Transcription factors continue to be of major interest in the field of pituitary research. Interestingly, emphasis is not anymore just on the role of transcription factors in pituitary development, but also on their role in tumorigenesis. For example, we have selected a paper suggesting Pit-1 is involved in breast cancer and a paper on the gene NUMB, which is known to be involved in cancer development, and is now shown to play a role in pituitary homeostasis. Aryl hydrocarbon-interacting protein (AIP) is involved in the development of familial isolated pituitary adenomas and a beautiful paper of Chahal et al. tracks an AIP mutation back to a common ancestor that lived in Ireland approximately 66 generations ago, using DNA from a skeleton of an Irish giant exhibited in a museum. Clinically important papers relate to the treatment of prolactinomas, albeit in adults, and to diagnostic outcome in a huge series of patients undergoing pituitary MRIs for sellar/parasellar tumors. We chose the involvement of microRNA in pituitary development as the mechanism of the year. This work shows that deletion of microRNA in the pituitary results in abnormal pituitary development and dwarfism, adding a new dimension to the genetic regulation of growth. Other developments include the presence of PIT-1 antibodies as a cause for combined TSH, GH and prolactin deficiency. The ‘Food for thought’ section includes a paper suggesting an important role for pituitary adenylate cyclase-activating peptide (PACAP) in post-traumatic stress disorder.

**Mechanism of the year – microRNA**

MicroRNAs regulate pituitary development, and microRNA 26b specifically targets lymphoid enhancer factor 1 (Lef-1), which modulates pituitary transcription factor 1 (Pit-1) expression

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*Background:* The authors hypothesized that microRNAs (miRNAs) play a role in pituitary development. 

*Methods:* MicroRNA was isolated from pituitary stem cell lines and hybridized on miRNA arrays to identify pituitary-specific miRNAs. *Dicer1*, an endonuclease essential for miRNA maturation, was conditionally knocked out using the *Pitx2-Cre* mouse, resulting in the loss of mature miRNAs in the anterior pituitary. ChIP assays were used to identify binding partners for lymphoid-enhancing factor 1 (Lef-1).

*Results:* The *Pitx2-Cre/Dicer1* mutant mice demonstrate growth retardation, and the pituitaries are hypoplastic with an abnormal branching of the anterior lobe, thus revealing a role for miRNAs in pituitary development. Growth hormone, prolactin, and thyroid-stimulating hormone β-subunit expression were decreased in the *Dicer1* mutant mouse, whereas proopiomelanocortin and luteinizing hormone β-subunit expression were normal. Pit-1 expression was decreased and Lef-1 expression increased in the mutant mouse pituitary, consistent with the repression of the Pit-1 promoter by Lef-1. Lef-1 directly targets and represses the Pit-1 promoter. MicroRNA-26b (miR-26b) was identified as targeting Lef-1 expression, and miR-26b represses Lef-1 in pituitary and non-pituitary cell lines. Furthermore, miR-26b upregulates Pit-1 and growth hormone expression by attenuating Lef-1 expression in GH3 cells.

*Conclusion:* MicroRNAs are critical for anterior pituitary development. miR-26b regulates Pit-1 expression by inhibiting Lef-1 expression and may promote Pit-1 lineage differentiation during pituitary development.
MicroRNAs (miRNAs) are short (usually 22 nucleotides), endogenous non-coding ribonucleic acids (RNAs) that post-transcriptionally regulate gene expression by targeting complement mRNA, either in the 3'UTR, resulting in inhibition of protein production, or in other regions of the mRNA, resulting in mRNA degradation. MiRNAs were identified in the 1990s but their biological significance was not recognized until the 2000s. They are now known to be involved in many physiological and developmental processes, including endocrine processes [1, 2]. Zhang et al. beautifully assessed the involvement of miRNA in pituitary development. In line with previous work in adult pituitary [3], expression of approximately 10 miRNA families was found in the anterior pituitary, including the miR-26 family. Pitx-2 (paired-like homeodomain transcription factor-2) is expressed at an early stage in pituitary development. Dicer is an endonuclease needed for miRNA maturation. Pitx-2-Cre mice and Dicer flox/flox mice were therefore used to downregulate Dicer and thus mature miRNA in all anterior pituitary cell lines. This resulted in a 20% reduction in size of the mice and severely reduced GH, PRL and TSH expression, suggesting suppressed Pit1 function. Indeed, further studies identified that miR26b represses lymphoid-enhancing factor 1 (Lef-1). Lef-1 represses Pit-1 directly and Lef-1 also binds β-catenin, a master ‘on-off switch’ in organogenesis, resulting in activation of c-myc and cyclin D1 promoters and proliferation. Physiological reduction of Lef-1 expression by miR-26b may shift more β-catenin to the Prop1-β-catenin complex and increase Prop1-specific targets like Pit-1 leading to promotion of cell differentiation. Regulation of Lef-1 and Pit1 expression by miR-26b adds further complexity to the regulation of pituitary development. miR-26 is only one of multiple miRNA families found in the pituitary, so we are far away from understanding pituitary development. The search is now for perturbations in miRNA expression in pituitary disease. Interestingly, downregulation of miR-26a expression is commonly found in nasopharyngeal tumors and increasing miR-26a expression inhibits their growth and tumorigenesis [4].

