The RAMS Registry/Repository: Biobanking for Microbiome Research in Women’s Reproductive Health and Pregnancy

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The Vaginal Microbiome Consortium at VCU

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Conflict of Interest Disclosure
Gregory A. Buck, Ph.D.

- Scientific Advisory Board for startup: Juno Bio, Ltd
  - Kemp House, City Rd, Old Street, London EC1V 2NX, UK
  - 2019-present
  - No salary or fees
~10 years ago:
NIH Launches the Human Microbiome Project (2 phases)

Objective: use high throughput genomic technologies to define the human microbiome:
from cradle to grave (”normal”)
in health and disease
In all body niches
~10 years ago: NIH Launches the Human Microbiome Project (2 phases)

Phase 1: split into two phases:
Phase 1A: define the ‘healthy’ human microbiome.
   What are the microbes that inhabit all human body niches?
Phase 1B: define the ‘abnormal’ human microbiome.
   What are the microbes in the human body that one finds in disease?

HMP 1 (2007-2013)
- ~40 projects
- ~20 institutions

Image source: http://commonfund.nih.gov/hmp
~10 years ago:
NIH Launches the Human Microbiome Project (2 phases)

Phase 2: Multi Omics
Objective: apply massively multi omic data intensive strategies to dissect the impact of the microbiome on a targeted disease state

HMP 2 (2013-present)
• 3 collaborative projects
• Massive increase in samples
• Massive increase in data
NIH invests millions in the HMP Data Coordination Center (U. Maryland)

The DACC “provides a common repository for diverse human microbiome datasets and minimum reporting standards established by the DCC, from both HMP1 and the second phase of the project, iHMP, providing researchers with the ability to query and retrieve metagenomic, metatranscriptomic, human genetic, microbial culture, and many other data types from each project.”

But, no accommodation for the massive number of samples being collected in the large scale longitudinal studies mandated by the program.
VCU Alone generated over a quarter million samples!

1. The Vaginal Human Microbiome Project (VaHMP):
   - A cross-sectional study
   - Samples from 6,063 visits; ~4500 women, ~1000 pregnant
     >60,000 vaginal, cervical, buccal, other samples to archive.

   - A longitudinal study of ~1500 pregnancies (1-10 visits each)
   - ~1500+ neonates (delivery, discharge, follow-up)
   - Yielded 45 Preterms that met criteria (spontaneous; <37 weeks)
   - >200,000 samples (vaginal, buccal, rectal, blood, birth products)

Over 250,000 irreplaceable samples generated.
   - An extremely valuable resource
   - Not only keep them alive, but keep them stable
     • Bacteria and Cells
     • DNA and RNA
     • Cytokines and metabolites/lipids
Samples collected at VCU

>250,000 collected/
~50,000 processed/analyzed

<table>
<thead>
<tr>
<th>Human DNA</th>
<th>cytokine</th>
<th>cells &amp; bacteria</th>
<th>metabolites</th>
<th>MTS</th>
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Each sample/stabilate was stored in a special medium or buffer.
Each was tested and validated for reproducibility after storage for extensive periods.
To manage this onslaught of samples/data:

We created a Microbiome Registry/Repository at VCU: the Research Alliance for Microbiome Science Registry (the RAMS Registry)

HISTORY OF THE RAMS REGISTRY

Founded in 2013 to "promote the health of all women, men and children by supporting research related to microbiome science."

The RAMS Registry has a goal of fostering "collaboration among researchers to accelerate knowledge and advance science by facilitating sharing, reuse and linking of data and samples."
Heart of the RAMS Registry: Freezer Farm

Started buying freezers

When we run out of room: we purchase another

Bootstrapped a monitoring system

One ‘extra’ freezer for emergency backup

Supported by NIH through the project grant

Total now: ~12 freezers (no more space in farm!)
Clinical and Survey Data is part of the RAMS Registry

- **Initial Visit**
  - New OB kit – 28 samples each
  - Health History Survey – 451 fields

- **Interim Prenatal**
  - Interim kit (2-10/subject) – 26 samples each
  - Review of Systems Survey – 173 fields

