Pituitary Transcription Factors

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Anterior Pituitary Development

Rathke’s Pouch

- PAX-5
- ISL-1
- BRN-4
- T/PIT
- NEUROD1
- LIF
- T/EBP
- BMP4
- WNT5A
- PTX1,2
- SIX3
- PROP-1
- SF-1
- GATA2
- DAX-1

α-subunit

- LHX-3, 4
- SOX2
- OTX2
- RPX (HESX1)

rostral caudal

- TEF
- POU1F1 (PIT1)

caudal

- ZN-15

GATA2

DAX-1

ISL-1

BRN-4

PTX1,2

SIX3

PROP-1

SF-1

TEF

POU1F1 (PIT1)

GH

Prl

TSH

LH/FSH

ACTH

Congenital Hypopituitarism

- Hypopituitarism is not rare:
  - incidence rate 4.2 cases per 100,000
  - prevalence rate of 45.5 per 100,000

- Transcription factor mutations are not uncommon:
  - 13.5% of 195 patients diagnosed with pituitary hormone deficiency had a genetic mutations in the 5 analyzed factors. The prevalence increased to 52.4% when 21 familial cases were considered.

Management of Congenital Hypopituitarism

• Who should have genetic screening?
• How should they be screened?
• When should they be screened?
• Which patients should be monitored for evolving hypopituitarism?
• How should patients with hypopituitarism be monitored?
Anterior Pituitary Development

- **Rathke’s Pouch**
  - PAX-5
  - ISL-1
  - BRN-4
  - T/PIT
  - NEUROD1
  - LIF

- **T/EBA**
- **BMP4**
- **WNT5A**

- **SOX2**
- **OTX2**
- **RPX (HESX1)**
- **PTX1,2**
- **SIX3**

- **PROP1**
  - **POU1F1 (PIT1)**
  - **ZN-15**

- **α-subunit**
- **TEF**

- **LH/FSH**
  - **NEUROD1**
  - **LIF**
  - **SF-1**
  - **GATA2**
  - **DAX-1**

- **ACTH**

- **TSH**

- **Prl**

- **GH**
POU1F1 (Pit-1)

• General
  - First cloned pituitary transcription factor
  - Member of the POU homeobox protein family
  - Contains a POU-specific and -homeobox domain required for DNA-binding and an activation domain in the N-terminus

• Clinical
  - CPHD (deficiency of GH, Prl, TSH)
  - Inherited as autosomal recessive or dominant

Cohen LE and Radovick S. Endocr Rev. 2002;23(4):431-42
POU1F1 Function

Development
POU1F1 gene

POU1F1
Prl gene
GH gene
TSH-β gene

Retinoids

Gene Activation and Regulation
POU1F1 DNA-Binding Sites

GH

Prl

TSH-β

POU1F1

distal

proximal
SOME OBSERVATIONS ON CRETINISM AND ITS TREATMENT*

DANIEL FEDERMAN, M.D.,† JACOB ROBBINS, M.D.,‡ J. E. RALL, M.D.§

BETHESDA, MARYLAND

THE present paper is a report of findings in a group of cretins who have been under observation at the National Institute of Arthritis and Metabolic Diseases. The patients, gathered within the course of a year, suggest that the condition is far from disappearing, and their histories reaffirm that tardy recognition remains a major problem of the syndrome.

The studies reported include those related to bone growth, cerebral activity and thyroid function. Seven of the 12 patients have been treated with L-thyroxine, and the results are reported below.

CLINICAL FINDINGS

The patients studied, 5 males and 7 females, ranged in age at the time of admission from four months to thirty-eight years. In all patients but 1, thyroid substitution had been allowed to lapse or was discontinued for at least three weeks before study.

