Gut microflora and estrogens: a new paradigm for breast cancer risk reduction

Dr. Kathleen Egan (Moffitt)

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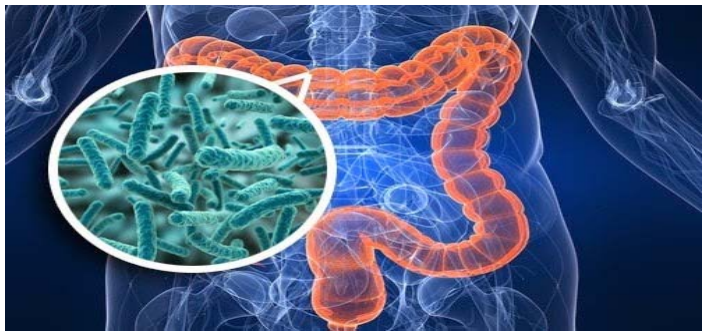
Background



- Approximately 100 trillion microorganisms live in our bodies and most are found in our intestines;
- The dynamic gut microbiota has myriad influences linked to breast cancer:
  - host immunity
  - metabolism and absorption of a wide range of compounds (steroid hormones, phytochemicals, nitrates, and xenobiotics)
  - integrity of the epithelial barrier and absorption
  - host energy balance and nutritional status
  - host susceptibility to oncogenic factors

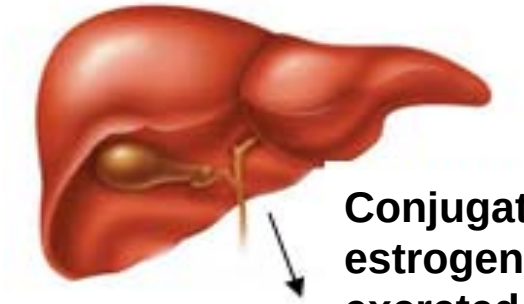
Background (continued)

- Evidence that the gut microbiome contributes to cancer risk in human populations is limited:
  - In **retrospective case-control studies** observed microbial profiles in cancer patients could reflect changes that occurred *as a result* of cancer diagnosis or treatment
  - In **prospective cohort studies** large numbers of subjects must be studied, long follow-up is required, and repeat sampling may be necessary over many years
- An alternative is to study intermediate risk biomarkers as a proxy, such as endogenous estrogen levels, which are linked to breast cancer.

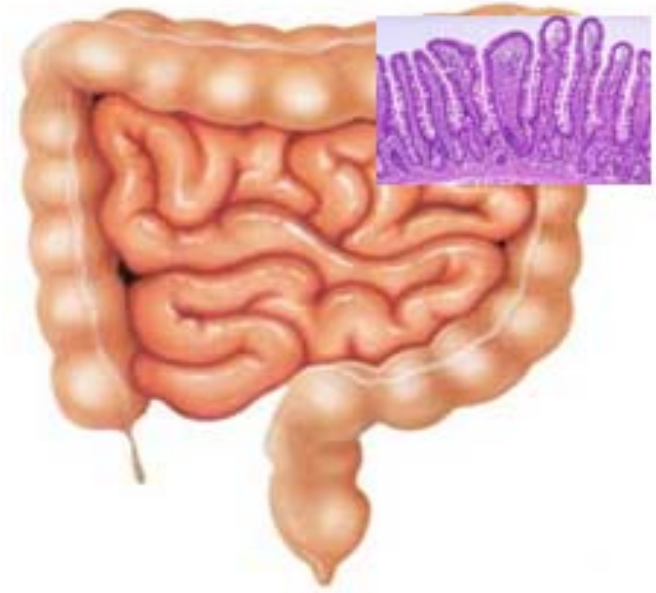
Background (continued)

- Two recent studies provided the first evidence that the intestinal microbiome may influence circulating estrogen levels in postmenopausal women;
- Studies are small (n=17 and n=60), however, and findings require confirmation in a large and well characterized study population.

Role of intestinal microbiota in estrogen metabolism



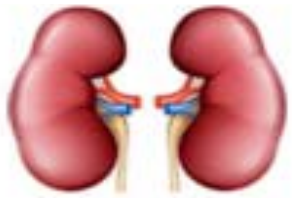
Conjugated estrogens excreted in bile



Excretion of conjugated estrogens in feces

Conjugated estrogens hydrolyzed and reabsorbed in intestines due to enzymatic activity of microbiome and intestinal mucosa

