Genomics in Pediatric Endocrinology: Genetic Disorders & New Techniques

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Limitation to the Present Presentation

- Not feasible to discuss all of the genetic/endocrinologic disorders within the constraints of the allotted time!

Only some exemplary disorders will be covered.
OUTLINE

- Introduction
  - Endocrinology: Past & Present
- Genetically determined Hypofunctional Endocrine Disorders
- Genetically determined Hyperfunctional Endocrine Disorders
- New Techniques and their Applications
  - Endocrinology: The Future
    - DNA Sequencing
    - Polymerase Chain Reaction
    - Positional Cloning and Linkage Analysis
    - DNA Microarray and Functional Genomics
The Explosion of Information which is occurring in the field of endocrinology is transforming what once was a relatively simple discipline in a discipline that has almost totally changed face.


**“Classical” Era:**

- **Definition** of hormones
- **Description** of the endocrine glands and their hormonal products
- **Description** of the physiologic effects
- Concept of the *target gland*
- Assumption that hormones were secreted from a *single gland* and that they had a *single* or predominant effect
“Neo-Classical” Era:

(Mechanistic Orient. : molecule/function)

- Discovery of more hormones
- Principles of synthesis, storage, secretion, transport
- Hormone assay (radioisotopes)
- Definition of the biochemical actions and of “feedback”
- More liberal definition of the “target gland”
- Introduction of the concept of receptor to understand the specificity/selectivity of hormonal actions
“Endocrinology” based on Physiologic studies
Hormone levels
Simplified concept

Endocrine Disorders

hormone deficiency
hormone excess
hormone resistance
Neoplasia
From the PAST to the Present

“Today”,
(although the classification still holds)
numerous conditions are explained by mutations in genes involved in \{Function, Growth\} endocrine tissue.

Endocrine Disorders

- hormone deficiency
- hormone excess
- hormone resistance
- Neoplasia
Recent advances in \{ Molecular biology, Genetics, Clinical research \} transforming our understanding of molecular mechanisms of human disease.
EVOLUTION of ENDOCRINOLOGY

Contemporary Era: (Molecular Orient.: molecule/function)

- Application of molecular biology to explain the mechanism of hormone action (genic expression)
- Multiple sites of hormone production and action
- First descriptions of the molecular basis of endocrine diseases
- Definition of the roles of oncogenes in the pathogenesis of tumors, including those of endocrine origin
- Large scale production of difficult to obtain hormones
- First descriptions of molecular causes of endocrine diseases
"Explosion" of our knowledge on:
- hormones
- receptors
- intracellular systems of activation

1) A clearer vision of the mechanisms involved in the synthesis and action of hormones

2) Identification of the molecular basis of many endocrine diseases
From the Past to the PRESENT

Genetics \( \xrightarrow{\text{dramatic transformation}} \) Basic investigator \( \xleftarrow{\text{tool}} \) Molecular genetics

Completion of sequencing of human genome

Development of new techniques to study DNA

Diagnosis \( \xrightarrow{\text{Covering all aspects}} \) Treatment

mainstream role in medical practice
Food for Thought

When
does an **endocrine disease**
stop being an endocrine illness
and becomes
a **genetic illness**?

the change
of a **single nucleotide** can induce
multiple and important alterations in
endocrine function
To better understand how the change of even a single nucleotide can induce multiple and important alterations in endocrine function, it is essential to clearly understand some basic concepts:

1) The complex metabolic pathways of hormone synthesis and action

2) The types of hormones and their characteristic cellular receptors.

3) The components of the signals for inter- and intra-cellular communication
1. Hormonal Control & Clinical Manifestations of endocrine illnesses

   a. Hypothalamus
   
   b. Pituitary
   
   c. target gland
2. Classes of Hormones

a. Protein

b. Non-protein
   - Steroid
   - Non-steroid
3. Inter- & Intra-Cellular Mechanisms of Communication

**Signal of Information**

Transmitting information between two cells: Cell “A” to Cell “B”

- **Inter-cellular**
- **Intra-cellular**

**Synthesis**

- **Structural**
- **Enzymatic**
- **Regulatory**

**Basic Concepts**
4. The action of a hormone, as well as its synthesis, is brought about through a cascade of cellular events.

   a. recognition of the “external” stimulus (receptors)

   b. intracellular transmission of the stimulus

   c. gene activation

   d. “hormone” synthesis (specific cellular action)
5. Conclusions

(“target cell”) Any cell in which a hormone binds to its receptor

b. Reversible and high affinity binding to protein receptors

☐ Cellular Membrane
☐ Cytoplasm
☐ Nucleus
Basic Concepts

Keep in mind!

