Roles of Estrogen Hormone Signaling in Ovarian Biology
It was unclear whether estrogen or ER plays any significant role in ovarian physiology.
“Estrogen action is essential for life in eutherian mammals”

“mutations which prevent either the synthesis of, or the response to, estrogens may be lethal at an early stage of development”

George & Wilson, Physiology of Reproduction, 1988
Estrogen Receptor Distribution in Mammalian Ovary

αERKO Mouse

Diagnosis: Estrogen Insensitivity
BW: 28.5 g
Length: 9.3 cm
Est: 340 pg/ml (norm 10-25)
Test: 7.2 ng/ml (norm 0.2-0.8)
LH: 6.7 ng/ml (norm 0.8-1.4)
FSH: 5.6 ng/ml (norm 6-8)

IRB Consent Form
You agree that you consent to the following investigative procedures performed in accordance with the best known experimental practices at the current time for the purpose of diagnosis and treatment of your syndrome.

subject signed
Ovarian Phenotypes in αERKO Mice

- All three functional components and cell types of the ovary are present
- Anovulatory and infertile
- Multiple hemorrhagic and cystic follicles first appear at 35d of age
- Cystic Phenotype and ovulation corrected by reducing elevated LH levels
- Serum hormone profile reflective of PCOS (↑LH, ↑T, normal FSH)
- Dysregulation of ovarian steroidogenic enzymes involved in androgen synthesis (Cyp 17, 17βHSD3)
- Thecal/Interstitial Cell dysmorphogenesis to Leydig cells
Ovarian Phenotypes in βERKO Mice

- All three functional components of the ovary are present
- No gross abnormal morphology, all follicle stages are present, but indication of follicular arrest
- Oligoovulatory and subfertile
- Ovulatory inefficiency with trapped follicles, reduced number of corpora lutea - can be rescued with forskolin
- Phenotype similar to LUF syndrome
- Follicle culture and isolated granulosa cells show lack of response to gonadotropins and estradiol synthesis
- Null mutations may be a source of idiopathic infertility
Ovarian Phenotypes in αβERKO Mice

- Initially all three cell types and functional components of the ovary are present
- Anovulatory and infertile
- Few hemorrhagic and cystic follicles
- Evidence of post-natal “sex-reversal” in the form of Sertoli-like cells and ectopic Sox9 expression
- Altered male versus female gonadal gene expression and granulosa cell differentiation
- Genetic susceptibility of phenotypic expression in background 129 vs BL6

Couse et al. (1999) Science 286:2328-2331
Clinical Case of ESR1 Insensitivity or Resistance

Estrogen Resistance Caused by a Mutation in the Estrogen Receptor Gene
E.P. Smith, et al

Delayed Puberty and Estrogen Resistance in a Women with Estrogen Receptor α Variant
S.D. Quaynor, et al

Familial Multiplicity of Estrogen Insensitivity Associated With a Loss of Function ESR1 Mutation
V. Bernard, et al
J Clin Endocrinol Metab. 2017 Jan 1; 102(1): 93–99
Phenotype and Genomic Analysis

A

B

C

c.1181G>A

D

Arg394

Human | I L M I G L V W R S M E H P G K
Gorilla | I L M I G L V W R S M E H P G K
Rhesus monkey | I L M I G L V W R S M E H P G K
Pig | I L M I G L V W R S M E H P G K
Cow | I L M I G L V W R S M E H P G K
Horse | I L M I G L V W R S M E H P G K
Rabbit | I L M I G L V W R S M E H P G K
Mouse | I L M I G L V W R S M E H P G K
Chicken | I L M I G L V W R S M E H P G K
Zebrafish | V L M I G I W R S I H S P G K

