A 35 Year Experience in Translational Research Funded by NICHD

Use of Human Disease Models to Define the Genetic Control of Human Reproduction

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Director of Clinical Research, MGH
Question #82: What triggers puberty?

“...no one knows exactly what forces childhood to end.”
Normal HPG Axis

HYPOTHALAMUS

GnRH

PITUITARY

LH  FSH

GONADS

Sex Steroids & Gonadal Proteins
Daunting Problems: Technical & Regulatory

- Small in size & # (~1,200 in humans)
- Widely dispersed network
- High species variability
- GnRH not measurable
- Must measure LH, FAS q10’
- Research in children
Why Work on GnRH Neurons?

**Big Opportunities!!:**

Unique Biology in the Human

- **One of ‘Big 3’ mandates of hypothalamus**
  - Nourishment => Growth (GHRH-IGF axis)
  - Deal with Stress => Adrenal (CRF-Cortisol axis)
  - Go forth & multiply => Reproduce (GnRH-Gonadal axis)

- **Master regulator of all ‘downstream’ reproduction**
  - “Pilot Light of Reproduction”

- **Critical role in evolution & survival of species**
  - adapts Reproductive “Fitness” in response to environmental changes
  - e.g. malnutrition, exercise, circadian changes, olfaction, predators, fertility

- **Human Genome Bioinformatics & New Genetic Tools:**
  - Remarkable lack of redundancy (unlike GPCRS, Tx. factors, 2nd mess.)
  - ? Implies its genetic control is above the hypothalamus & complex
  - Known genetic heterogeneity (X-linked; AR; AD)
Hypogonadotropin Hypogonadism = UBO

Key Findings in 1943
- Undescended testes
- Small phallus
- Absence of any puberty
- No sense of smell

Pituitary

Gonads

LH & FSH
Gonadal Fx
Normal
Hyper ↑↑
1° Failure
Hypo ↓↓
2° Failure
Normal Adult Male Range

IHH Male: Baseline

T = 20ng%

Normal Adult Male

T = 500ng%

Hoffman & Crowley, NEJM 1980
### GnRH Analogues = Agonists

<table>
<thead>
<tr>
<th>Amino Acid Sequence</th>
<th>In Vitro Potency</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pGlu His Trp Ser Tyr Gly Leu Arg Pro Gly-NH₂</td>
<td>1</td>
<td>GnRH</td>
</tr>
<tr>
<td>------------------------------------------------------------- N -EtNH₂</td>
<td>4</td>
<td>Fujino</td>
</tr>
<tr>
<td>-------------------------------- D-Ala------------------------------------</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>--------------------------------- D-Ala--------------------- N -EtNH₂</td>
<td>14</td>
<td>Decapeptyl</td>
</tr>
<tr>
<td>--------------------------------- D-Trp-------------------------------------</td>
<td>144</td>
<td>Nafarelin</td>
</tr>
<tr>
<td>--------------------------------- D-Nal 2------------------- N -EtNH₂</td>
<td>15</td>
<td>Leupron</td>
</tr>
<tr>
<td>--------------------------------- D-Leu---------------------N -EtNH₂</td>
<td>20-40</td>
<td>Buserelin</td>
</tr>
<tr>
<td>--------------------------------- D-Ser(tBu)--------------- N -EtNH₂</td>
<td>144</td>
<td>Deslorelin</td>
</tr>
<tr>
<td>--------------------------------- D-Trp----------------------N -EtNH₂</td>
<td>210</td>
<td>Histrelin</td>
</tr>
</tbody>
</table>
Attempted Replacement Rx in GnRH Deficiency with GnRHa: Homologous Desensitization of GnRHR

GnRHa 5 μg QD

GnRHa 10 μg QD

GnRHa 10 μg BID

GnRHa 10 μg QOD

Crowley W, Vale W, Rivier J. NEJM, 1979
### Potential Rx Applications of GnRHa-Induced Homologous Desensitization of GnRHR

<table>
<thead>
<tr>
<th>Application</th>
<th>Alternative Rx.</th>
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</thead>
<tbody>
<tr>
<td>Prostate Cancer</td>
<td>Surgical Castration</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>Synthetic Progestins</td>
</tr>
<tr>
<td>IVF Timing</td>
<td>Oral Contraceptives</td>
</tr>
<tr>
<td>Precocious Puberty</td>
<td>None</td>
</tr>
</tbody>
</table>
GnRH\(_a\) => Reversible Biochemical Castration of Pituitary-Gonadal Axis in Children with Precocious Puberty

JCEM; NEJM, 1981

4 yr. old
Pre-Rx

+ 6 mo. Rx
GnRHa Sales in $B/yr: 1990-2009

Source: www.evaluatepharma.com
Literature Impact of NICHD Initiated Program

Google Ngram Search Tool: “Central Precocious Puberty”

