Insights into Pubertal Regulation from Patients with Hypogonadotropic Hypogonadism

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Objectives

- Physiology of puberty
- What triggers puberty?
- How do you uncover genes regulating puberty?
  - Studies in humans with extremes of pubertal timing
    → Human GnRH deficiency
    → Central Precocious Puberty
- Fertility induction in hypogonadotropic hypogonadism

Hypothalamic-pituitary-gonadal axis

Gonadal feedback loops

HPG Axis across development:
Fetal/Infancy

Disclosure

In compliance with ACCME Standards for Commercial Support of CME activities

Ravi Balasubramanian
I have no relevant financial relationships to disclose

Studies in humans with extremes of puberty

Mini-puberty (Boys: ~6 months; Girls: ~2y)

Penile growth
Testicular descent

Adapted from Winter et al. JCEM 1976
HPG Axis across development: Reactivation at puberty

Reactivation at puberty

Penile growth
Testicular descent
Mini-puberty
Inhibitory factors

DAY
NIGHT

Fetal Infancy Childhood Puberty Adult

Adapted from Winter et al. JCEM 1976

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What triggers puberty?

Uncover the ontogeny of the GnRH neurons, the “pilot” light of reproduction!

Embryonic joint origin of olfactory and GnRH neurons

vno vomeronasal organ
gt ganglion terminale
ob olfactory bulb
poa preoptic area

Schwanzel-Fukuda & Pfaff, Nature 1989
Olfactory epithelial origin of GnRH neurons

Is this true in humans? If so, what genes/pathways govern them?

Genes governing puberty: The human model

Problems
- Only ~1,200 in human
- Small/widely dispersed
- Can’t measure GnRH
- Measure LH, FAS q10'
- Research in children

Solution?
Study of human phenotypic extremes

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Idiopathic hypogonadotropic hypogonadism

Characterized by:
- absence of pubertal maturation by age 18y in both sexes

Overall prevalence:
1 in 30,000 in males
1 in 120,000 in females

Male: Female ratio: 4:1

Idiopathic hypogonadotropic hypogonadism

Normal

IHH

GnRH

HYPOTHALAMUS

PITUITARY

LH

FSH

GONADS

Normal Adult Male Range

IHH Males: Measuring
Pulsatile GnRH Rx: Re- Constitutes Normal HPG Axis in IHH


Idiopathic hypogonadotropic hypogonadism

Normal

IHH

HYPOTHALAMUS

GnRH

PITUITARY

LH

FSH

GONADS

Normal

Kallmann Syndrome

Non-syndromic presentation:
- Normosmic form of Human GnRH deficiency

Syndromic presentation:
- Simple syndromic form
  - Anosmia and other minor abnormalities = Kallmann Syndrome
- Severe syndromic forms:
  - Moebius Syndrome; CHARGE syndrome

Human Isolated GnRH Deficiency: Simple Syndromic form

KALLMANN SYNDROME
- Undescended and small testes
- Small phallus
- Absence of any puberty
- No sense of smell

Using Human Phenotypes to Guide Gene Discovery and Provide Biological Insights

Extreme Phenotypes
Identifying genes governing puberty from rare and complex trait genetics

Gene discovery challenges in IGD: Heterogeneous inheritance patterns

Given these challenges how do you discover new genes?

Given these challenges how do you discover new genes?

Extreme phenotypes = Rare genetic variants

Mendelian Genetics

• Phenotypic Extremes:
  • Familial inheritance
  • Transmission risk to offspring
  • Often due to inherited or in rare cases spontaneous de novo mutations
  • Phenotypes very severe (e.g. syndromic)

Gene discovery challenges in IGD

• Nearly 70% of Human Isolated GnRH deficiency patients are sporadic presentations
  - Reproductive disorder, so lack of transmission
  - ? De-novo mutations
  - ? Incomplete penetrance of inherited mutations

Taking advantage of genomic alterations
• Your favorite candidate gene!
• Strength of familial inheritance
• Bioinformatic pathway analysis
• Power of syndromic presentations
• Guided by non-human models
• Power of next-generation genomics

Balasubramanian et al, Neuroendocrinology 2010;92:81–99
First gene for human GnRH deficiency: Insights from an infant with a deletion

Karyotype analysis

Mother

Looking for

Chromosomal Rearrangements

-Typically visible in karyotypes


First human insights into GnRH neuronal ontogeny

Scheussel-Falude et al. Brain Res Mol Brain Res. 1989

Summary from the KAL1 discovery story

- Human genetics and clinical investigation – Highly valuable tool for novel biology
  - Chromosomal rearrangements in humans – in this case a contiguous gene deletion, led to the discovery
  
  - Clues from the phenotypes:
    - Consider contiguous gene rearrangement if multiple clinical phenotypes were to be present
    - Complemented the basic discoveries known at that time
    - KAL1 was a X-linked gene - Could this be the reason for the male predominance for this disease??
      - Are they all going to be deletions??

KAL1 mutations in humans

Only Accounted for 5-7% of cases!!