Bernard et al, JCEM 2017
### Family Members

<table>
<thead>
<tr>
<th></th>
<th>IV-3 (Proband)</th>
<th>IV-5 (Affected Sister)</th>
<th>IV-6 (Affected Brother)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Age, y</td>
<td>25</td>
<td>21</td>
<td>18</td>
<td></td>
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<tr>
<td>Height, cm</td>
<td>156</td>
<td>175</td>
<td>163</td>
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<tr>
<td>Weight, kg</td>
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<td>BMI, kg/m²</td>
<td>26.3</td>
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<td>Birth weight, g</td>
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<td>3300</td>
<td>2600</td>
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<td>Puberty onset, y</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Tanner stage</td>
<td>P5B1</td>
<td>P5B1</td>
<td>P2G1</td>
<td></td>
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<tr>
<td><strong>Hormonal parameters</strong></td>
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<td></td>
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<tr>
<td>17β-estradiol, pmol/L</td>
<td>9476</td>
<td>9233</td>
<td>218</td>
<td>F (EFP): 120–300; M: 10–50</td>
</tr>
<tr>
<td>LH level, IU/L</td>
<td>24</td>
<td>18</td>
<td>13</td>
<td>F (EFP): 2–8; M: 2–8</td>
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<tr>
<td>FSH level, IU/L</td>
<td>13</td>
<td>15</td>
<td>56</td>
<td>F (EFP): 2–10; M: 2-10</td>
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<tr>
<td>Testosterone, nmol/L</td>
<td>6.5</td>
<td>7.3</td>
<td>3</td>
<td>F (EFP): 0.3–1.5; M: 12.1–29.5</td>
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<tr>
<td>DHEAS, µmol/L</td>
<td>1.4</td>
<td>1.7</td>
<td>1.4</td>
<td>F (EFP): 0.95–10.9; M: 2.2–12.2</td>
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<tr>
<td>AMH, pmol/L</td>
<td>19</td>
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<td>14-48</td>
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<td><strong>Imaging</strong></td>
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<td>Ovarian ultrasonography</td>
<td>Multicystic ovaries</td>
<td>Multicystic ovaries</td>
<td>NA</td>
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<tr>
<td>Bone age evaluation, y</td>
<td>13 at 19 yrs old</td>
<td>13 at 21 yrs old</td>
<td>11 at 18 yrs old</td>
<td></td>
</tr>
</tbody>
</table>

Bernard et al, JCEM 2017
Functional Analysis of R394H Mutation

Bernard et al, JCEM 2017
Delayed Puberty & Estrogen Insensitivity

Physical Exam: age 18
BMI = 16.4 kg/m2
163.2 cm/44.1 kg
Tanner 1 breasts
Tanner 3 pubic hair
No abdominal mass

US: cystic, enlarged ovaries
E2 = 3,500 pg/mL
LH/FSH mildly elevated

ESR1 mutation? Q375H

Quaynor et al. NEJM 2013;369:164-171.
Case Presentation

- Adopted Caucasian female (DNA analysis showed parents First cousins)
- Abdominal pain at age 15
- Age 18: no breasts & primary amenorrhea
- Markedly high serum estrogens, elevated androgens
- Mildly elevated FSH and LH; Ovarian cysts

Quaynor et al. NEJM 2013;369:164-171.
Complete Estrogen Insensitivity Syndrome

Clinical Features (Female)

Open Radial & Ulnar Epiphyses

Bone Age 13.5 yrs

DXA

Open Growth Plates

Closed Growth Plates

Osteocalcin
65.1 ng/mL
[4.9-30.9]

Bone-Specific Alkaline Phosphatase
45.1 μg/L
[0-21.3]

C-Telopeptide
1060 pg/mL
[112-738]
Clinical Conclusions

Female Patients (Estrogen Insensitivity)

- Presented with lack of breast development & ovarian cysts
- Open epiphysis and osteopenia
- High serum estrogen and elevated androgen levels
- No estrogen induced growth spurt
- Impaired response to physiologic levels of estradiol
- Known mutations in ESR1 gene in highly conserved regions in helix 3 or helix 5 of ligand binding domain
- Mutation of ESR1 gene is not a clinically lethal mutation
Q375H Functional Characterization