JCEM & NEJM Articles on Central Precocious Puberty
Pulsatile GnRH Rx: Re-Constitutes Normal HPG Axis in IHH

Normal Adult Male
$T = 500\text{ng}\%$

Normal Adult Male Range

IHH Male: Baseline
$T = 20\text{ng}\%$

Normal Adult Male Range

GnRH IV q 2hr

$T = 500\text{ng}\%$

+ Generates Experimental Opportunities:
Dose-Response Curves
(Spratt et al, JCEM)
Pulsatile GnRH Administration via Mini-Pump
(Dean Kamen)
Hypogonadism with $\Rightarrow\Downarrow$ Gonadotropins

Anosmia
(Defective Olfactory Bulbs)

Kallmann syndrome (KS)

Normosmic Idiopathic Hypogonadotropic Hypogonadism (nIHH)

Isolated GnRH Deficiency

Developmental theme

Neuroendocrinetheme
Critical Observation #1 (Mouse Development): *GnRH Cells of Origins are Extramural to CNS*

Schwanzel-Fukuda & Pfaff, *Nature*

Diagram labels:
- vno: vomeronasal organ
- gt: ganglion terminale
- ob: olfactory bulb
- poa: preoptic area
Critical Observation #2: **Contiguous Gene Syndrome**  
(Male) Del p22.31: **KS, Ichthyosis, Ca++ Disorder**

- ↓ LH, FSH, T during neonatal ‘minipuberty’ window
- microphallus + cryptorchidism
- absent olfactory bulbs & tracts

Mother 46XX  
shared terminal deletion Xp22.31  
Son 46XY

GnRH Immunostaining of Human Fetal Brain at 19 Weeks Gestation

Normal Fetus

KS Fetus: term del of Xp22.31

Median Eminence (no GnRH staining)

GnRH Neurons

Schwanzel-Fukada et al. Molec Brain Res 1989
KAL1 = Gene for GnRH Neural Migration: Establishes Neurodevelopmental Theme

Normal

KAL1
The GPR54 Gene as a Regulator of Puberty

Kisspeptin Signaling System: Neuroendocrine Theme

Metastin-> \( \uparrow \text{GnRH} \)

1st Direct query of a Hypothalamic neuron
Also desensitizes

50% of all KISS1Rs are on other hypothalamic Neurons

Who ?What

All GnRH neurons Have KISSIRs

Kisspeptin Neurons

Bob Steiner, U. of Washington
“most important discovery in Reproductive Endocrinology Since GnRH

Dungan et al, Endocrinology 147(3): 1154, 2006
Kisspeptin Administration to Normal Volunteers
(Chan & Seminara)

Kisspeptin (Metastin 45-54) Administration
Healthy Male Volunteers - Conditional Analysis

LH (mIU/mL)

Time (hrs)
0 1 2 3 4 5 6 7 8 9 10 11 12

Pituitary Units (mIU/mL)

Urinary Standard (IU/L)

n=13
GnRH Neuronal Ontogeny: A Story Told by Patients with GnRH Deficiency

- Olfactory Placode
- KAL1
- NELF
- Olfactory Bulb
- Cribiform Plate
- Olfactory Tract
- GnRH Neurons
- Kiss Neurons
- GnRH
- GnRH_R
- KISS
- KISS1R
FGFR System Problem: 4 Receptors + 23 Ligands

- III b
- III c
- III a

Exon 8a
Exon 8b
Exon 7 lg

3 IgG Domains

Ligand Binding Domains

Cell Membrane

Protein Kinase Domains
Mutations in KAL2 (FGFR1)

- nIHH
- Kallmann

- SP
  - G48S
  - Y99C (1)
  - N117S

- D1
  - R78C (2)
  - Y99C (1)
  - V102I
  - S107L
  - T112T

- D2
  - D224H
  - G237D
  - R250Q (1)
  - V273M (1)
  - G237S
  - R254Q
  - E274Q
  - V102I
  - S107L
  - T112T

- D3
  - P285R
  - Y339C
  - L342S
  - S346C
  - I347fsX19
  - T357I (Ex.8A)
  - P366L
  - I378I
  - V429E
  - P483T

- TM
  - FRS2

- PTK
  - V427L
  - R470L
  - I538V
  - K618N
  - R622X (1)
  - A671P
  - Q680X
  - L689L
  - P722H
  - N724K
  - Q764H
  - D768Y
  - Y585X
  - R622X (1)
  - G687R
  - E692G
  - G703R
  - G703S
  - P745S
  - L754L (16)
L/S 343 in FGFR1 eliminates FGF8 binding without changing FGF2 (M. Mohammedi).
First Inkling of Oligogenicity: 
**NELF & FGFR1**