**Not so new gene of the year**

**AIP mutation in pituitary adenomas in the 18th century and today**


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**Background:** Gigantism results when a growth hormone-secreting pituitary adenoma is present before epiphyseal fusion. Mutations in the aryl hydrocarbon-interacting protein (*AIP*) can be the underlying cause for such adenomas. In 1909, when Harvey Cushing examined the skeleton of an Irish patient who lived from 1761 to 1783, he noted an enlarged pituitary fossa.

**Methods:** DNA was extracted from the patient’s skeleton’s teeth and sequenced.

**Results:** A germline mutation in the aryl hydrocarbon-interacting protein was identified. The same mutation and associated haplotype was present in four contemporary Northern Irish families who presented with pituitary adenomas. Coalescent theory was used and it was inferred that these patients share a common ancestor approximately 57–66 generations ago.

**Conclusion:** A single mutation in *AIP* is responsible for the formation of pituitary adenoma in many Irish families. The mutation has been identified in DNA extracted from the tooth of a 200-year-old skeleton of an Irish giant.
Characterization of aryl hydrocarbon receptor-interacting protein (AIP) mutations in familial isolated pituitary adenoma families

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Hum Mutat 2010;31:950–60.

Background: Familial isolated pituitary adenoma (FIPA) is an autosomal dominant disease with variable genetic background and incomplete penetrance. Germline mutations of the aryl hydrocarbon receptor-interacting protein (AIP) gene have been reported in 15–40% of FIPA patients. Limited data are available on the functional consequences of the mutations or the regulation of the AIP gene.

Methods: Mutations in a large cohort of FIPA families are described and all mutations are characterized using minigene constructs, luciferase and β-galactosidase assays, as well as in silico predictions.

Results: A promoter showed reduced in vitro activity corresponding to lower mRNA expression in patient samples. Stimulation of the protein kinase A-pathway positively regulates the AIP promoter. Silent mutations led to abnormal splicing resulting in truncated protein or reduced AIP expression. A two-hybrid assay of protein-protein interaction of all missense variants showed variable disruption of AIP-phosphodiesterase-4A5 binding. In addition, it was found that patients with AIP mutations had a lower mean age at diagnosis (23.6 ± 11.2 years) than AIP mutation-negative patients (40.4 ± 14.5 years).

Conclusion: Exonic, promoter, splice-site, and large deletion mutations in AIP are implicated in 31% of families in this large FIPA cohort. Functional characterization of AIP changes is important to identify the functional impact of gene sequence variants.

AIP appears to act as a tumor suppressor gene and normally decreases cell proliferation. Heterozygous germline mutations are found in 15–40% of patients with familial pituitary adenomas, most often somatotroph or somatolactotroph adenomas. The first paper is very interesting from both a scientific and historic point. The skeleton of a 231-cm tall giant, originally from Ireland, is kept at the Huntarian Museum in London. William Harvey examined its skull many years ago and described that the pituitary sella was expanded, suggestive of an adenoma, and in the 1980s a bone age showed epiphyses had not fused, likely due to secondary hypogonadotrophic hypogonadism. Korbonits’ team extracted DNA from the tooth and found a mutation in AIP, which has also been found in other Irish families. Four contemporary acromegalic Irish families carried the same mutation. Microsatellite data and coalescent simulation are techniques that can be used as a retrospective model of genealogies for a sample from a population with a defined demographic history. The demography for the Irish families and the family of the giant were known and well defined. Using this technique it was inferred that a common ancestor that carried the mutation lived approximately 1,400–1,650 years ago.