Increase in the level of circulating and urinary estrogens



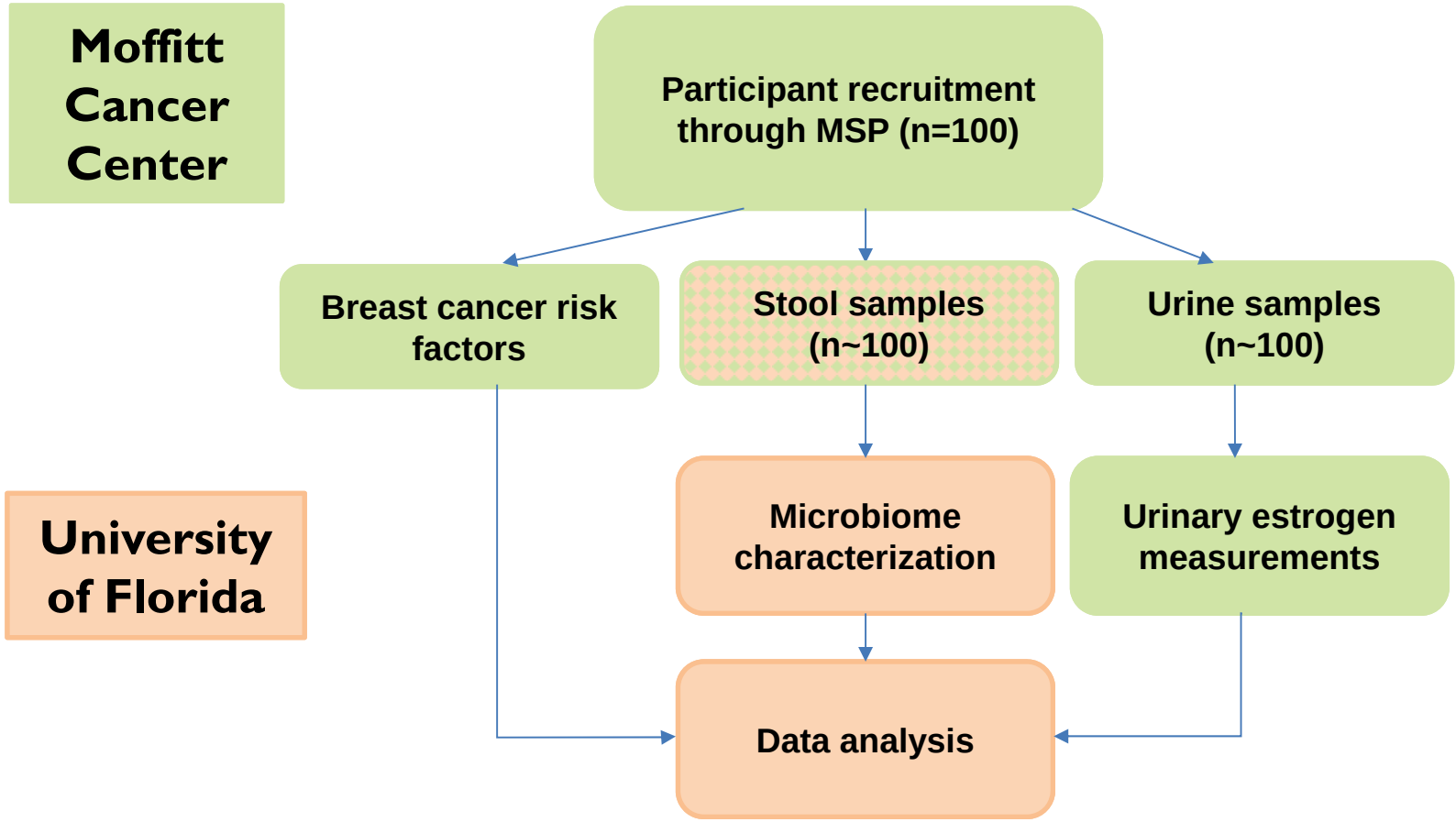
FACCA Investigation

- **Hypothesis:** Specific microbiome profiles are associated with endogenous hormone levels as measured in urine
- **Specific Aim:** To examine associations of the fecal microbiome with individual urinary metabolites, total urinary estrogens, and metabolite to parent compound (estrone and estradiol) ratios that are reflective of the estrogen conversion rate

Significance …

- The project will provide new insights on the role of intestinal microbiome in endogenous estrogen metabolism;
- Findings will be assist in planning large-scale investigations testing the hypothesis that the gut microbiome represents an important contributor to breast cancer via modulation of endogenous estrogen metabolism;
- As the gut microbiome is a potentially modifiable risk factor, the research may ultimately lead to specific dietary guidelines or even bio-therapeutic interventions to reduce breast cancer risk.