The pregnancy of generation in each case was reported as normal, and there was a history of familial thyroid disease in only 2 subjects, who were nine and thirteen years of age and who were siblings. They were both athyreotic cretins and the only children of parents with no other history of thyroid disease. A suggestion that there might be a familial factor in

 athyreotic cretinism has been noted previously.¹ ² ³

None of the children were born or brought up in an area of frequent goiter. Ten of the patients were thought to be normal at birth. Of the other 2, 1 (K.S.) was noted to be quite yellow and had an umbilical hernia. The other (S.G.) had acute respiratory distress because of a large mass in the neck that was thought to be a goiter. A tracheostomy was considered, but the infant did well when kept in a sitting position and given oxygen, and no surgery was performed.

Indication of abnormality occurred within the first three months of life in 8 of the patients. There was a striking uniformity of symptoms, inactivity, marked reduction of food intake, weak sucking, failure to gain weight and constipation being reported in virtually all cases. One infant (S.G.) was admitted to another hospital at five weeks of age because of diarrhea, despite which her appearance of distention and apathy suggested the diagnosis of cretinism. Two children had repeated respiratory infections as early symptoms.

PREVIOUS TREATMENT

In all subjects desiccated thyroid had been used as thyroid replacement. In 8 cases treatment had been interrupted because of uncertainty of diagnosis (R.R.), parental laxity (J.L.) or signs interpreted as toxicity (T.R.). From the standpoint of dosage, 5 of the subjects received 0.12 gm. (2 gr.) or more of desiccated thyroid per day, which might be considered inadequate (Table 1). The remaining 4 received by any criteria inadequate dosage. From the standpoint

Fig. 1. Patient W.T.R., age 10 4/12 years.
Congenital hypothyroidism in a young man with growth hormone, thyrotropin, and prolactin deficiencies

A growth-retarded, mentally deficient young man is described with diminished secondary response of growth hormone, thyrotropin, and prolactin to the pharmacologic stimuli of insulin, arginine, chlorpromazine, and thyrotropin-releasing hormone. Growth hormone and ACTH functions were normal both basal and upon pharmacologic stimulation. Additionally, the patient was unresponsive to exogenous thyrotropin injections. These data suggest that the hypothyroidism in this patient was due to combined thyroid dysgenesis and pituitary insufficiency, i.e., primary and secondary hypothyroidism.

Alan D. Rogol, M.D., Ph.D.,* and C. Ronald Kahn, M.D., Bethesda, Md.

Hypothyroidism may be subdivided according to the level at which the hypothalamic-pituitary-thyroid axis is altered: in primary hypothyroidism there is a disturbance of the thyroid gland, and in secondary hypothyroidism there is a disturbance of production or secretion of TSH by the pituitary. Secondary hypothyroidism itself can be subdivided into pituitary TSH deficiency, in which the pituitary fails to respond to thyrotropin-releasing hormone, and hypothalamic hypothyroidism in which there is a presumed disturbance of production or secretion of TRH. These usually may be distinguished by measurements of plasma TSH, response of the pituitary to exogenous TRH, and response of the thyroid to exogenous TSH.

The present report is of a patient who presented with athyreotic cretinism with severe growth retardation. Growth response to therapy with thyroid hormone was poor, although bone age rapidly matured. On re-evaluation 15 years later the patient was found to have, in addition to thyroid dysgenesis, deficiencies of TSH, prolactin, and growth hormone. Thus this patient presents both primary and secondary hypothyroidism. Furthermore, in contrast to previous studies† of patients with

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GH- and TSH-deficient dwarfism, the lack of response to TRH in this patient suggests a pituitary rather than a hypothalamic origin of the TSH deficiency.

<table>
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<th>Abbreviations used</th>
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<tr>
<td>TSH: thyroid-stimulating hormone</td>
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<td>TRH: thyrotropin-releasing hormone</td>
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<td>GH: growth hormone</td>
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<td>HPL: human prolactin</td>
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<td>MUU: mouse uterine units</td>
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<tr>
<td>HGH: human growth hormone</td>
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<td>TRIG: testosterone-binding globulin</td>
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<td>LH-RH: luteinizing hormone-releasing hormone</td>
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CASE REPORT

Patient W. T. R., a 1,000 gms, full-term infant, was born May 16, 1945; pregnancy, labor, and delivery were uncomplicated. The mother was a 25-year-old, gravida 3, para 2 woman.