The second paper is impressive because it examines the functional consequences of all mutations in AIP described so far, including promoter mutations and splicing mutations, and also describes six new mutations. Importantly, some missense mutations did not have functional consequences and may therefore not be related to the phenotype. This emphasizes the need to assess functional consequences of sequence variants before they can be assumed to be disease-causing mutations. Very little information is available about regulation of the promoter of AIP and these studies also revealed that cAMP-PKA signaling is a positive regulator of the promoter. The paper also highlights the need for Multiplex Ligation-dependent Probe Amplification (MLPA) analysis, used to detect copy number changes, to exclude AIP deletions in the work-up of families with GH adenoma. Some patients with familial adenomas may present in childhood, and this is therefore important knowledge for pediatric endocrinologists.
Deregulation of the Pit-1 transcription factor in human breast cancer cells promotes tumor growth and metastasis

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Background: The transcription factor PIT-1 plays a critical role in cell differentiation during organogenesis of the anterior pituitary in mammals. Expression of PIT-1 however has also been reported in human breast cancer cells. Here the authors investigate the role of PIT-1 in breast cell tumorigenesis and metastasis.

Methods: Human breast cancer cell lines were used to overexpress and knock down PIT-1 to assess in vitro effect on cell migration, BrdU incorporation, apoptosis and epithelial-mesenchymal transition. SCID mice, injected with PIT-1-positive or PIT-1-negative breast cells, were used to assess the in vivo effect of PIT-1 on tumor growth and metastasis. Human breast cancer biopsies were rated for PIT-1 immunostaining to assess a correlation between PIT-1 expression and prognostic factors.

Results: PIT-1 overexpression or knock down in human breast cancer cells induced profound changes in the expression of proteins involved in cell proliferation, apoptosis, and invasion. Some of these pro-tumorigenic effects of PIT-1 were mediated by upregulation of SNAI1, known to induce epithelial-mesenchymal transition. In immunodeficient mice, Pit-1 overexpression induced tumor growth and promoted pulmonary metastasis. In patients with invasive ductal carcinoma of the breast and lymph node-positive tumor, high expression of PIT-1 was significantly correlated with SNAI1 positivity. Notably, in these patients, elevated expression of PIT-1 was significantly and independently associated with the occurrence of distant metastasis.

Conclusion: PIT-1 is able to regulate tumorigenesis and metastasis of breast cancer cells. The findings may suggest that PIT-1 could be used as a marker to help define the prognosis in patients with node-positive breast cancer and may represent a new therapeutic target.
with breast cancer as it may pave the way for the development of a new prognostic marker as well as a new therapeutic target. The possibility of directly targeting Pit-1 has been opened by a recent in vitro study, showing a direct inhibition of the DNA-binding activity of Pit-1 by a small molecule (DB293) [5].

**New concept – important for clinical practice**

**Adult combined GH, prolactin, and TSH deficiency associated with circulating PIT-1 antibody in humans**


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**Background:** A genetic defect in the *PIT-1* gene results in congenital growth hormone (GH)-, prolactin (PRL)-, and thyroid-stimulating hormone (TSH) deficiency but antibody-related combined GH, PRL and TSH deficiency has not been described.

**Methods and Results:** Three cases of adult-onset combined GH-, PRL-, and TSH deficiencies were investigated. The endocrine phenotype in each was linked to autoimmunity directed against the PIT-1 protein. Anti-PIT-1 antibody as well as other autoantibodies were detected in the patients’ sera. An ELISA-based screening revealed that the antibody was highly specific to the disease and absent in control subjects. Immunohistochemical analysis showed that PIT-1-, GH-, PRL-, and TSH-positive cells were absent in the pituitary of 1 of the patients, who also had a range of autoimmune endocrinopathies and fulfilled the criteria for autoimmune polyendocrine syndrome (APS). However, the main manifestations of APS-I – hypoparathyroidism and *Candida* infection – were not observed and the pituitary abnormalities were different from the hypophysitis associated with APS.

**Conclusion:** The patients described define a unique ‘anti-PIT-1 antibody syndrome’, related to APS.

Autoimmune polyendocrinopathy syndrome can be divided into three groups: (1) APS-I caused by defects in the *AIRE* gene, (2) X-linked IPEX syndrome (immune dysfunction, polyendocrinopathy, X-linked), due to *FOXP3* mutations, and (3) APS-II, which includes overlapping groups of disorders. Hypophysitis can be part of the syndrome and several antigens including GH, α-enolase and tudor domain containing protein-6 (TDRD-6) have been proposed as candidate antigens. Three patients are described with GH, PRL and TSH deficiency and features of autoimmune disease including IDDM and atrophic gastritis, but without mutations in *PIT-1, PROP1* and *HESX1*. When the sera of the patients were used as primary antibodies, a band the size of *PIT-1* was detected in lysates from pituitary and GH3 cells, whereas control sera did not. Patient’s sera also detected PIT1-positive cells in immunohistochemistry in pituitary slices. Autopsy in 1 of the patients shows typical lymphocytic infiltrates in multiple tissues including the pituitary and absence of specifically the GH-, PRL- and TSH-producing cells. The authors postulate that the pituitary pathology is different from the hypophysitis described in APS, partly because MRI did not show an enlarged pituitary. Antibodies against PIT-1 have not been described before and are unusual, since PIT-1 is a nuclear antigen, and further analysis is therefore needed to elucidate the mechanism. The authors propose that this is a novel anti-APS-related syndrome and have named it anti-PIT-1-antibody syndrome.
Pituitary magnetic resonance imaging for sellar and parasellar masses: 10-year experience in 2,598 patients