Study design



Project Team …

- **Cancer epidemiology**: Dr. Lusine Yaghjyan (*UF*) and Dr. Kathleen Egan (*Moffitt*), Co-Principal Investigators
- **Gut microbial ecology**: Dr. Volker Mai (*UF*) and Dr. Christine Pierce-Campbell (*Moffitt*)
- **Mass spectrometry**: Dr. John Koomen (*Moffitt*)
- **Statistics and "big data" analysis**: Dr. Mattia Prosperi (*UF*)

Study Population …

- Eligibility criteria:
  - postmenopausal status
  - no history of hormone use within the prior 6 months
  - no history of breast cancer, metabolic or hepatic disorders, or chronic intestinal problems
  - BMI ≤30 kg/m^2
- Exclusion criteria:
  - any oral/IV antibiotics within 30 days and/or more than two separate antibiotic regimens within the previous three months

Recruitment and data/sample collection (Moffitt)

- Patients are recruited under the 'Lifetime' protocol, a long-running effort to enroll healthy persons for pilot- and prospective cancer-themed investigations;
- Survey data, body measurements, blood sample and breast density;
- FACCA grant – spot urine (estrogen measures) and stool samples (gut microbiome)

Microbiota Profiling (UF)

- Bacterial genomic DNA is isolated and amplified according to standard methods;
- Sequence reads are generated using the Illumina MiSeq platform and processed through Dr. Mai's in-house pipeline for gut microbial classification;
- Each stool sample is processed to derive quantitative microbial signatures using an integrated suite of computational and statistical algorithms;
- Microbiome diversity and overall microbiome structure can be evaluated.

Quantification of urinary estrogens (Moffitt)

- Proteomics Core at Moffitt under Dr. John Koomen;
- Urine samples are processed in parallel for extraction of estrogens;
- Liquid chromatography-selected reaction monitoring mass spectrometry (LC-SRM) is used to quantify each target molecule, using a published methods;
- Creatinine levels in urine will be measured in to adjust for hydration and inter-individual differences in kidney function.

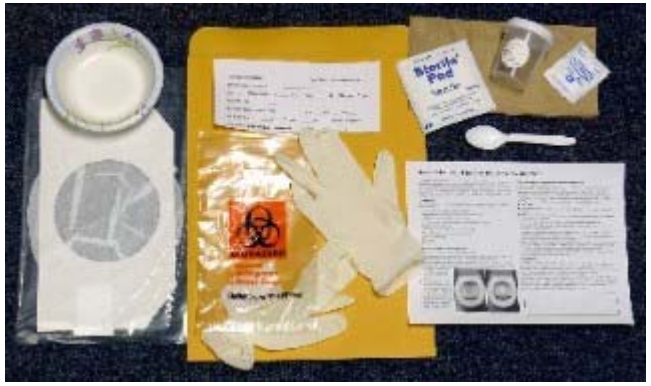
Statistical Analysis (UF)

- Microbiome profiling will yield categorical variable (high/low) based on the proportions in different taxonomic units (Firmicutes, etc) and also the dominant bacterial signature;
- Associations with:
  - total urinary estrogens
  - Individual estrogen metabolites
  - metabolites to parent compound ratios
- GLM adjusting for potential confounders (BMI, parity, alcohol use, smoking, etc)

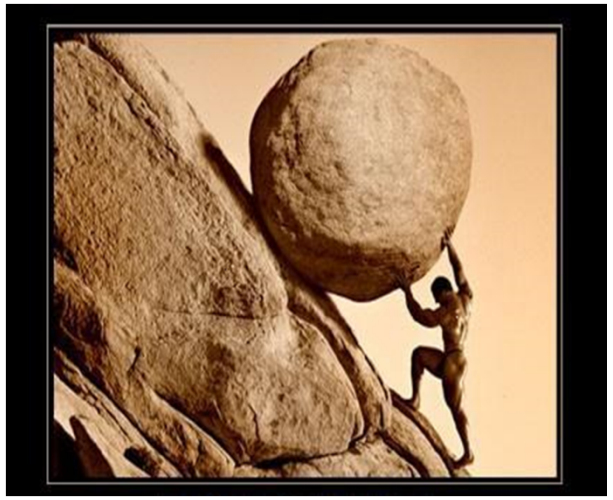
Progress to date …

Since funding in August:

- IRB approval at both centers;
- Established & pilot-tested protocols for urine sampling and home-stool collection;
- Began official recruitment in November with 35 women consented, 8 eligible, and 6 sampled.



Challenges …



- EPI studies are complex!
- IRB approval took ~3mos;
- Vagaries of recruitment/ staffing;
- No-cost extension period will be needed to complete scientific aims of the investigation;
- Two-year project (or option for 2nd year) recommended for EPI projects.

Future Directions …

- Grant proposals submitted/ in development on diet/ breast density;
- Knowledge transfer to other start-ups including oral microbiome in H&N cancer (Co-I: Christine Pierce-Campbell);
- Feasibility using FOB cards in lieu of stool samples in clinics.

Please attend this afternoon's discussion "Viruses, Bacteria, and the Microbiome". Discussants: Christian Jobin, PhD [UF];

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Questions?