1. Number of known hormones \( \approx 50 \)
2. Number of differentiated cells \( \approx 200 \)
3. Number of cells that respond to hormones ("target cell") \( \approx 75 \times 10^{12} \)
The pediatrician must be placed in the position to be able to recognize the existence of a complex problem that could need an evaluation from a specialist in pediatric endocrinology.

Today, the “pediatrician” can no longer pretend to hold dear those “primordial” concepts which were at the heart of the “classical” era of endocrinology.
Regardless of which of the situations exists, the result is an inability to synthesize a functional peptide and the patient will present with clinical characteristics of a hormone deficiency state.
Intracellular Membrane

**Cellular Localization and Class of Hormone**

- **Intracellular**
  - Androgens
  - Estrogens
  - Glucocorticoids
  - Iodotyronines
  - Mineralcorticoids
  - Vitamin D

- **Membrane**
  - Protein hormones
  - Neurotransmitters *(monoamine type)*
  - Prostaglandins
  - Growth Factors *(EGF; FGF; PDGF; NGF, ecc)*
### General Characteristics of Hormones on the basis of the Cellular Localization of their Receptors

<table>
<thead>
<tr>
<th>Characteristics of the Hormone</th>
<th>Localization of the Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class of Hormone</td>
<td>Intracellular</td>
</tr>
<tr>
<td></td>
<td>Membrane</td>
</tr>
<tr>
<td>Solubility</td>
<td>Lipophilic</td>
</tr>
<tr>
<td></td>
<td>Hydrophilic</td>
</tr>
<tr>
<td>Plasma half-life</td>
<td>Long (hrs, days)</td>
</tr>
<tr>
<td></td>
<td>Short (minutes)</td>
</tr>
<tr>
<td>2° messenger</td>
<td>the &quot;Hormone-Receptor&quot; complex</td>
</tr>
<tr>
<td></td>
<td>Ca^{++}</td>
</tr>
<tr>
<td></td>
<td>cAMP, phosphatidylinositol</td>
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</tbody>
</table>

G - Proteins
G - Proteins

(Heterotrimeric Proteins which bind a guanine nucleotide)

1. Discovered » 30 years ago
2. In the last 10 yrs marked increase information on
   a. Structure
   b. Function
   c. Dysfunction as a cause of disease in man
HORMONE + NH₂

COOH

Promoter

Codifying sequencing

Transcription

Translation (Hormone Synthesis)

Activation of G-Protein

GTP

GDP

ADENYL CYCLASE

HORMONE (Shankar RR, Pescovitz OH: Adv Endocrinol Metab 1995;6:55)
Endocrine Disorders

MUTATIONS

in

G - Proteins

Receptors coupled to G - Proteins

NH\textsubscript{2}

COOH

GDP
CLASSIFICATION OF MUTATIONS OF G-PROTEINS

<table>
<thead>
<tr>
<th>GENES</th>
<th>MUTATION</th>
<th>EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha</td>
<td>Gain of Function</td>
<td>Activation of Intracellular signals (in absence of Ligand)</td>
</tr>
<tr>
<td>beta</td>
<td>Loss of Function</td>
<td>Resistance to the action of the hormone</td>
</tr>
<tr>
<td>gamma</td>
<td></td>
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</tr>
</tbody>
</table>

GDP
Activation Inactivation

MUTATIONS

G - Proteins

Activation

Inactivation

of the corresponding pathway for signal transduction

Hyper-

CLINICAL SYMPTOMS

Hypo-
Examples of Mutations

- Loss of Function
  + Hypo-secretion
Pseudo-Hypo-Parathyroidism

1. Typical example of a mutation with loss of function (inactivation)

2. First pathology recognized to be due to Resistance to a hormone

3. Characterized by:
   a. normal renal function
   b. hypocalcemia
   c. hyperphosphatemia
   d. increased PTH levels
Pseudo-Hypo-Parathyroidism

Other characteristic signs:

- % with papilledema: 18%
- % with subcut. soft tissue calcifications: 60%
- % with mental retardation: 63%
- % with round face, short stat. (obesity): 50–75%
- % with short metacarpals (stubby fingers): 50–75%
Pseudo-Hypo-Parathyroidism

Laboratory findings

- Ca: low
- P: high
- PTH: high
- Alk P'ase: low/high
- Mg: nl

Diagnostic triad
PHP Type I

No increase in cAMP levels

Defect in the Transduction of the signal before the generation of the 2° messenger

