5c. For Project 2 (vaginal microbiome) Randomly select 75 controls with cervical cytokines results but no persistent hrHPV
5d. For Project 3 (GWAS) – All 13,500 controls are enrolled

For Project 3 (Candidate gene association studies) – 640 women with cervical cytokines results (from 4b and 5b) and 150 women with vaginal microbiome results will be allocated into categories depending on the results of their specific analysis and their association with specific SNPs tested

Study procedures and participants’ flow for ACCME projects Cases-pink; Controls-blue
Data Management Center

How old are you? : Refresh Plot

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Lowest values: 18, 19, 19, 20, 20
Highest values: 59, 60, 60, 60, 61

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HPV and Cervical Cancer

Record Status Dashboard (all records)

Displayed below is a table listing all existing records responses and their status for every data collection instrument (and longitudinal, for every event). You may click any of the colored buttons in the table to open a new tab/Window in your browser to view that record on that particular data collection instrument. Please note that if your form-level or user privileges are restricted for certain data collection instruments, you will only be able to view those instruments, and if you belong to a Data Access Group, you will only be able to view records that belong to your group.

Record ID: N00001NAH

Legend for status icons:
- Incomplete
- Unstarted
- Complete

Displaying record #4 through #15 of 1921 records

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Laboratory resources
Laboratory resources
Acknowledgement

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• IHVN Research Department Staff and collaborators