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melmed@csmc.edu

Background: The presentation of sellar and parasellar masses can vary enormously. Patients can be asymptomatic or present with symptoms resulting from mild hormone anomalies to local compression effects of the tumor mass. Pituitary masses are diagnosed with increased frequency due to increased availability and advances of magnetic resonance imaging (MRI), but indications and diagnostic outcomes of MRI screening for sellar lesions are not defined. Pituitary adenomas are the most frequently encountered sellar mass lesions in adults, but other etiologies should be considered in the differential diagnosis.

Methods: The study retrospectively reviewed 2,598 subjects that had had at least one pituitary MRI scan from 1999 to 2009 in a single tertiary pituitary center. Prevalence and diagnosis of specific sellar and parasellar masses as screened by pituitary MRI were assessed.

Results: Hyperprolactinemia and hypogonadism were the most common indications for pituitary imaging (excluding known mass follow-up). 47% of subjects had a normal pituitary gland. Prolactinoma (40%), non-functioning adenoma (37%), and GH adenoma (13%) were the most common pituitary adenomas initially identified by MRI. Non-adenomatous sellar masses accounted for 18% of visible lesions, of which the most common were Rathke’s cleft cyst (19%), craniopharyngioma (15%), and meningioma (15%). Metastases accounted for 5% of non-pituitary lesions and breast cancer was the most common primary source.

Conclusions: Nearly half of all pituitary MRI scans performed in a large adult population were reported as normal. Non-adenomatous pituitary lesions should be considered in the diagnosis of sellar masses, and a high clinical suspicion is required to exclude the presence of a non-functioning pituitary adenoma.

The incidence of clinically active pituitary adenomas is 1:1,000–1,250, and in autopsies pituitary adenomas have been reported in as much as 1.5–27% of people. Over the last decade, the incidence of pituitary adenomas has increased substantially, at least partly due to an increase in incidental detection using advanced MRI techniques. This paper describes an enormous case series, likely the largest to date, of MRIs of patients with sellar and parasellar masses.

The most common indication for MRI was follow-up of a known mass, followed by endocrinological work-up (most commonly for hyperprolactinemia, suspected Cushing’s disease or hypogonadism), followed by neurological symptoms (most commonly headaches and visual loss/blur). Approximately half the MRIs were normal. Of the identified sellar and parasellar masses, approximately 10% could not be diagnosed, and approximately 75% were pituitary prolactinomas and non-functioning adenomas (40% prolactinomas). The remaining 18% were mostly Rathke’s cysts, craniopharyngiomas and meningiomas (19, 15 and 15% respectively) and the remainder was due to an extremely wide variety of rare abnormalities, including metastases and not further defined pituitary hyperplasia, apoplexy, lymphoma and even syphilis (see table 1). The 282 incidentalomas were most often non-functioning adenomas (117), followed by Rathke’s cyst (22), craniopharyngioma (15) and meningiomas (18). Even though the incidence and etiology of sellar masses in children is different compared to adults, this study is of value for pediatricians. It will not be possible to build a similarly large patient cohort in children, and many of the rare pituitary abnormalities reported in this study, may be found in children. Sellar and parasellar masses are rare in children but like in adults, incidentalomas are becoming more common. Physiological pituitary enlargement during puberty complicates assessment of pathology and decisions regarding the need for intervention in children and young people with sellar masses. This paper may help with some of the diagnostic dilemmas.
**Table 1.** Diagnoses in 2,598 patients who underwent pituitary MRI [adapted from Famini et al.: J Clin Endocrinol Metab 2011;96:1633–1641]