PHP Type Ia

Mutations (in the germinal cell line) of the gene that encodes the alpha subunit causing a decrease (loss) of its function

Resistance to: PTH; TSH; LH; FSH
The most common mutation in the $\text{Gs}\alpha$ gene with loss of function identified in non-related families with AHO is:

deletion of 4 base pairs in exon 7
Pseudo-Hypo-Parathyroidism

PHP Type Ia with precocious puberty (PP)
( Gonadotropin-independent PP )

A single mutation in \( Gs_\alpha \)
rendering the \( G \)-protein temperature sensitive

At normal body temperature (37\(^\circ\) C),
the \( Gs_\alpha \) is degraded,
resulting in PHP

in the cooler temperature of
the testes (33\(^\circ\) C),
the \( Gs_\alpha \) results in constitutive activation of the LH receptor
causing PP
Examples of Mutations

\[
\begin{align*}
\text{Loss of Function} & \quad \text{Gain of Function} \\
\quad + & \quad + \\
\text{Hypo-secretion} & \quad \text{Hyper-secretion} \\
\end{align*}
\]

- Pseudo-Hypo-Parathyroidism
- Temperature Sensitive
- Precocious Puberty
1) Found in ~ 20% of patients with Hypogonadotropin Hypog.

2) Most mutations are compound heterozygous changes that reduce:
   a) GnRH binding and/or
   b) Activation of signaling pathways
      ① Inositol tri-phosphate (or)
      ② Phospholipase-C
c) Mutations in the GnRH receptor

- Q106R/S217R
- Q106R/R262Q
- A129D/R262Q
- R262Q/Y284C

**Pathways:**
- Inositol Triphosphate
- Phospholipase C
- A129D (complete loss of function)
- S168R (complete loss of R-mediated signaling)
GnRH – Receptor Mutations

3) Clinical features are highly variable (spectrum)
   a) **Complete** loss of Function (A129D or S168R)
      ① micropenis
      ② cryptorchidism
      ③ Complete failure of pubertal development
      ④ Resistance to pulsatile GnRH treatment

   b) **Mild** loss of Function
      ① Partial GnRH resistance
      ② Basal Gn’s detectable
      ③ Modest Gn response after GnRH stimulation
      ④ Incomplete pubertal development
Examples of Mutations

Loss of Function + Hypo-secretion

Gain of Function + Hyper-secretion
Mutation which leads to an Increase in Function of the G–Protein

Mutation in position 201 or 227 of the gene for the alpha subunit reduces the intrinsic activity of the hydrolysis of GTP, resulting in continuous stimulation.
1. Typical example of a mutation which leads to increase in function (activation)

2. Characterized by the clinical triad:

- Cafe-au-lait skin lesion
- Polyostotic fibrous dysplasia
- "Precocious puberty"
McCune Albright Syndrome

G-Protein:
Mutation in position 201
(histidine / cystine for arginine)

Translation (Hormone Synthesis)

Transcription
Codifying Sequencing
Promoter

ADENYL CYCLASE
ATP → cAMP

GTP → GDP
GDP → GDP
GDP
GTP
γ
β
α
Necessary Criteria to Evaluate when to Suspect an Endocrine Disorder due to a Mutation of the G – Protein or of the Coupled Receptors of G – Proteins

<table>
<thead>
<tr>
<th>Clinical Signs and Symptoms</th>
<th>Loss of function</th>
<th>Gain of function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypo-function</td>
<td>Hyper-function</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode of Transmission</th>
<th>Recessive (homozygotic)</th>
<th>Dom./Sporad. (heterozygotic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/High</td>
<td>Low/Absent</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Circulating level of the hormone</th>
<th>Normal/not reduced</th>
<th>Normal/not increased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/High</td>
<td>Low/Absent</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Biologic Activity of the endogenous hormone</th>
<th>Normal</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/not reduced</td>
<td>Normal/not increased</td>
<td></td>
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</tbody>
</table>

<table>
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<tr>
<th>Activity of exogenous hormone</th>
<th>Non-responsive</th>
<th>--------</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Associated Autoimmune Diseases</th>
<th>Absent</th>
<th>Absent</th>
</tr>
</thead>
</table>
Examples of Mutations

- **Loss of Function** + **Hypo-secretion**
- **Gain of Function** + **Hyper-secretion**
- **Loss of Function** + **Hyper-secretion**
HYPOGLYCEMIA from HYPERINSULINISM

(Persistent Hyperinsulinemic Hypoglycemia of Infancy - PHHI)
Presence of Urinary Ketones?

