# MicroRNA expression in adolescents and young women with endometriosis

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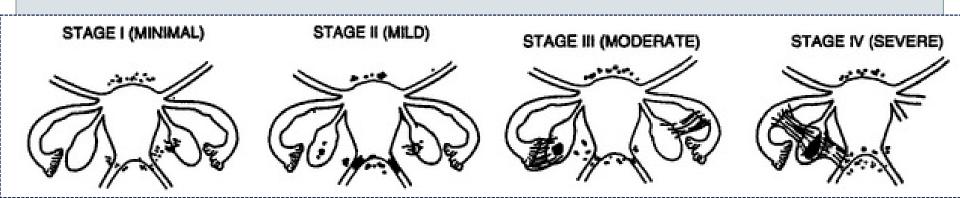


#### **Disclosure**

• I have no financial relationship with a commercial entity producing health-care related products and/or services

#### **Endometriosis**

- Implantation of endometrial glands/stroma outside of the uterus, in up to 10% of women.
- Associated with pelvic pain, dyspareunia, dysmenorrhea, and bladder and bowel dysfunction.
- Infertility in 30-50%: Altered pelvic anatomy, inflammatory and immune function, oocyte quality.



#### **Endometriosis**

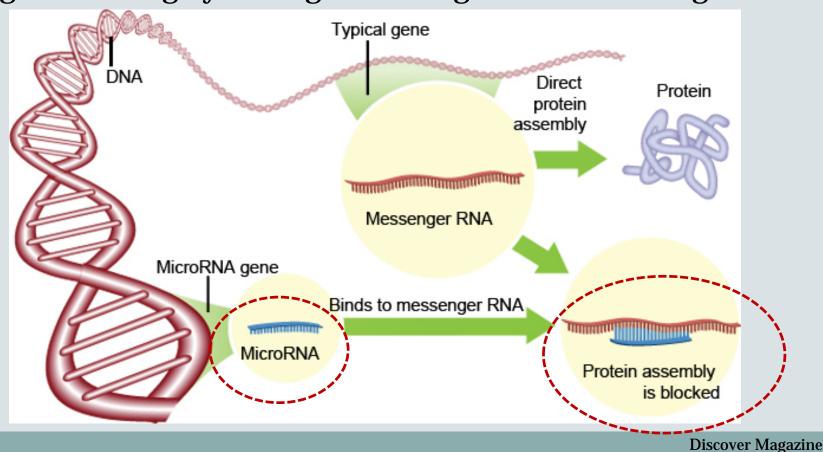
- Symptoms often start at menarche, but diagnosis is often delayed (on average 6.7 years)
- Early identification and intervention can slow disease progression and may improve fertility and functional outcomes.
- Currently, definitive diagnosis requires surgical visualization

#### A Non-Invasive Test for Endometriosis

- Earlier diagnosis and referral to specialists
- Diagnosis for patients with geographic, insurance or other barriers to specialist referral
- Patients could potentially avoid unnecessary surgery
- Monitoring endometriosis activity and recurrence (avoid reoperation)
- Insights into the currently enigmatic pathogenesis of disease
- In the future: miRNA profile of endometriosis may allow for personalized precision medical treatment

#### **MiRNA**

• Small noncoding RNA involved in epigenetic gene regulation largely through messenger RNA silencing.



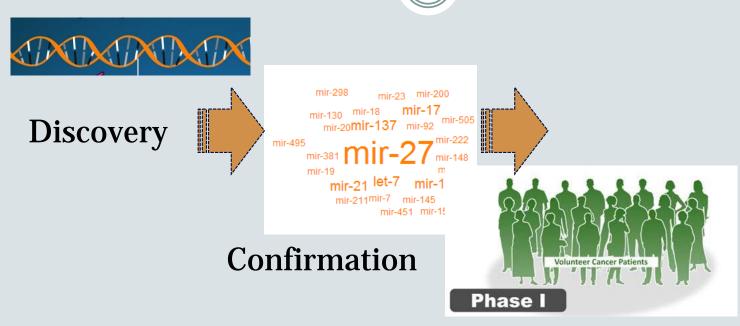
# MiRNA Utility

- MiRNA are identified in a wide variety of biologic samples, including tissues, serum, plasma, saliva and urine
- RNAase-resistant, therefore biologically stable.
- MiRNA profiles of endometriosis and ectopic pregnancies, among other gynecologic conditions, are starting to be elucidated

#### MiRNA and Endometriosis

- Expression of over 20 specific miRNA shown to be altered in adults with endometriosis.
  - miRNA 16, 17, 18a, 20a, 21, 22, 29c, 34c, 100, 125a, 126, 141, 145, 195, 199a, 200a, 200b, 200c, 202, 222, 424, 449
- Inverse correlations are observed between certain miRNA dysregulated in endometriosis and
  - VEGF-A (upregulating angiogenesis)
  - COX-2 (an enzyme involved in inflammation)
  - TGF-beta (regulating cell growth, proliferation, differentiation and apoptosis).