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Total number</th>
<th>Pathological dx available</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anterior pituitary tumors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prolactinoma</td>
<td>395</td>
<td>54</td>
</tr>
<tr>
<td>Non-functioning adenoma</td>
<td>364</td>
<td>164</td>
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<tr>
<td>GH adenoma</td>
<td>127</td>
<td>43</td>
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<tr>
<td>ACTH adenoma</td>
<td>84</td>
<td>34</td>
</tr>
<tr>
<td>GH/prolactin mixed adenoma</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Nelson’s syndrome</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pituitary carcinoma</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>LH/FSH functioning adenoma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TSH adenoma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>GH/TSH mixed adenoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cysts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rathke’s cleft cyst</td>
<td>42</td>
<td>25</td>
</tr>
<tr>
<td>Craniopharyngioma</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>Arachnoid</td>
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<tr>
<td>Epidermoid</td>
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<td>1</td>
</tr>
<tr>
<td>Pineal cyst</td>
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<td>0</td>
</tr>
<tr>
<td><strong>Non-adenomatous neoplasms</strong></td>
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<td></td>
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<tr>
<td>Meningioma</td>
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<td>13</td>
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<tr>
<td>Chordoma</td>
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<tr>
<td>Pituitary lymphoma</td>
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<td>Chondrosarcoma</td>
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<td>Embryonal rhabdomyosarcoma</td>
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<td>Germinoma</td>
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<tr>
<td>Granular cell tumor</td>
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<td>1</td>
</tr>
<tr>
<td>Hemangiopericytoma, malignant</td>
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<td>1</td>
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<td>Leiomyosarcoma</td>
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<td>1</td>
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<td>Mucoepidermoid carcinoma</td>
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<td>Xanthogranuloma</td>
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<td>1</td>
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<td><strong>Inflammatory and vasculitides</strong></td>
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<td>Lymphocytic hypophysitis</td>
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<td>Amyloidosis, primary</td>
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<td>Sarcoidosis</td>
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<tr>
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<tr>
<td>Syphilis</td>
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<tr>
<td><strong>Metastases</strong></td>
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<tr>
<td>Breast</td>
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<td>1</td>
</tr>
<tr>
<td>CNS lymphoma, to pituitary stalk</td>
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</tr>
<tr>
<td>Nasopharyngeal lymphoma</td>
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<tr>
<td>Lung, adenocarcinoma</td>
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<td>0</td>
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<tr>
<td>Pineal germinoma/dysgerminoma</td>
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<td>Plasmacytoma</td>
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<tr>
<td>Prostate, adenocarcinoma</td>
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<tr>
<td>Sinusoidal squamous cell carcinoma</td>
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</tbody>
</table>
Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline

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Background: The aim of this work was to formulate a practical guideline for the diagnosis and treatment of hyperprolactinemia in adults.

Methods: A Task Force developed the guideline using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system describing both the strength of recommendations and the quality of evidence.

Results: Consensus was met to present evidence-based approaches to assessing the cause of hyperprolactinemia, treating drug-induced hyperprolactinemia, and managing prolactinomas in pregnant and non-pregnant patients. Indications and side effects of drugs used in the treatment of prolactinoma are discussed.

Conclusion: Guidelines are presented for the diagnosis and treatment of patients with elevated prolactin concentrations.

This work was the joint effort of the Endocrine Society, European Society for Endocrinology and The Pituitary Society. The guideline is written and intended for adult patients and adult endocrinologists, but it contains interesting points for pediatric endocrinologists. The guideline recommends the use of dopamine agonist therapy to lower prolactin concentrations, decrease tumor size and restore gonadal function and specifically recommends cabergoline rather than bromocriptine because of its higher efficacy and fewer side effects. Follow-up should include regular prolactin measurements, a repeat MRI in 1 year (or in 3 months in patients with macroprolactinoma) and visual-field examinations. Cessation of treatment is a difficult issue, also in pediatric patients. The guideline suggests that with careful clinical follow-up, therapy may be tapered and stopped after 2 years of treatment if prolactin concentrations have normalized and no tumor remnant is visible on MRI. The guideline also recommends surgical intervention only for those that fail to respond to increased doses of cabergoline, and this may not be common practice in pediatrics. Interestingly, the guideline recommends first-line cabergoline treatment for all patients independent of tumor size or chiasmal impingement,
factors that may be taken into consideration in pediatric prolactinomas. Radiotherapy is reserved for those who fail surgical treatment or harbor aggressive or malignant prolactinomas. Maybe this guideline can be used to assess and review the management of pediatric prolactinomas in various centers.

New genes – Usp39 and Numb in pituitary development

Zebrarfish usp39 mutation leads to rb1 mRNA splicing defect and pituitary lineage expansion

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Background: Loss of retinoblastoma (Rb) tumor suppressor function is associated with human malignancies. Molecular and genetic mechanisms responsible for tumorigenic Rb downregulation are not fully defined.