Pedigree 1

**NELF**

1. **++**
2. **+/Del**

**FGFR1**

3. **+/S**
4. **+/Del**
5. **++**
6. **++**
7. **++**
8. **+/S**

- Midline Defects
- IHH
- An/hyposmia
- Clinodactily
- Delayed Puberty

Pitteloud, PNAS, 2007
Oligogenicity in 376 GnRH Deficient Probands (11%)

<table>
<thead>
<tr>
<th># of alleles with non-synonymous rare variants</th>
<th>GnRH Def. patients (n=397)</th>
<th>controls (n=179)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>78%</td>
<td>90%</td>
</tr>
<tr>
<td>1</td>
<td>17%</td>
<td>10%</td>
</tr>
<tr>
<td>2: same gene</td>
<td>2.5%</td>
<td>0%</td>
</tr>
<tr>
<td>≥2: different genes</td>
<td>2.5%</td>
<td>0%</td>
</tr>
</tbody>
</table>

- **FGFR1**: 8.5% with rare non-synonymous variants
  - Digenic: 4.7%
  - Bi-allelic: 3.7%
  - Mono-allelic: 0.1%

- **KAL1**: 4.7%
  - Digenic: 4.7%
  - Bi-allelic: 0%
  - Mono-allelic: 0%

- **PROKR2**: 4.2%
  - Digenic: 0%
  - Bi-allelic: 4.2%
  - Mono-allelic: 0%

- **GNRH**: 3.7%
  - Digenic: 3.7%
  - Bi-allelic: 0%
  - Mono-allelic: 0%

- **FGF8**: 1.6%
  - Digenic: 1.6%
  - Bi-allelic: 0%
  - Mono-allelic: 0%

- **KISS1R**: 1.3%
  - Digenic: 1.3%
  - Bi-allelic: 0%
  - Mono-allelic: 0%

- **NELF**: 1.0%
  - Digenic: 1.0%
  - Bi-allelic: 0%
  - Mono-allelic: 0%

- **PROK2**: 0.8%
  - Digenic: 0.8%
  - Bi-allelic: 0%
  - Mono-allelic: 0%
GnRH Neuronal Ontogeny: A Story Told By a Human Disease Model: GnRH Deficiency

Neuro-Developmental Genes

Overlap???

Neuro-Endocrine Regulatory Genes
Complexity of Genetic Architecture of GnRH Neuronal Network over 15 years

1995
- KAL1 11%
- GNRHR 5%
- Unknown 84%

2003
- KAL1 9%
- GNRHR 4%
- FGFR1 10%
- KISS1R 1%
- NELF 1%
- Unknown 75%

2008
- KAL1 7%
- GNRHR 3%
- FGFR1 10%
- FGF8 2%
- KISS1R 1%
- NELF 1%
- PROKR2 3%
- PROK2 1%
- Unknown 72%

Present
- KAL1 6%
- GNRHR 4%
- GNRH1 1%
- FGFR1 12%
- FGF8 2%
- KISS1R 2%
- PROKR2 5%
- NELF 2%
- PROK2 1%
- PROK2 1%
- CHD7 9%
- TACR3 6%
- TAC3 1%
- Unknown 47%

Exomic Sequencing => RSVs
Segregation & Validation in Gene (-) Pool
Functional Analyses: Dicty, Worm Fly

Gene (-) Pool Functional Analyses:
Dicty, Worm Fly
GnRH: Timeline of Landmark Discoveries

- **1971**: GnRH Sequenced
- **1977**: Nobel Prize
- **1980**: Hpg mouse
- **1986**: GnRH Rx in IHH
- **1989**: GnRH Cloned; Gene Rx
- **1990**: GnRH Neurons Mapped
- **1995**: DAX
- **1996**: KISS1R
- **2003**: TAC3/R
- **2004**: PROK2/R
- **2005**: FGF8
- **2006**: CHD7
- **2007**: NbL
- **2011**: KISS1

**Human**

**Non-Human**
MGH Reproductive Endocrine Unit Training Program (T32) = 80

- >60% = Women
- 80% in Academics = peer-reviewed funding
- 50% >10 years = Prof.
- Other 50% = Assoc. Prof.
- 3 Presidents of Endocrine Society
- 10 Members of Endo Society leadership
## NICHD: Keys to Our Success

<table>
<thead>
<tr>
<th>Programs</th>
<th>Visionary Leadership</th>
</tr>
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<tbody>
<tr>
<td>R01 Grant Program</td>
<td>Marvin Karten</td>
</tr>
<tr>
<td>U54 Specialized Center</td>
<td>Michael McClure</td>
</tr>
<tr>
<td>T32 Training Grant</td>
<td>Lou DePaolo</td>
</tr>
<tr>
<td></td>
<td>Yvonne Maddox</td>
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<tr>
<td></td>
<td>Susan Taymans</td>
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Translational Research is Expensive