Slow development was first noted at 8 months when he was unable to hold his head against gravity. Evaluation at 11/12 years revealed an enlarged "hydrocephalic-appearing" head and marked delay in growth and development. Bilateral subdural aspirations yielded only clear fluid. At 11 1/2 years, a nondepressed linear fracture of the pelvic bone was noted. Urinalyses, glucose tolerance test, intravenous pyelogram, STS, nonprotein nitrogen, serum alkaline phosphatase, calcium, and phosphorus concentrations were within normal limits. The serum concentration of cholesterol was 187 mg/dl.

W. T. R. History

• 51 years old
• Severe mental retardation
• Short stature
• Undetectable GH
• Undetectable TSH
• Low prolactin
• Normal cortisol response to insulin
• Normal gonadotropin response to GnRH
Human POU1F1 Mutations

POU-specific

- ++
- K145X
- A158P
- R172X
- W193R
- E174G

POU-homeo

- ++
- E250X
- P239S
- K216E
- V272X
- R271W (11)
- R271A

OH

- N
- Q4X
- P24L
- F135C
- R143Q
- R172X
- K145X
- A158P
- W193R
- E174G

C

- C
- V272X
A Mutation in the POU-Homeodomain of Pit-1 Responsible for Combined Pituitary Hormone Deficiency

Sally Radovick, * Michelle Nations, Yuefen Du, LaVonne A. Berg, Bruce D. Weintraub, Fredric E. Wondisford†

Pit-1 is a pituitary-specific transcription factor responsible for pituitary development and hormone expression in mammals. Mutations in the gene encoding Pit-1 have been found in two dwarf mouse strains displaying hypoplasia of growth hormone, prolactin, and thyroid-stimulating hormone-secretion. In the anterior pituitary, a point mutation in this gene identified on one allele in a patient with combined pituitary hormone deficiency. Mutant Pit-1 binds DNA normally but acts as a dominant inhibitor of Pit 1 action in the pituitary.

The patient (W.T.R.) studied in this report was previously documented to have a deficiency of GH, PRL, and TSH, which was manifested as severe growth retardation and short stature (9). Both GH and TSH were undetectable in the plasma before or after provocative stimuli. PRL in the plasma was low (1 µg per liter) and did not respond to stimulation by clonidine or TRH. Baseline gonadotropin amounts, however, were normal (stimulating hormone (LH), 15 IU per liter, and follicle-stimulating hormone, 7 IU per liter) and responded appropriately to stimulation by gonadotropin-releasing hormone. Serum cortisol levels were normal (8 a.m., 376 nmol per liter; and 4 p.m., 190 nmol per liter); steroid precursors increased appropriately after menses. The patient's mother (E.R.) is of normal stature and has normal pituitary hormone levels. Unfortunately, the remaining family members were inaccessible for study; but, according to historical reports, they all have normal stature, which suggests that this patient may represent a sporadic case of combined pituitary hormone deficiency (CPHD).

The polymerase chain reaction (PCR) was used to amplify genomic DNA fragments from human Pit-1 (10). The Pit-1-specific and POU-homeodomain were amplified separately by means of specific oligonucleotides (11). The Pit-1 domains amplified in this study correspond to exons 4 through 6 of the mouse Pit-1 gene (9) and contain most of the Pit-1-specific and the entire POU-home domain. As a control in these experiments, genomic DNA from three normal patients was also included. We found no abnormalities in the Pit-1-specific domain after sequencing at least seven independent clones from two separate PCR amplifications from either the patient or normal controls. A C to T mutation of codon 271, however, was found in approximately one-half of independent clones we sequenced from the patient (three of seven cloned fragments); the