Methods and Results: Forward genetic screening and positional cloning was used to search for genes involved in pituitary homeostasis in zebrafish. This led to the identification of ubiquitin-specific peptidase 39 (usp39), the human homolog of which encodes a component of RNA splicing machinery. Zebrafish usp39 mutants exhibit microcephaly and adenohypophyseal cell lineage expansion of POMC cells without apparent changes in major hypothalamic signals. Gene expression profiling of usp39 mutants revealed decreased rb1 and increased e2f4, rbl2 (p130), and cdkn1a (p21) expression. Rb1 mRNA overexpression, or antisense morpholino knock down of e2f4, partially reversed embryonic pituitary expansion in usp39 mutants. Analysis of pre-mRNA splicing status of critical cell cycle regulators showed misspliced Rb1 pre-mRNA resulting in a premature stop codon, likely due to abnormal RNA splicing machinery.

Conclusion: These studies unravel a novel mechanism for rb1 regulation by a neuronal mRNA splicing factor, usp39. Zebrafish usp39 regulates embryonic pituitary homeostasis by targeting rb1 and e2f4 expression, respectively, contributing to increased adenohypophyseal sensitivity to these altered cell cycle regulators. These results provide a mechanism for dysregulated rb1 and e2f4 pathways that may result in pituitary tumorigenesis.

Previous studies have shown that retinoblastoma (Rb)+/− mice develop pituitary adenomas, however RB1 mutations have not been found in human pituitary tumors. In this study, a novel genetic pathway was uncovered that may lead to Rb downregulation through RNA splicing mediated by usp39, a gene involved in assembly of the spliceosome. A forward genetic study in zebrafish, using ENU-induced mutagenesis, showed that loss of usp39 resulted in aberrant rb1 mRNA splicing, which likely caused elevated expression of its target e2f4, a key regulator known to have oncogenic activity when overexpressed. The authors showed that e2f4 upregulation is a main factor responsible for the adenohypophyseal cell lineage hyperplasia observed in the zebrafish usp39 mutant. This work has revealed a new mechanism for the regulation of zebrafish rb1 by the mRNA splicing factor usp39, specifically expressed in the brain, pituitary and eyes. Loss of usp39 results in dysregulation of rb1 and e2f4 leading to loss of pituitary cell homeostasis. Human USP39 may play a similar role in human pituitary development or tumorigenesis.
**Numb deletion in POMC-expressing cells impairs pituitary intermediate lobe cell adhesion, progenitor cell localization, and neuro-intermediate lobe boundary formation**

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**Background:** Formation of pituitary hormone-secreting cells during development relies on Notch signaling to prevent progenitors from prematurely differentiating. Tight regulation of Notch activity is important for cell fate determination in progenitor cells, including, potentially, stem cells. The nature of the signal curtailing Notch signaling in the pituitary is unknown, but a good candidate is the endocytic adaptor protein NUMB, which acts as a Notch antagonist. The adaptor protein NUMB was first identified as a mediator of asymmetric cell division in *Drosophila*. NUMB targets Notch for proteolytic degradation, but it also has a broad range of actions, including stabilizing adherens junctions through interactions with cadherins and influencing cell proliferation by stabilizing expression of the tumor suppressor protein p53.

**Methods:** In situ hybridization on embryonic and adult mouse pituitaries was performed. *Numb* and its homolog *Numblike* were conditionally deleted using Pomc-Cre mice and Numb and Numblike floxed mice.

**Results:** NUMB and NUMBLIKE are expressed in undifferentiated cells during development and later in gonadotropes, and melanotropes in the intermediate lobe. Conditionally deleting Numb and Numblike in the intermediate lobe melanotropes with *Pomc-Cre* mice led to disruption of the border between the posterior and intermediate lobe and mixing of AVP-axon terminals and POMC cells. The mice also had disorganized progenitor cells, marked by SOX2, and impaired localization of adherens junction proteins. Interestingly, Notch signaling was unaffected.

**Conclusion:** *Numb* is critical for maintaining cell-cell interactions in the pituitary intermediate lobe that are essential for proper cell placement, at least partly independent of Notch.

This is an interesting study investigating the roles of the proteins NUMB and NUMBLIKE in pituitary development. The Notch signaling pathway is critical for early pituitary formation, but becomes largely undetectable after E14.5 in the mouse, and can only be found in isolated pituitary stem cells. NUMB is believed to play a role in the regulation of Notch signaling, however these studies also reveal a NOTCH-independent role for NUMB. In the postnatal pituitary, NUMB staining was observed in gonadotropes as well as sporadically in the intermediate lobe of the pituitary. The authors show that loss of NUMB in the mouse intermediate lobe melanotropes dramatically altered cell adhesion and progenitor cell localization, and results in posterior and intermediate lobe cell intermixing, identifying NUMB and NUMBLIKE as putative regulatory proteins during early pituitary development as well as during adulthood.