- **YES**: "Ketotic" Hypoglycemia
- **NO**: Hyperinsulinemia (Carnetine deficiency)
Hyperinsulinemia

Increased “tissue” growth

LGA baby

Visceromegaly

Increased “body” growth

(Gigantism)

Macroglossia

Beckwith – Wiedemann Syndrome
1. **Characteristics:**
   a. Omphalocele
   b. Macroglossia
   c. Macrosomia (Gigantism, visceromegaly)
   d. Hypoglycemia
   e. Characteristic linear ear lobe crease

2. **Etiology:**
   □ anomaly of locus 11p15.5
HYPOGLYCEMIA from HYPERINSULINISM
(Persistent Hyperinsulinemic Hypoglycemia of Infancy - PHHI)

An Effective Medical Treatment requires a global understanding of the cellular pathophysiology of Persistent Hyperinsulinemic Hypoglycemia of Infancy (PHHI) which was missing up to recently.
HYPOGLYCEMIA due to HYPERINSULINISM

Classification of Medical Therapy

- Mobilization of Glycogen
  *Glucagon*

- Intracellular generation of ATP

- Opening of the $K^+$ channel

- Closure of the $Ca^{++}$ channel
The β-cell contains an ATP-sensitive $K^+$ (K$_{ATP}$) channel, which consists of an alpha subunit (Kir 6.2) and a beta subunit (SUR1). A mutation in one of the two subunits of the ATP-sensitive $K^+$ (K$_{ATP}$) channels of the β-cell acts as a receptor for sulfonylureas.
Glucose → G - 6 - P → depolarization

Insulin

K⁺ → ATP → ADP

Ca⁺⁺ → exocytosis → Insulin → HYPOGLYCEMIA

(Kane C. et al: J Clin Invest 1997;100:1888)
HYPOGLYCEMIA due to HYPERINSULINISM

Classification of Medical Therapy

- Mobilization of Glycogen
  - Glucagon

- Intracellular generation of ATP
  - Leucine–free diet + Diazoxide
  - Dichloroacetate

- Opening of the K⁺ channel
  - Diazoxide
  - Somatostatin
Glucose

$\text{G - 6 - P}$

$\text{K}^+\rightarrow\beta\text{-cell}$

$\text{ATP}\rightarrow\text{ADP}$

$\text{Ca}^{++}\rightarrow\text{Insulin}$

Diazoxide
Somatostatin

Repolarization

(Kane C. et al: J Clin Invest 1997;100:1888)
HYPOGLYCEMIA due to HYPERINSULINISM

Classification of Medical Therapy

- **Mobilization of Glycogen**
  
  *Glucagon*

- **Intracellular generation of ATP**
  
  *Leucine–free diet* + *Diazoxide*
  
  *Dichloroacetate*

- **Opening of the K⁺ channel**
  
  *Diazoxide* + *thiazides*
  
  *Somatostatin*

- **Closure of the Ca⁺⁺ channel**
  
  *Niphedipine*
Glucose → G - 6 - P → depolarization → K⁺ → ATP → ADP → Insulin → Ca²⁺ → Niphedipine

Diagnostic Approach

Endocrine Pathology on a GENETIC basis

Absence/Altered  Defective  Altered/Absent  Altered/Absent

Gene  Hormone  Receptor  intracellular Effect (2° messenger)

Pit-1  GH  GH  PTH  Pseudo-Hypo-P
CAH  LHβ  Gn  Gn  McCune Albright
Cong H  FSHβ  ADH  ADH  Diab.Insip.
      TSHβ  ACTH  GH  Acromegaly
      AMH  Insulin  Thyroid
      Insulin  Androgens

Gene Hormone Receptor
intracellular
Effect (2° messenger)

Pit-1  GH  GH  PTH  Pseudo-Hypo-P
CAH  LHβ  Gn  Gn  McCune Albright
Cong H  FSHβ  ADH  ADH  Diab.Insip.
      TSHβ  ACTH  GH  Acromegaly
      AMH  Insulin  Thyroid
      Insulin  Androgens
      Insulin

Laron Syndrome  Resistant Ovarian S.  Nephrogenic D.  I.  Familial Deficit of gluco.  Leprecaunism & others  Refetoff Synd.  Androg Insens S.  Vit D Resist Rickets