More genes to be found
Given these challenges how do you discover new genes?

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**Candidate gene search for IGD**

- 50% of patients with IGD are normosmic
- Therefore, there ought to be mutations that are disrupting the migration of the GnRH neurons
- No mutations in KAL1 were seen in normosmic patients
- It was also observed that not all IGD patients responded to GnRH with pituitary gonadotropin secretion

→ There seemed to be resistance at the level of the pituitary could this be GnRH – receptor inactivity?

**GNRHR-Inactivating compound heterozygous mutations**

![Diagram of GNRHR mutations with gene names and genotypes](image)

De Roux et al., NEJM, 1997; 337:1597-1603

**Genetic defects in Isolated GnRH deficiency patients circa 1998**

- Kallmann Syndrome
  - KAL1: Discovery by chromosomal rearrangement
  - Defect in GnRH migration
- Normosmic IGD
  - GNRHR: Discovery by candidate gene screening
  - Defective GNRHR signaling

**Genes discovered by homozygosity mapping/linkage analysis: Example 1**

![Diagram of linkage analysis with gene names and genotypes](image)

Genes discovered by homozygosity mapping/linkage analysis: Example 1

Discovery of the kisspeptin signaling pathway as gatekeeper of puberty

KISS1R knockout in mice recapitulates human phenotype

Kisspeptin signaling and GnRH secretion

- KISS1R is a G-protein coupled receptor, an obligate receptor for kisspeptin, a novel neuropeptide secreted by KISS1 neurons in the hypothalamus
- Kisspeptin subsequently shown to be robust stimulator of GnRH secretion in all mammalian species
- Kisspeptin pathway additionally mediates control of fertility by integrating metabolic and environmental (e.g. photoperiod) cues

Kisspeptin signaling and GnRH secretion

Adapted from Leonor Pinilla et al. Physiol Rev 2012;92:1235-1316

The Kisspeptin-Neurokinin B-Dynorphin (KnDY) neuronal control of GnRH secretion:

Adapted from Leonor Pinilla et al. Physiol Rev 2012;92:1235-1316
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**Chromosomal rearrangement yields**

*FGFR1* gene: 8p11.2-p12 deletion

**FGFR1** mutations cause both Kallmann and normosmic forms of IGD

**Mapping mutations to crystal structure of FGFR1 yields insights into ligand, FGF8**

Adapted from Leonor Pinilla et al. Physiol Rev 2012;92:1235-1316

Dode et al Nature Genetics; 2003; 33: 1-3

Nelly Pitteloud et al. PNAS 2006;103:6281-6286

PNAS 2006; 1
**FGFR1 discovery → Systems Biology**

- Autosomal Dominant Inheritance
- Associated Skeletal Abnormalities
- Both KS & IHH Syndromes
- Striking variability of expression
- Reversal of KS/IHH, i.e., Congenital GnRH Deficiency
- Clues as to which FGF ligand is involved

→ SYSTEMS BIOLOGY APPROACH

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**Moebius Syndrome**

- 19y M with GnRH deficiency
  - complete absence of signs of puberty
  - Microphallus and cryptorchidism noted at birth
  - Anosmia
  - Birth history notable for:
    - III, IV, VI and VII cranial nerve deficits
    - vocal cord paralysis, needed tracheostomy

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**Labs**

- T: <10 ng/dL
- LH 0.1  FSH: 1.2
- TFT’s, prolactin, cortisol axes: Normal
- Ferritin, iron studies: Normal
- MRI: Olfactory bulb agenesis; corpus callosal agenesis

Severe syndromic form of GnRH deficiency

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**Moebius Syndrome**

- Birth defect characterized by congenital ophthalmoplegia and VII Nerve palsy

<table>
<thead>
<tr>
<th>Classical (~50%)</th>
<th>Atypical (~50%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral</td>
<td>Unilateral</td>
</tr>
<tr>
<td>facial weakness</td>
<td>facial weakness</td>
</tr>
</tbody>
</table>

*Sequencing Images: Mendeley J.A. 2016: KIńskiego Institute of Immunology and Experimental Therapy*
**TUBB3 mutations cause atypical Moebius syndrome**

- **TUBB3** gene
  - R26Q
  - R262H

**Exquisite genotype-phenotype correlation**

<table>
<thead>
<tr>
<th>Inheritance</th>
<th>Familial AD</th>
<th>Familial AD</th>
<th>Familial AD</th>
<th>De novo</th>
<th>De novo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypes</td>
<td>Severe isolated Ocular dysmotility</td>
<td>Severe Ocular dysmotility + Corpus callosum agenesis + Abnormal basal ganglia + Develop Delay</td>
<td>Severe ocular dysmotility with polyneuropathy</td>
<td>Atypical Moebius Syndrome + Congenital limb contractures; Lower extremity weakness; Sensory neuropathy</td>
<td></td>
</tr>
</tbody>
</table>