NUMB affects p53 degradation and can both promote and inhibit progenitor cell division, and also controls epithelial- to mesenchymal-like transitions and alters cell migration; it is therefore not surprising that loss of NUMB is associated with a number of cancers. Keeping in mind the role of Notch proteins in cancer and pituitary adult stem cell maintenance, studies such as these will illuminate further the increasing links between cancer and development.
Increased transactivation associated with SOX3 polyalanine tract deletion in a patient with hypopituitarism

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Background: Correct gene dosage of SOX3 is critical for the development of the hypothalamo-pituitary axis. Previous studies have shown that overdosage of SOX3, as a result of gene duplication, and loss of function resulting from expansion of the first polyalanine (PA) tract (p.A234_A235ins11, referred to as_+11PA; p.A240_A241ins7, +7PA) are associated with variable degrees of hypopituitarism, with or without mental retardation. A single in-frame deletion (p.A240_A248del9, del9PA) has been described in 2 brothers with mental retardation but without a clearly defined pituitary phenotype; the significance of this finding was unknown because functional studies were not performed and the deletion was also observed in the unaffected maternal grandfather. Sox3 null mutant animals have a variable phenotype, showing poor growth, craniofacial defects, and variable endocrine deficits. They have a small anterior pituitary with additional clefts and dysgenesis of the corpus callosum. The aim of this study was to further investigate the contribution of SOX3 in the etiology of hypopituitarism and the mechanisms involved in the phenotypic variability.

Methods: 154 patients with congenital hypopituitarism and an undescended posterior pituitary were screened for mutations in SOX3 and variability in the length of the first PA tract. In addition, 300 patients with variable septo-optic dysplasia were screened for variability of the PA tract.

Results: A novel 18-base-pair deletion (p.A243_A248del6, del6PA) was identified in a female patient with hypopituitarism resulting in a twofold increase in transcriptional activation in vitro, compared with wild-type SOX3; the del9PA was also associated with a similar increase in transcriptional activation. The paper also reports a previously identified 7-alanine expansion (p.A240_A241ins7, +7PA) in 2 male siblings with isolated GH deficiency and a distinct phenotype, in addition to the non-synonymous variant p.R5Q in an unrelated individual; this appears to have no functional effect on the protein. In contrast to +7PA, del6PA and del9PA maintained their ability to repress β-catenin-mediated transcription in vitro.

Conclusion: This is the first study to report that PA tract deletions associated with hypopituitarism have functional consequences in vitro, possibly due to increased activation of SOX3 target genes. In addition, the phenotypic spectrum associated with PA tract expansion (+7PA) mutations has now expanded to include panhypopituitarism or isolated GH deficiency, with or without mental retardation.

This study describes a novel mechanism of hypopituitarism associated with SOX3 mutations, namely increased transcriptional activation associated with deletion within a polyalanine tract. The phenotype is also novel, with an enlarged anterior pituitary in association with a normally descended posterior pituitary; previous mutations have been associated with anterior pituitary hypoplasia in association with an undescended posterior pituitary. The increased transactivation may reflect a gain in function, which is reminiscent of the SOX3 duplications associated previously with hypopituitarism. The presence of a phenotype associated with an X-linked gene in a female patient is intriguing and counterintuitive; however, Rizzoti et al. [6] have previously shown the presence of structural defects in the hypothalamo-pituitary region and abnormal pouch morphology in 3 of 4 female adult XX; Sox3/gfp mice without growth failure, reflecting incomplete penetrance of a hypothalamo-pituitary phenotype in females. The paper also underlines the considerable phenotypic variability observed with SOX3 mutations, particularly with respect to the extent of hormonal deficiencies and learning difficulties.
Novel heterozygous nonsense GLI2 mutations in patients with hypopituitarism and ectopic posterior pituitary lobe without holoprosencephaly

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Background: GLI2 is a transcription factor downstream in sonic hedgehog signaling, acting early in ventral forebrain and pituitary development. Previously, GLI2 mutations were reported in patients with holoprosencephaly and pituitary abnormalities. This paper reports three novel frameshift/nonsense GLI2 mutations and the phenotypic variability in the three families.

Methods: The GLI2 coding region of patients with isolated GH deficiency (IGHD) or combined pituitary hormone deficiency was amplified by PCR using intronic primers and was sequenced.