## Clinical application of miRNA



Clinical trial/use

 Though none are currently used in patient care, miRNA-based diagnostic tests for clinical use are in development.

#### Need for research

- Existing studies of miRNA profiles in endometriosis:
  - Small
  - Span endometriosis stages I-IV
  - Include only adults
  - Inconsistent in biologic sample analyzed (serum, plasma, peritoneal fluid, eutopic or ectopic endometrium, endometriomas)
- These issues limit comparisons among studies, and to adolescents

## **Specific Aims**

- To test the hypothesis that certain miRNA are differentially expressed in adolescent patients with surgically-confirmed endometriosis, as compared to age-matched healthy controls.
- **Specific Aim 1:** "Discovery"—To perform quantitative real-time PCR using plasma from a representative subset of adolescent patients and age-matched controls.
- **Specific Aim 2:** To externally validate dysregulated miRNAs identified in Specific Aim 1, using the FirePlex<sup>™</sup> Circulating microRNA Assay (Abcam®, Cambridge, MA), in a replication set of case samples and age-matched controls.

### Study samples

- Case samples: Females 13 26 years with surgically-confirmed endometriosis, enrolled in The Women's Health Study: From Adolescence to Adulthood, a longitudinal cohort study of endometriosis across the lifespan.
- Controls: Healthy volunteers, age 13-26, without signs or symptoms of endometriosis



### Study samples

 Plasma collected and processed according to World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonization Project (EPHect) guidelines

> World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project: III. Fluid biospecimen collection, processing, and storage in endometriosis research

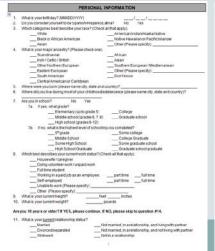
Nilufer Rahmioglu, Ph.D., <sup>a</sup> Amelie Fassbender, Ph.D., <sup>b.c</sup> Allison F. Vitonis, S.M., <sup>d.e</sup> Shelley S. Tworoger, Ph.D., <sup>f.g</sup> Lone Hummelshoj, <sup>h</sup> Thomas M. D'Hooghe, M.D., Ph.D., <sup>b.c</sup> G. David Adamson, M.D., <sup>b.i</sup> Linda C. Giudice, M.D., Ph.D., <sup>b.l</sup> Christian M. Becker, M.D., <sup>b.l</sup> Krina T. Zondervan, D.Phil., <sup>a,k,1</sup> and Stacey A. Missmer, Sc.D., <sup>d.e,f,g</sup> for the WERF EPHect Working Group

Fertility and Sterility.

• Blood centrifuged 2,500 x g for 10 minutes at 4°C; the plasma was aspirated, aliquoted into cryovials, then frozen to -80°C

### Phenotypic data

- An expanded WERF Endometriosis Phenome and Biobanking Harmonisation Project clinical questionnaire was completed upon enrollment.
  - Includes extensive medical, surgical and family history
  - Symptom onset, location, severity
  - Endometriosis treatments past and present (NSAIDs, opiates, hormonal)



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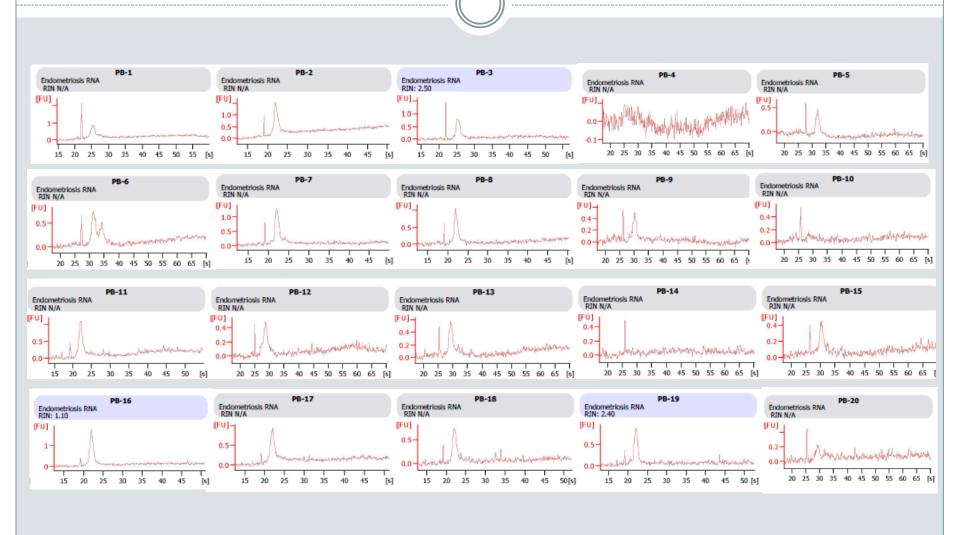
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#### Discovery phase