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Human POU1F1 Mutations

- W261C (Snell mouse)
- R271W
- R271A
- K216E
- E250X
- P239S
- F135C
- A158P
- E174G
- R172X

Model of Dominant Negative Effects of Pit-1 Mutants
M. R. History

- Birth weight 2.9 kg, 36 weeks gestation
- “Prematurity” jaundice, normal neonatal screen
- Microphallus
- High-arched palate
- 2 months, CT normal
- 6 months, fall off in length and weight
  - GH provocative testing (GH max 1.4 µg/l)
  - NI TFTs
- 22 months:
  - TSH deficiency: T4 39nmol/l (58-193); TRH test (TSH max 1.7mU/l, basal Prl 5.4µg/l, no increase), MRI small pituitary
Human POU1F1 Mutations

- **POU-specific:**

- **POU-homeo:**
Human POU1F1 Mutations

- R172X
- A158P
- E174G
- F135C
- R143Q
- K216E
- E250X
- P239S
- W261C (Snell mouse)
- R271W
- R271A
R271W Does Not Dimerize
K216E Forms a Strong Dimer

CTATACATTATATTCATG
GATATGAATAAAGTAC

R217W
K216E

CTATACATTATATTCATG
GATATGAATAAAGTAC

H D S
H D S

R271W Does Not Dimerize
K216E Forms a Strong Dimer
How does K216E cause hypopituitarism?
Defective Transactivation by a Mutant Pit-1/RAR Complex

[Diagram showing the interaction of RA with the Distal Pit-1 enhancer and the formation of the Pit-1/RAR complex.]

- RA induces the formation of the Pit-1/RAR complex on the Distal Pit-1 enhancer.
- A mutant Pit-1/RAR complex results in defective transactivation.

[Diagram showing the expression of GH, PRL, and TSH-β in response to Pit-1 complex interaction.]

- Pit-1 complex activates the expression of GH, PRL, and TSH-β.
- Mutant Pit-1 complex shows a reduced ability to activate these genes.

Additional notes:
- Distal Pit-1 enhancer is crucial for gene expression regulation.
- RA (Retinoic Acid) plays a role in inducing the formation of the regulatory complex.
Effect of K216E on Somatotroph Function

Hypothalamus

- GHRH
- SRIF

SOMATOTROPHE

- Gs
- Gi
- cAMP
- PKA
- CBP

CBP

- K216E
- Wt

GH

Effect of K216E on Somatotroph Function
Anterior Pituitary Development

Rathke's Pouch

- SOX2
- OTX2
- RPX (HESX1)
- PTX1, 2
- SIX3

α-subunit

- LHX-3, 4
- (PROP-1)
- SF-1
- GATA2
- DAX-1

- TEF

- rostral
- caudal

- T/PIT NEUROD1
- LIF

- POU1F1 (PIT1)

- ZN-15

- T/EBP
- BMP4
- WNT5A

- PAX-5
- ISL-1
- BRN-4

- ACTH

- LH/FSH

- TSH

- Prl

- GH
RPX (Rathke’s pouch homeobox) or HESX 1

- Earliest known pituitary gland marker and restricted to Rathke’s pouch
- Prop-1 represses Rpx expression later in development
- Septo-optic dysplasia with absence of optic nerves, corpus callosum and panhypopituitarism
- Heterozygous or homozygous mutation with GH, TSH, prl and LH deficiencies; ACTH deficiency reported

**Diagram:**
- Q6H
- I26T
- R53C
- 103 insAG
- Homeodomain
- R160C
- S170L
- T181A
- 185
- 184delG
- 103 insAG
- TLE1-interacting
J. R.

