The gut microbiome, inflammation and metabolic disease in individuals infected with HIV

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HIV Microbiome Meeting
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Metabolic health of HIV patients

- HIV patients often suffer from metabolic derangements
  - Dyslipidemia (High triglycerides, Low HDL)
  - Insulin Resistance
  - Increased risk of Cardiovascular Disease and Diabetes

- Unique symptoms and driving factors in the context of HIV (Pao et al. 2008)

- HIV-related risk factors
  - Lipodystrophy
    - Re-distribution of fat
      - Lipoatrophy: face, limbs, buttocks
      - Lipohypertrophy: trunk, “buffalo hump”
    - Associated with first generation ART drugs
  - Lower CD4 T cell count in treated infection
  - Untreated HIV infection (dyslipidemia)
  - Certain ART drugs (protease inhibitors)
Metabolic Endotoxemia

Serum LBP associated with chronic inflammation (CRP and IL-6) and Metabolic syndrome in Hemodialysis patients (Lim et al. Blood Purif. 2019). Results in obesity are mixed.

Bacterial translocation may also be a driver of chronic inflammation and metabolic disease in MSM.

Li et al, PloS Pathogens. 2019

Palmer et al, AIDS. 2014
Metabolic abnormalities linked with microbially produced metabolites

- Branched Chain Amino Acids (BCAA) (*Prevotella copri*) (Pederson et al. Nature 2016)
- Trimethyl Amine (TMA -> TMAO) (Chen et al. Cell metabolism 2019)
MSM microbiome is highly altered in composition

An exploration of Prevotella-rich microbiomes in HIV and men who have sex with men.
Armstrong AJS, Shaffer M, Nusbacher NM, Griesmer C, Fiorillo S, Schneider JM, Preston Neff C, Li SX, Fontenot AP, Campbell T, Palmer BE, Lozupone CA.

MSM microbiome is pro-inflammatory in vitro and in gnotobiotic mice

Fecal Microbiota Composition Drives Immune Activation in HIV-infected Individuals.
Neff CP, Krueger O, Xiong K, Arif S, Nusbacher N, Schneider JM, Cunningham AW, Armstrong A, Li S, McCarter MD, Campbell TB, Lozupone CA, Palmer BE.

Gut microbiota from high-risk men who have sex with men drive immune activation in gnotobiotic mice and in vitro HIV infection.
Li SX, Sen S, Schneider JM, Xiong KN, Nusbacher NM, Moreno-Huizar N, Shaffer M, Armstrong AJS, Severs E, Kuhn K, Neff CP, McCarter M, Campbell T, Lozupone CA, Palmer BE.
We hypothesize that HIV infected and HIV negative MSM with worse metabolic health will harbor a distinct microbial signature.

We will further investigate the potential influence of these microbes via immune mechanisms, effects of bacterial translocation, or through microbiologically derived metabolites.
Cohort and data types

- **Cohorts (116)**
  - HIV negative (54)
    - MSW (22)
    - MSM (32)
  - HIV positive – MSM (62)
    - Untreated (15)
    - ART Treated (47)
      - Lipodystrophy (26)
      - No-Lipodystrophy (21)

- **Data types measured**
  - Microbiome (16S)
  - Cytokines (ELISA)
  - Diet (FFQ)
  - Clinical measures (survey)
  - Metabolome (untargeted mass spec)
    * ART treated HIV+ only
Metabolic score
Metabolic score interpretation

Score >3 outside healthy ranges of triglycerides, fasting glucose/insulin, HDL
Proportion of individuals with an unhealthy metabolic score increased in Lipodystrophy

<table>
<thead>
<tr>
<th>HIV negative MSW</th>
<th>HIV negative MSM</th>
<th>HIV untreated</th>
<th>HIV treated</th>
<th>HIV treated LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>19%</td>
<td>28%</td>
<td>8%</td>
<td>25%</td>
<td>52%</td>
</tr>
</tbody>
</table>

Age distribution for different HIV status categories.
Relationship between metabolic score and age across study cohorts

Metabolic Score ~ Cohort + Age + Cohort*Age
Driving factors of metabolic disease in HIV-infected and high risk populations

Orientation
HIV
Treatment
Lipodystrophy

Exposures

Metabolic Health

Outcome

Immune Activation
Microbiome
Metabolome
Diet

Measures
Overview of computational approach

- Perform a systems level analysis to predict drivers of metabolic score in HR and HIV+ MSM
- Handle a high number of measured variables (>4000) across multiple data types
- Understand inter-relations between predictive variables