- Matched 10 pairs of cases and controls
- 5 pairs using hormonal medications at the time of sample collection, 5 without hormone use for at least 30 days prior to collection
- Samples collected before any surgery performed, or ≥ 6 months postoperatively
- Cases and controls age-matched within 2 years
- For discovery phase, excluded patients with known inflammatory or autoimmune disease

## MiRNA extraction and quantification

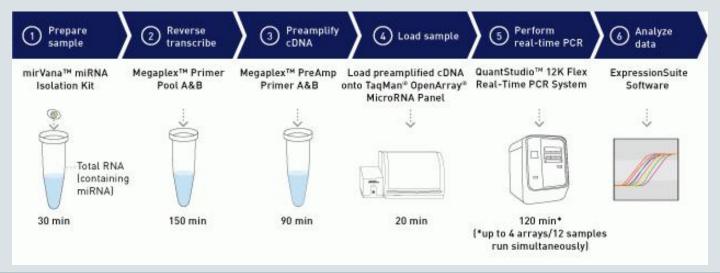


#### Quantitative real-time PCR

- Applied Biosystems TaqMan<sup>®</sup> Array MiRNA 384 well cards x 2
- Analyze 760+ miRNA in each sample



• Relative miRNA expression between cases and controls calculated using the  $2-\Delta\Delta Ct$  method.

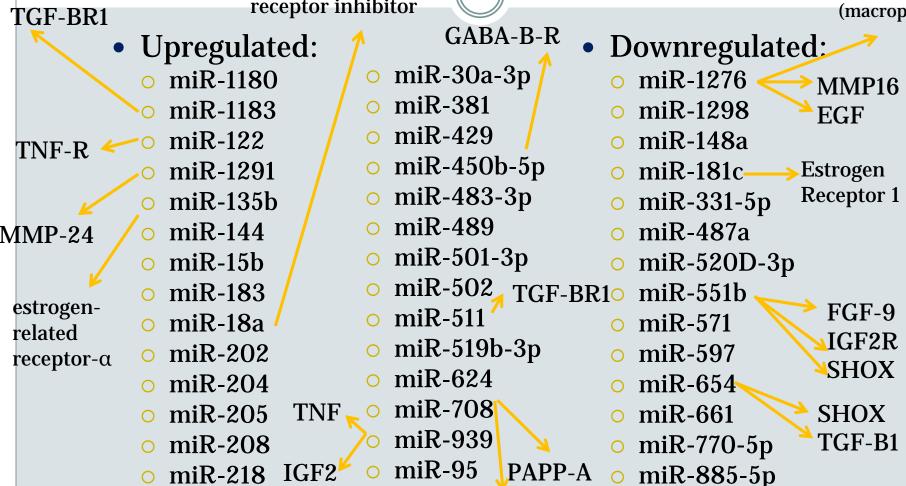


# Preliminary results

Prostaglandin F2 receptor inhibitor

miR-25

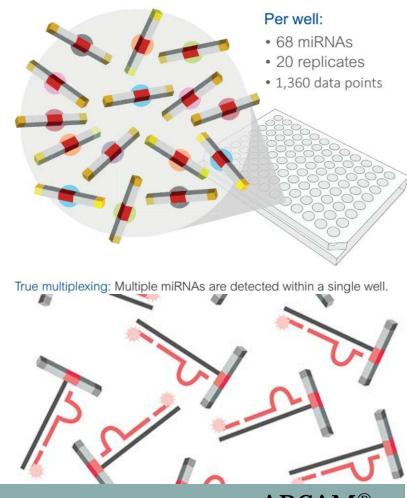
colony stimulating factor 1 (macrophage)



prostaglandin G/H synthase and cyclooxygenase

### **Next Step: Confirmation**

- FirePlex: Multiplex circulating miRNA assay
- 68 miRNA per well, 96 wells
- Hydrogel technology—each well contains particles with code to identify individual miRNA
- Readout is performed on flow cytometer



# Thank you

- New England Fertility Society
- Ferring
- Stacey Missmer, ScD
- Allison Vitonis, ScM
- Sigitas Verselis, PhD