Molecular Defects

ERα Transactivation

Significantly Impaired Activity of Q375H on ERE Reporter (HepG2 & Cos-7 cells)

Q375H activation is right shifted 2 orders of magnitude

Normal E2 Serum Conc (Human) = 100 pg/mL
Q375H Mutant (Human) = 10,000 pg/mL

[Coons/Korach unpublished; Quaynor 2013]
Markedly impaired estrogen signaling due to interference with coactivator binding except during the administration of high doses of estradiol, exceeding physiological limits consistent with transactivation assay.
Estradiol vs Diethylstilbestrol (DES)

73 compounds screened for ER reporter activity

Ishikawa Cells
Human endometrial adenocarcinoma cell line

SKBR3 Cells
Human breast adenocarcinoma cell line

Coons/Korach unpublished
Coactivator Interactions with DES and E2

**E2**

**ERα M2H**

SxGal4-TATA-Luc - HepG2

10/16/2014

**Wild Type**

**Mutant**

---

**DES**

**ERα M2H**

SxGal4-TATA-Luc - HepG2

10/16/2014

**Wild Type**

**Mutant**
Estrogen Receptor Gene Polymorphisms and Ovarian Dysfunction in Women

Estrogen Receptor-α (ESR1):


Estrogen Receptor-β (ESR2):


ERα Mutant Patients

- ESR1 mutant female patients are infertile. Significant cystic ovarian effects due to hormonal imbalance, elevated steroid and trophic hormone levels. Thin or absent endometrial stripe.

- Male patients have low sperm counts and sperm viability. Retracted or cryptorchid testis.

- Mutations not spontaneous, originates from consanguineous family relationship (first cousins)

- Heterozygous family members show no clinical signs

- Analysis of mutant receptors shows DES an active agonist from \textit{in vitro} assays, but not \textit{in vivo}.

Potential that idiopathic Infertility could be a result of polymorphism or mutations in the estrogen receptor genes
Acknowledgements

- John Couse
- Karina Rodriguez
- Judith Emmen
- Fuminori Tanaguchi

- Lawrence Layman
- Sophia Christin-Maitre

- John Katzenellenbogen

- Page Meyers
- James Clark

- Kyathanahalli Janardhan

Microarray Core, Genotyping Core, Bioinformatics Group, Protein Expression Core, Histology, IHC Core, X-Ray Crystallography, Confocal-Imaging Ctr, CMB, KO Core
Je vous remercie
Thank You
ERα Mutant Male Patient

- Skeletal Alterations (genu valgum), open epiphysis, low BMD, low sperm count and viability, elevated steroid and trophic hormone levels

- Unresponsive to estrogen therapy, premature atherosclerosis, compromised vascular dilatory response

- Loss of function point mutation in ERα gene produces premature stop codon (no protein expression)

- Mutation not spontaneous, originates from consanguineous family relationship (first cousins)

- Heterozygous family members show no clinical signs

E.P. Smith et al, NEJM 1994
Karotype: 46, XY

Height: Age 16 = 178 cm (75%
Age 28 = 204 cm (>95%

Genu Valgum

Treatment: Estraderm Patch
- 14/wk @ 100 ug ethinyl-E2/patch
- 6 months duration

Serum Hormones after 6 mos
E2: 250 pg/ml (prior 119)
LH: 34 mIU/ml (prior 37)
FSH: 30 mIU/ml (prior 33)
PRL: 5.9 ng/ml (prior 5.9)

Bone Age and Density: No Change

Side Effects: No Nausea
No fluid retention
No gynecomastia

Male Patient expressed no ERα protein, but expressed ERβ
CELLULAR ESTROGEN MECHANISMS

Early Tissue Responses

Direct DNA Binding
Activation

Tethering
Activation

mRNA

Polysomes

Protein

Genomic Tissue Responses