- **L&D Triage**
  - Triage kit – 22 samples each
  - Review of Systems Survey – 173 fields

- **Delivery**
  - Superkit (5 sub-kits) – 68 total samples each
  - Delivery & Discharge Survey – 88 fields

- **Post-Partum**
  - Follow-Up kit – 18 samples each
  - Follow-Up Survey – 143 fields

Plus: Medical Record Abstraction: one for each of ~6,000 subjects
Electronic Data Capture (LIMS)

Momspi.vmc.vcu.edu

Clinical App (Django, Apache)

Tracking DB (Auth, Participant Mapping, Sample Tracking, Results etc)

Lab App (Django, Apache)

P App (Django, Apache)

LimeSurvey App (PHP, Apache)

Participant Assignment, Temp Survey

Cerner EMR

VCUHS IDX

Smomspi.vmc.vcu.edu

Clinical DB (Contact, Consent, IDX, Survey)

Research Coordinator Tablet

Technical Staff

Participant Tablet

Mom survey.vmc.vcu.edu
MOMS-PI Sample/Data Management & Tracking System

Sample Collection
- VCU Clinics
- GAPPS

Clinical Metadata System (Tablet Interface)

Lab Sample Processing

Sample Tracking System

Raw Data
- 16S Gene Metagenomics
- WMGS
- Bacterial Sequences
- Meta Transcriptomics
- Immunomics
- Metabolomics
- Interactomics

Data Analysis
- RDP Classifier
- STIRRUPS
- Metaphlyn, HUMAnN
- Velvet, CLC Bio assembler
- Software pipeline
- Gene Expression Profils
- Cytokine Profiles
- Lipid Profiles
- Protein Interactions

Results

MOMS-PI Database

Database APIs

File Storage

Query Portal

Browse Portal

Bulk Download
- Raw Data
- Analysis Files

External Upload System

Public Portal
- Protocols
- Info for participants

Results File Parsers

Web Portal

Controlled Access to VMC members and NIH approved teams

Existing

New

Input/Output

16S, WMGS, Meta Transcriptomics

NCBI SRA

NCBI dbGap

EBI IntAct

NCBI Genbank

MOMS-PI Data Management System
Now holds over 250,000 samples from VaHMP (HMP1) and MOMS PI (HMP2) clinical survey data & medical record abstractions from over 6,000 participants multi omic data from ~50,000 samples

Bacterial Vaginosis (dysbiosis of the vaginal microbiome)
- ~29% (23-51%) prevalence in women (CDC).
  - Decreased *Lactobacillus*, increase in ‘unhealthy anaerobes’
  - ‘Clue cells’: vaginal epithelial cells coated with bacterial biofilm
  - Symptoms include thin, grayish discharge and vaginal odor
- Often recurrent and non-responsive to treatment
- Increased risk of STI, HIV, trichomoniasis, etc.
- *Increased risk of preterm birth*

Preterm birth:
- 1 in 10 births in the USA are preterm (<37 weeks gestation)
  - As high as 1 in 6 in some populations
- Annually over 12 million preterm births worldwide (2005 WHO)
  - Mortality rate as high as 42%.
  - Survivors have short and long term health issues
- Over $26 billion/yr in US for prematurity associated healthcare.
- 30-40% caused by poorly defined bacterial agents.
  - Microbial etiology is not clear (which bacteria, mechanism, host factors...)

What have we learned?
Why are we interested in the vaginal microbiome?
--a clear role in women’s urogenital health--
Preterm birth rates worldwide (ranges from <10% to over 15% of live births)

countries – where are the highest rates?

Adapted from: Jim Litch, GAPPS

Preterm rate, 2010

- <10%
- 10-15%
- >15%
- Not available
- Not applicable

A global challenge… ~1 of 10 neonates worldwide.
Over 12 million annually.

11 countries with preterm birth rates over 15%:
1. Malawi
2. Congo
3. Comoros
4. Zimbabwe
5. E. Guinea
6. Mozambique
7. Gabon
8. Pakistan
9. Indonesia
10. Mauritania
11. Botswana
In contrast to gut and oral microbiomes, health of the vaginal microbiome is defined differently.