Chew, Balasubramanian et al, Brain, 2013

**TUBB3 protein is a critical component of mammalian microtubules**

- **TUBB3** encodes “neuronal” class-3 β-tubulin
- Key component of mammalian microtubules ~20% of neuronal tubulin
- Forms a heterodimer with α-tubulin and tandem repeats of these polarized heterodimers form the building blocks for microtubules

**Microtubules-motor protein interactions**

Mammalian microtubules interacts with kinesin and dyenins- motor proteins to produce motility to transport vesicles/organelles travel along “monorails” provided by the cytoskeleton

**TUBB3 E410K is a dominant negative mutation**

- **TUBB3** E410K mutant β-tubulin
  - forms heterodimers
  - Incorporates into microtubules

**Impaired Vesicular and Mitochondrial Axonal Transport**

Tischfield et al, Cell 2010; News et al, EMBO J. 2013
Summary

- **TUBB3 E410K** mutation exclusively causes a severe syndromic form of human GnRH deficiency
- First evidence of a cytoskeletal protein that impairs axonal transport and growth cone pathfinding specific to GnRH neurons
- May have roles in other hypothalamo-pituitary axes beyond the HPG axis

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Puberty gene discovery guided by murine knockouts: **PROK2/PROKR2**

PROK2/PROKR2 mutations in IGD

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**Insights into regulation of puberty from rare and complex trait genetics**

Balasubramanian et al, Neuroendocrinology 2010;92:81–99

**Genetics of central precocious puberty**

A GPR54 Activating Mutation in a Patient with Central Precocious Puberty

B. INGLI | MD, PhD | WWW.NEJM.ORG | FEBRUARY 14, 2008

Whole exome sequencing

Central Precocious Puberty Caused by Mutations in the Imprinted Genes MKRN3

A. FUCI | J HUM GENETICS | NWJM 0RC | JUNE 27, 2013

**Maternal imprinting of MKRN3: Parent-of-origin effect**

Makorin 3: Novel inhibitor of GnRH secretion

Highly conserved putative ribonucleoprotein with:
- centrally located RING finger motif
- two amino-terminal C3H zinc finger motifs
- conserved Cys–His residues called a Makorin zinc finger motif
- carboxy-terminal C3H zinc finger motif


**HPG Axis across development: Reactivation at puberty**

Adapted from Winter et al. JCEM 1976

Adapted from Stamou et al, Endo Rev 2015 Sep 22:e20151045
Piecing together the ontogeny of GnRH neurons as told by patients

1998
Unknown

2002
Unknown

2008

2015

Piecing together the genetic control of puberty: 40 genetic hits in GnRH deficiency

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Fertility induction in IHH patients
Conventional approach to fertility induction in IHH

- Pulsatile GnRH therapy
  - Labor intensive
  - Mostly in a research environment
- Gonadotropin induction
  - Human chorionic gonadotropin 1500 Units s/c 2-3 times weekly –
    - Target trough Testosterone: lower third of normal range
    - May suffice to induce spermatogenesis in those with baseline TV > 8 ml
    - If azoospermia or severe oligospermia after 6-12 months of therapy, add recombinant FSH: 75-150 Units 3 times a week
    - Monitor testicular volume and semen analysis

Non-responders: Defects beyond the hypothalamus

Testicular descent

Adapted from Winter et al. JCEM 1976

Two waves of Sertoli cell proliferation: Role of FSH

Adapted from Winter et al. JCEM 1976

HPG Axis across development: Reactivation at puberty

Adapted from Winter et al. JCEM 1976
Sequential gonadotropin therapy to optimize response

- Hypothesis:
  - As spermatogenic success, FSH pre-treatment will be superior to conventional therapy

Does genetic basis determine fertility success?

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N (N=46)</th>
<th>N (N=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAL1</td>
<td>14/27</td>
<td>6/15</td>
</tr>
<tr>
<td>CCDC28</td>
<td>19/27</td>
<td>6/15</td>
</tr>
<tr>
<td>NDUFA2</td>
<td>16/27</td>
<td>6/15</td>
</tr>
<tr>
<td>FSHR</td>
<td>17/27</td>
<td>6/15</td>
</tr>
<tr>
<td>KEG11</td>
<td>17/27</td>
<td>6/15</td>
</tr>
<tr>
<td>PROCE2</td>
<td>16/27</td>
<td>6/15</td>
</tr>
</tbody>
</table>

Sykiotis et al., JCEM 2010, 95, 3019-3027

Summary: From N=1 to the population

- The human model of GnRH deficiency has been prismatic to provide insights into:
  - Neuroendocrine regulation of human reproduction
  - Genes and pathways that control puberty in humans
  - These genetic pathways, in addition to their Mendelian phenotypes also contributes to the variation in timing of puberty in the general population

Going from N=1 to cohorts

http://reprogenes.partners.org

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Center for Human Genetics, MGH
Jim Gusella PhD

FSH pre-treatment followed by GnRH pump therapy (open circles)

Vs

GnRH pump therapy (close circles)

Dayer et al., JCEM 2013, 98, E1790-E1795.