PTH  Gp  Pseudo-Hypo-P
Gn  McCune Albright
ADH  Diab.Insip.
GH  Acromegaly

Pseudo-Hypo-P  McCune Albright  Diab.Insip.  Acromegaly
Distinctive Characteristics of an Endocrine Disease due to "Loss of Function" on the basis of the molecular defect

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Lack of Hormone Recognition</th>
<th>Receptor Pathology</th>
<th>Pathology of 2° Messenger</th>
</tr>
</thead>
<tbody>
<tr>
<td>absence of Gene</td>
<td>Hypofunction</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Defective Hormone</td>
<td>Hypofunction</td>
<td>Normal (no reduced)</td>
<td>Normal (not reduced)</td>
</tr>
</tbody>
</table>

Clin Signs / Sympt

Level of circulating hormone

Biologic Activity of the endogenous hormone

Biologic Activity of exogenous horm.

Autoimmune Disease

<table>
<thead>
<tr>
<th>Low</th>
<th>Normal/High</th>
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<tbody>
<tr>
<td>Low</td>
<td>Normal/High</td>
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<tr>
<td>Absent</td>
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<td>Absent</td>
<td>Absent</td>
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<td>Absent</td>
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</table>
Polymerase Chain Reaction:

1. Allows rapid logarithmic amplification of targeted DNA sequences, obtaining in a few hours up to a million copies of the sequence to study.
2. Important application: identifying a specific sequence of DNA in a biologic sample.

   Medical
   Infectious disease
   Forensic
   Research

DNA Sequencing:

1. Identification of mutations.
2. Knowing gene sequence is important but it is of fundamental importance also to know its organization in the context of the additional information contained in the surrounding DNA, which regulate gene expression.
New Techniques & Their Applications

- **Positional Cloning and Linkage analysis**:  
  1. Identification of a gene for a specific phenotype with only its approximate chromosomal location known ("candidate region")  
  2. Identification of polymorphisms (with identification of the carrier state of mutations for a variety of disorders)

- **DNA Microarray and Functional Genomics**:  
  1. DNA microarrays and SAGE allow the simultaneous testing of the expression of thousands of genes and allows one to automatically identify the gene of interest  
  2. Major impact of this technology is in the gene analysis of tumors which have led to the discovery of genes involved in pathogenesis of pituitary and adrenal tumors  
  3. Functional genomics: capability to understand the function of each gene, its product, the regulation of its expression and tissue specificity in order to understand in a comprehensive and integrated manner the structure and function of "people"
Expansion of Internet has allowed storage, analysis, and exchange of information worldwide, rapidly available for consultation to all scientists.
Databases Available On-Line

Containing information on:

- Genetic defects
- Allelic variants
- Modes of transmission
- Clinical features

- Genomics and its impact on medicine and Society

- Gene Map

- Gene Tests
Databases Available On-Line

- National Organizations for Rare Disorders
  - http://www.rarediseases.org

- National Human Genome Research Institute
  - http://www.genome.gov

- Online Mendelian Inheritance in Man
  - http://www.oncbi.nlm.nhi.gov/Omim

- Policy Statements from the American College of Medical Genetics
  - http://www.faseb.org/genetics/acmg/polmenu.htm

- Policy Statements from the American Academy of Pediatrics
  - http://www.aap.org/policy/pprgtoc.cfm
EVOLUTION OF ENDOCRINOLOGY

FUTURE:  ?  (cure/prevention)

- Precise description of the mechanism of action of hormones
- Hormones “made to order” to alter specific processes
- Detailed understanding of the etiology of endocrine illnesses
- Medicines “made to order” to treat specific disorders
- Rational Approach to the prevention of endocrine illnesses
The expanding knowledge in the field of **Molecular Biology**
is attributing the correct importance to what should be considered an **holistic** vision of medicine.
Instead of the traditional **disciplinary** vision.

When

*does an endocrine disease stop being and endocrine illness and becomes a genetic illness?*
Conclusions
CONCLUSIONS

- New discoveries in genetics on a daily basis
  Continuous changes in the way medicine has been practiced up to now

- New molecular technologies and acquisition of increased information from the human genome
  Rapidly advancing knowledge of the cause of disease
  Elucidating the molecular and genetic pathways that regulate normal physiological endocrine processes

Sum of this information will allow

- Accurate diagnosis of endocrine disorders
- Novel, molecular-oriented pharmacologic therapies to treat them
Although all of these advances are scientifically directed to improve the “quality of life”, the powerful nature of the continuously developing new molecular technologies in uncovering every aspect of past, present, and future health problems will raise important ethical implications that also need to be addressed.
Thank you for your Attention