Gut microbiome
Oral Microbiome
Skin microbiome

Dogma:
*Lactobacillus* dominated vaginal microbiome is healthy;
Diverse vaginal microbiome considered less healthy.
Population matters…

HMP Consortium Healthy Cohort (143 healthy young women)

Mostly *Lactobacillus*:
- Very healthy (carefully screened)
- Young (college age)
- Mostly of European ancestry (Caucasian)

Populations matter!!!
More recently:
- analysis of more samples (e.g., VaHMP)
- more community state types (vagitypes)
- ~10 major, more minor

Surprisingly, there is not a very good correlation with health:
- Some complex vagitypes in apparently healthy women
- Some homogeneous vagitypes in women with symptoms

Five community state types

Jacques Ravel et al. PNAS 2011
What happens to the vaginal microbiome during pregnancy?

Non-Pregnant ~2,935 women
Pregnant ~880 women

- Increase in prevalence of *Lactobacilli* (p < 0.05) (mostly *L. iners*)
- Decrease in prevalence of *G. vaginalis* (p < 0.05) and other vagitypes (e.g., *Sneathia*, *Atopobium*, etc.)
- No decrease in BVAB1

Pregnancy drives a ‘healthier’ (more *Lactobacillus* dominant microbiome?)

This cohort: take all comers (no case-matching, preterm birth, etc., not excluded)

Aagaard et al. Plos One (2011)
Romero et al. Microbiome (2014)

Lower numbers (less significance)
Focused cohort (e.g., Caucasian only)
Targeted age group (college age)
What specific taxa are altered in abundance in pregnancy?

~10 taxa affected (q<0.05)

Increased:

L. iners

At the expense of (decreased):

A. vaginalis
G. vaginalis
Sneathia sp.
Prevotella sp.
Dialister sp.
BVAB2

Note: BVAB1 not decreased

Mann-Whitney U

Changing pregnancy microbiome is driven by changes in Pregnant African-Americans

Non-African-Americans:
- proportion with *Lactobacillus* dominated microbiome unchanged.
- Decreased *L. crispatus*, increased *L. iners* (p<0.05)

African-Americans:
- increased *Lactobacillus* (mostly *L. iners*) in pregnancy (p<0.05).
- Less *G. vaginalis* and other vagitypes (p<0.05).

In terms of total *Lactobacillus*, pregnant African American women: profiles begin to resemble non-African profiles

Vaginal microbiome: differences based on populations
- Racial, socioeconomic, other environmental factors

Longitudinal Full Term Pregnancies

Vagitype transitions tend to occur relatively early in pregnancy.

African-American

These results show:
1. The vaginal microbiome transitions to a less complex, Lactobacillus-dominated profile during pregnancy.
2. The bulk of these changes occur early in pregnancy and are generally complete by the 2nd trimester.
3. The changes are more prevalent in our cohort of women of African ancestry (not shown).

What about Preterm Birth?

45 preterm births (from ~1000 pregnancies)
Only *spontaneous* preterm births included
90 case matched (age, race, SES) controls

**Table 1 | Description of cohort studied in this project**

<table>
<thead>
<tr>
<th></th>
<th>Preterm delivery &lt;37 weeks (n = 45)</th>
<th>Term delivery ≥39 weeks (n = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>26 (5.68)</td>
<td>25.9 (5.43)</td>
</tr>
<tr>
<td>Ancestry/ethnicity (no. (%))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>35 (77.8)</td>
<td>71 (78.9)</td>
</tr>
<tr>
<td>European</td>
<td>6 (13.3)</td>
<td>13 (14.4)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3 (6.7)</td>
<td>5 (5.6)</td>
</tr>
<tr>
<td>Native American</td>
<td>1 (2.2)</td>
<td>1 (1.1)</td>
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<tr>
<td>Household income (no. (%))</td>
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</tr>
<tr>
<td>&lt;US$20,000</td>
<td>29 (72.5)</td>
<td>66 (77.7)</td>
</tr>
<tr>
<td>US$20,000–59,999</td>
<td>9 (22.5)</td>
<td>15 (17.6)</td>
</tr>
<tr>
<td>US$60,000+</td>
<td>2 (5.0)</td>
<td>4 (4.7)</td>
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<tr>
<td>Vaginal delivery (no. (%))</td>
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<tr>
<td>38 (84.4)</td>
<td>74 (82.2)</td>
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<tr>
<td>Previous preterm (no. (%))</td>
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<td>14 (31.1)</td>
<td>9 (10.0)</td>
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<tr>
<td>Preterm premature rupture of the membranes (no. (%))</td>
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<tr>
<td>26 (57.8)</td>
<td>0 (0)</td>
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</tbody>
</table>

