Host Genetic Determinants of the Vaginal Microbiome and Bacterial Vaginosis in Kenyan Women

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Background

• Bacterial vaginosis: High diversity and lack of *L. crispatus*
  • 20-50% prevalence in Eastern and Southern African countries
• Meta-analysis: 1.6x increased risk of HIV acquisition
  • Population attributable fraction: 15%
• Risks for BV: sexual practices, male partner circumcision status, vaginal hygiene practices
• Unexplained variability and persistent racial differences suggest genetic component

Background

• Limited number and scope of candidate gene studies identify host differences in genes for inflammatory immune factors (e.g., genes encoding TLRs, IL-1β)

• No genome wide association study (GWAS) of vaginal microbiome traits and BV

• **Objective:** Conduct GWAS on BV and vaginal microbiome traits to broadly elucidate biological mechanisms of these complex traits
Methods: Study Sample, BV

• Afya Jozi, Afya Jamii: Prospective cohort of 252 heterosexual couples in Kisumu, Kenya
• Baseline samples from 200 women, selected to maximize the number of women with and without BV
• BV assessed at baseline, 1-, 6-, and 12- months: Nugent scoring of Gram stained vaginal smear; dichotomized 7-10 vs. 0-6
• Microbiome characterization in cervicovaginal lavage samples: 16s rRNA gene amplicon sequencing of the V3-V4 regions (UIC)
• Quality control and taxonomic annotation UMD Institute for Genomic Science
Methods: Genotyping and Quality Control

• Oral buccal swabs for genotyping

• Samples genotyped using the Illumina Global Screening Array (~654,000 markers)

• Removed SNPs: MAF < 1% and missingness > 5%

• Imputed dataset using the Kenyan reference panel from 1000 Genomes Project via Minimac3
Methods: Analysis

• Data from 176 women after QC of microbiome and SNP data

• Linear and logistic regression (PLINK, Mach2DAT, Mach2QTL), adjusting for age and first 3 principal components for association between SNPs and:
  • Proportion of BV-positive visits across follow-up (82% ≥3 visits)
  • Relative abundances of *L. crispatus*, *L. iners*, and *G. vaginalis*
  • Shannon diversity index

• Pathway analysis (SKAT-O, WebGestalt) to identify putative biological processes associated with microbiome traits and BV
# Results: Sample Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in years (IQR)</td>
<td>22 (20-25)</td>
</tr>
<tr>
<td>HIV positive</td>
<td>9.8%</td>
</tr>
<tr>
<td>HSV-2 seropositive</td>
<td>54%</td>
</tr>
<tr>
<td>BV at baseline</td>
<td>22%</td>
</tr>
<tr>
<td>BV at any point in follow-up</td>
<td>43%</td>
</tr>
<tr>
<td>Multiple visits with BV</td>
<td>25%</td>
</tr>
<tr>
<td><em>L. crispatus</em> present / mean relative abundance</td>
<td>24% / 30%</td>
</tr>
<tr>
<td><em>L. iners</em> present / mean relative abundance</td>
<td>83% / 46%</td>
</tr>
<tr>
<td><em>G. vaginalis</em> present / mean relative abundance</td>
<td>74% / 23%</td>
</tr>
<tr>
<td>Median Shannon diversity index (IQR)</td>
<td>0.93 (0.30 – 1.82)</td>
</tr>
</tbody>
</table>
Results of GWAS

**Lactobacillus crispatus**

- \( P = 4.99 \times 10^{-6} \)
- \( \text{OR} = 4.53 \) (A)
- \( \text{MAF} = 0.36 \)

**Lactobacillus iners**

- \( P = 5.20 \times 10^{-7} \)
- \( \beta = 1.05 \) (A)
- \( \text{MAF} = 0.06 \)
Results of GWAS

Probability of Bacterial Vaginosis

- $P = 7.38 \times 10^{-7}$
- $\beta = 0.72$ (A)
- MAF = 0.01

Shannon Diversity Index

- rs115869045
  - $P = 3.70 \times 10^{-7}$

- $P = 9.89 \times 10^{-7}$
- $\beta = 0.67$ (A)
- MAF = 0.20
Results of GWAS

\[ P = 2.15 \times 10^{-6} \]
\[ \beta = 0.58 \text{ (A)} \]
\[ \text{MAF} = 0.45 \]
Pathway Analysis: *L. crispatus*

- Abnormality of the genitourinary system
- Abnormality of the urinary system
- Abnormality of the female genitalia
- Hypoplastic labia minora
- Abnormal vitamin B12 level
- Decreased methylcobalamin
- Hyperhomocystinemia
- Abnormality of Krebs cycle metabolism
- Decreased methionine synthase activity
- Decreased adenosylcobalamin
- Hypomethioninemia
- Decreased methylmalonyl-CoA mutase activity
Pathway Analysis: G. vaginalis

Ontology

Abnormality of metabolism/homeostasis

Abnormality of the genitourinary system

Abnormality of the urinary system

Number of Genes

size

- 1400
- 1500
- 1600
- 1700
- 1800

FDR

- 0.0450
- 0.0455
- 0.0460
Pathway Analysis: *L. iners*
Pathway Analysis: Proportion of Visits with BV

- I-kappaB kinase/NF-kappaB signaling
- Regulation of I-kappaB kinase/NF-kappaB signaling
- Positive regulation of I-kappaB kinase/NF-kappaB signaling
- Positive regulation of NF-kappaB transcription factor activity
- Alcohol dehydrogenase activity, zinc-dependent
Discussion

• First GWAS of host genetic contribution to vaginal microbiome traits and BV

• We identified genetic loci and biologically relevant pathways associated with these traits, adding to evidence of host genetic influences on vaginal microbiome composition and BV

• Limitations:
  • Pathway results still a jump from true biological mechanisms
  • Methodologic challenge to analysis of categorical traits, such as community state type
Conclusions and Next Steps

• Further studies: Larger samples with comparison across microbiome sites within individuals, and across populations:
  • African women, African American women
  • ❶ Broader evaluation of pathobiant taxa, ❷ Confirm candidate SNPs, ❸ Gene x Environment Interactions

• Potential applications: Better understanding of, and predict susceptibility to: ❶ BV occurrence, treatment failure or recurrence; ❷ optimal benefit of probiotic treatment; ❸ potentially aid in preventing other pathological conditions related to vaginal dysbiosis
Thank you! Questions?

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