• 6 months: dx SOD, blindness and nystagmus
  • MRI: absent corpus callosum, thin optic nerves, small anterior pituitary, absence of posterior bright spot
• 3 6/12 years: ht 98.2cm (-.014SDS) wt 16.8kg (+.68SDS)
  • IGF-I 50ng.ml (17-28), BA 4 years. TFTs nl, no DI, ACTH stim (5 to 21.7µg/dl)
• 4 9/12 years: decreasing growth velocity (2.2cm/yr)
  • IGF-1 22 ng/ml, TFTs nl, TRH test nl, Insulin stim: GH 4.2 µg/l, cortisol 20.8µg/dl.
• 6 years: TSH 0.13mU/l, T4 4.4 µg/dl, (6-12.3)
175 176 177 178 179 180 181 182 183 184 185      Codon

GCG  AAA  AAA  AAT  TTC  AAC  ACA  AAT  CTG  CTG  GAA        Wt
Ala  Lys  Lys  Asn  Phe  Asn  Thr  Asn  Leu  Leu  Glu

GCA  AAA  AAA  ATT  TCA  ACA  CAA  ATC  TGC  TGG  AAT  AGA  1684delG
Ala  Lys  Ile  Ser  Thr  Gln  Ile  Cys  Trp  Asn  Arg
Abnormal timing of PROP1-dependent Pituitary Program

Pattern of HESX1 and PROP1 expression during pituitary development

Stimulation of gene expression

PROP1-Dependent Cell Lineages

Abnormal Pituitary Development

Pattern of HESX1 and PROP1 expression during pituitary development

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Pattern of HESX1 and PROP1 expression during pituitary development

Stimulation of gene expression

PROP1-Dependent Cell Lineages

Abnormal Pituitary Development
Combined Pituitary Hormone Deficiency (CPHD)

• Hormone deficiencies can evolve in patients with mutations in pituitary developmental factors

• Can be vertically transmitted
Can evolve in patients with mutations in pituitary developmental factors

Can be vertically transmitted
S.K.

- 20y/o post partum, unable to lactate
- Newborn with hypoglycemia and jaundice
- Hypothyroidism noted at 6 months of age and treated with levothyroxine
- Poor growth: diagnosed with GH deficiency at 16 months (0.9ng/ml post clonidine) and treated with GH until 16yrs
- Normal puberty
- Normal pregnancy, TFTs monitored

Pine-Twaddell E, et.al. in preparation.
S.K. TFTs during pregnancy

• 13 weeks:
  - TSH 0.058 μIU/ml, T4 7.8 μg/dL (4.5-12.0), FT4 0.8 ng/dL (0.61-1.76)

• 17 weeks:
  - TSH 0.067 μIU/ml, decreased levothyroxine

• 21 weeks:
  - FT4 0.44 ng/dl (0.61-1.76)

Pine-Twaddell E, et.al. in preparation.
J.A.K. – son of S.K.

• 36 week newborn
• Neonatal hypoglycemia
• Undetectable TSH
• Undetectable GH
• Normal cortisol
• Normal gonadotropins
• FMHx: mother treated with T4 and previously with GH
J.A.K. Interval History

Age: 9 months

• Normal brain MRI
• Bilateral hearing loss
• Developmental delay
• Continues on levothyroxine, begun on GH
J.A.K. and S.K. Pit-1 Mutation

Vertical transmission of a dominant negative POU1-F1 gene mutation with newborn sequelae.
Management of Congenital Hypopituitarism

• Who should be screened? How should they be screened?
  • All patients with congenital hypopituitarism should have a genetic evaluation; especially those with a family history and/or severe short stature.

• When should they be screened?
  • Genetic screening should occur after the clinical diagnosis of hypopituitarism.

• Which patients should be monitored for evolving hypopituitarism?
  • All patients with congenital hypopituitarism should have continued monitoring of pituitary function.
Management of Congenital Hypopituitarism

• How should patients with hypopituitarism be monitored?

The phenotype for patients with some genetic forms of CPHD is evolving and unpredictable.

These patients require lifelong follow-up.
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