*Standard deviation listed in parentheses. *Missing values n = 5 (PTB), n = 5 (TB).

Clear differences in PTB samples (q < 0.05):
less: L. *crispatus*
more: BVAB1/2, *S. amnii/sanguinegins*, *Dialister* sp
*Prevotella* sp
No diff: *Gardnerella vaginalis*

Model using microbiome data alone predicts risk of preterm birth

False positives: those falsely predicted to experience PTB

False negatives: those falsely predicted to not experience PTB (need to minimize)

Improve with:
- Clinical Demographics (race, SES)
- Microbiome
- Cytokine
- Proteome
- Metabolome
- Lipidome

~77% sensitivity and ~76% specificity (slightly better than current clinical parameters)

Promise for better predictive measures to permit early intervention.
What about the host immune response?

Integrative sparse canonical correlation analysis (cytokines vs taxa)

Strong negative correlation between *L. crispatus* and pro-inflammatory cytokines and taxa associated with dysbiosis and PTB.

*L. iners* associated with IP10 (induces chemotaxis of immune cells and is considered to be pro-inflammatory). (Jespers et al. 2017)

In women who would experience PTB, dysbiotic taxa and pro-inflammatory cytokines form a tighter cluster. IP10 and *L. iners* no longer correlated

Preterm Birth group shows tight clustering of dysbiotic taxa and proinflammatory cytokines.
What do we believe now?

1. Pregnancy tends to homogenize the vaginal microbiome toward a more *Lactobacillus*-dominant composition. This is more evident in women of certain racioethnic groups. Precision microbiome: population dependent.

2. This homogenization reduces the prevalence of a finite panel of bacterial taxa that are often associated with dysbiosis.

3. This homogenization occurs relatively early in pregnancy.

4. An overlapping panel of anaerobic bacteria are associated with (and possibly causative of) preterm birth. Again, this is more evident in women of certain racioethnic background.

5. Prevalence of these taxa generally decrease as pregnancy progresses even in women who will experience preterm birth.

6. Early in pregnancy, pro-inflammatory cytokine expression is correlated with bacteria that are associated with preterm birth.

Can we improve on our accuracy of prediction of risk of preterm birth? Can we predict risk of preterm birth even prior to pregnancy?
Early in pregnancy, potentially pathogenic bacteria are present in the vaginal microbiome. A model for how the vaginal microbiome could cause preterm birth is shown in the diagram.

Complex pro-inflammatory microbiome early in pregnancy:
- As pregnancy progresses, *Lactobacillus* taxa are favored.
- However, some pro-inflammatory bacteria persist, or ascend. Infection or inflammation causes labor and premature birth.

The diagram shows the progression of the microbiome from the first trimester to the postpartum period, highlighting the pro-inflammatory stage early in pregnancy and infection-mediated preterm labor.
In summary:

1. RAMS Registry: an important asset to our efforts:
   - Incredible investment of time and effort.
     - Establishment, validation, digital investment, longevity
   - Many places to ‘go wrong’.
   - Limited financial support (what happens when grants end?).

2. That said, it has largely worked for us:
   - Over 250k samples stored in validated protocols
   - ~50,000 samples processed
   - Digital tracking of sample processing and storage works
   - Published many manuscripts
   - Succeeded in new funding to continue our work.
The Vaginal Microbiome Consortium at VCU

Thank you!

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Others.....

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Craig Rubens
Others....
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Hagit David (NIAID)
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The ~8,000 women who have generously enrolled in our studies