**Step 1:** Identify and summarize highly co-correlated modules of features.

**Step 2:** Identify features that predict metabolic score

Random Forest: VSURF

**Step 3:** Determine relationship of important features with metabolic score and with each other

Correlation and network Visualization

SCNIC: [github.com/shafferm/SCNIC](https://github.com/shafferm/SCNIC)
Step 2: Random Forest for Variable Selection (VSURF)

- Takes a random subset of the data many times (30k) and builds decision trees that can be used to predict an unknown samples outcome
- Selects important variables and reduces variables down to remove redundant features
- Variable reduction: 4000+ variables
  - In MSM only: predictors of Metabolic score among microbiome + diet + immune + metadata
  - 80 important variables (11 non-redundant)

Genuer et al. 2010. Pattern Recognition Letters
Step 3: Visualizing Relationships with Networks

Var1

Var 2 → Var 3

Metabolic Score

Correlation Direction

Variable Correlation

R value

-0.53

+0.67

Variable Correlation

FDR P-value

0.05

10^-10
Diet Module 12
Total sugars
Carbohydrates

Diet Module 6
Total polyunsaturated fatty acids
20:1 eicosenoic acid (MFA)
18:2 octadecadienoic acid (PFA)

Diet Module 3
Food folate
Lutein zeaxanthin
Vitamin K
Beta carotene

Variable Correlation
R value
-0.53 to +0.67

Variable Correlation
FDR P-value
0.05 to 10^-10
LPS binding protein (LBP) neighbors

- LBP negatively correlates with butyrate producers
  - Coprococcus cactus/ sp.
  - Butyrivibrio

- LBP positively correlates with:
  - Dorea: Can degrade sialic acid on mucins (Crost et al, PlosOne, 2013)
  - Oscillospira + Ruminococcaceae
Metabolic health predictors within a population of HIV+ and HR MSM

• Traditional risk factors (Age, BMI, diet)
• HIV, ART and lipodystrophy status not selected
• Evidence for importance of translocation
  – An importance of LPS Binding Protein and associated plasma inflammation markers.
  – A lack of certain butyrate producers and increase mucolytic bacteria in Dorea genus
What about the metabolome?

• Untargeted LC/MS for HIV+ ART+ (47)
  – Lipodystrophy (26)
  – No-Lipodystrophy (21)
• Microbe associated metabolites
  – GF vs. humanized mice fed a HFD
  – 766 metabolites/modules that have a metabolites significantly different; 187 in human data
Inflammatory cytokines linked with *Dorea* sp. and diverse triglycerides

\[ y = \text{Metabolic score}; \quad x = \text{measures (microbiome, metabolome, diet, immune, clinical data)} \]

121 important variables

TG = triglycerides

UnAn = Unannotated metabolite

Dorea sp.

IL-12/23p40

GM-CSF

TNF-beta

IL-17A

D

Cohibin

UnAn

Montecristin

TG

UnAn

TG

TG

TG

TG

121 important variables
Diet Interventions

- Diet interventions
  - 4 week dietary intervention with either high-fiber/low-fat agrarian type diet or control
  - 98 total: 50 HIV+ ART, 24 HR-MSM, 24 women/MSW
  - Same readouts (inflammation, microbiome, demographics)
- Personalized approaches to metabolic health in HIV and high-risk populations
  - Can we use decision trees to determine which factors predict response to diet interventions
  - Better tailor diet interventions

<table>
<thead>
<tr>
<th>Diet Composition</th>
<th>Western</th>
<th>Agrarian</th>
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</thead>
<tbody>
<tr>
<td>Protein</td>
<td>15% (62g)</td>
<td>15% (66.75g)</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>55% (228g)</td>
<td>70% (311.5g)</td>
</tr>
<tr>
<td>Fat</td>
<td>30% (124.5g)</td>
<td>15% (66.75g)</td>
</tr>
<tr>
<td>Fiber</td>
<td>18g</td>
<td>45g</td>
</tr>
<tr>
<td>Sugar</td>
<td>114g</td>
<td>67g</td>
</tr>
<tr>
<td>Sodium</td>
<td>3650g</td>
<td>1325g</td>
</tr>
<tr>
<td>Energy</td>
<td>2000kcal</td>
<td>2000kcal</td>
</tr>
<tr>
<td>Processed foods</td>
<td>Yes</td>
<td>No</td>
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The Lozupone Lab
Thanks!

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