Results: Three novel heterozygous GLI2 mutations were identified: c.2362_2368del p.L788fsX794 (family 1), c.2081_2084del p.L694fsX722 (family 2), and c.1138 G>T p.E380X (family 3). All predict a truncated protein with loss of the C-terminal activator domain. The index case of family 1 had polydactyly, hypoglycemia with seizures, developmental delay, short stature and absence of puberty with GH, TSH, prolactin, ACTH, LH, and FSH deficiencies. Her mother and 7 relatives harboring the same mutation had polydactyly, including 2 uncles with IGHD and 1 cousin with GH, TSH, LH, and FSH deficiencies. In family 2, a boy had cryptorchidism, cleft lip and palate, and GH deficiency. In family 3, a girl had hypoglycemia, seizures with developmental delay, poor growth, excessive thirst and polyuria, and GH, ACTH, TSH, and antidiuretic hormone deficiencies. Magnetic resonance imaging of 4 patients with GLI2 mutations and hypopituitarism showed a hypoplastic anterior pituitary and an ectopic or undescended posterior pituitary lobe without holoprosencephaly. In family 3, MRI showed anterior pituitary hypoplasia with a non-visualized posterior pituitary.

Conclusion: Three novel heterozygous frameshift or nonsense GLI2 mutations, predicting truncated proteins lacking the activator domain, are partly penetrant and associated with IGHD or combined pituitary hormone deficiency and an ectopic/undescended posterior pituitary without holoprosencephaly.

Direct and indirect requirements of Shh/Gli signaling in early pituitary development

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Background: Oral ectoderm-derived sonic hedgehog (Shh) is required for specification and/or proliferation of early pituitary progenitors. Shh signaling is mediated by the Gli transcription factors, Gli1, Gli2, and Gli3. Gli2 is the major activator of Shh signaling, and previous studies have revealed a variable loss of the pituitary in Gli2 mutants at E12.5 [7]. However, the mechanism of action whereby different Gli genes mediate Shh signaling to control pituitary progenitor development has not yet been determined.

Methods: Pituitary-specific Gli mutant embryos were generated, using either the FoxG1Cre or Pitx2Cre. FoxG1 is expressed initially in the anterior neural ridge and in the early telencephalon, whereas Pitx2 is largely expressed in the anterior pituitary. Gli2 mosaic mutant embryos were also generated.

Results: Gli2 is required for proliferation of specific groups of pituitary progenitors but not for initial dorsoventral patterning. Most Gli2–/– embryos showed smaller pituitaries than the wild-type mice, with a reduction in corticotropes, somatotropes and lactotropes. Rathke’s pouch and the infundibulum were morphologically normal but contained fewer cells, suggesting a requirement for Gli2 for cell proliferation in both anterior and posterior pituitary. Gli1 and Gli3 mutants had no pituitary phenotype, suggesting that Gli2 is the primary Gli transcription factor in the pituitary. Gli2/Gli3 double mutants, in which there is also loss of expression of Gli1, completely lack a pituitary. Conditional transgenesis using an active form of Smo resulted in an increase in cell proliferation in the anterior dorsal region of Rathke’s pouch, and at E14.5, the pituitaries were much bigger than in WT controls, confirming a role...
for Shh in proliferation. Lastly, Shh/Gli2 signaling controlled the diencephalic expression of bone morphogenetic protein 4 (Bmp4) and fibroblast growth factor 8 (Fgf8), two genes known to play critical roles in patterning and growth of Rathke’s pouch. In keeping with these data, all Gli2 mutants had no posterior pituitary.

Conclusion: The results suggest both cell-autonomous and non-cell-autonomous requirements for Gli2 in regulation of pituitary progenitor specification, proliferation and differentiation.

These two papers by Franca et al. and Wang et al. shed novel insights on the role of GLI2 in normal hypothalamo-pituitary development in mouse and human. Wang et al. report a series of murine mutants and show that Gli2 loss of function is associated with variable pituitary hypoplasia of the anterior pituitary with absence of the posterior pituitary. The patterning of the pituitary in the conditional mutants is normal, but there is a clear proliferation defect. Removal of Gli2 function at an early stage using the FoxG1Cre leads to an abnormal pituitary phenotype whereas later removal using Pitx2Cre does not affect the pituitary, suggesting a critical window of Shh/Gli2 signaling in the control of pituitary progenitors. Gli2 is also implicated in the development of the posterior pituitary and this effect is probably mediated via effects on Bmp4 and Fgf8 expression in the diencephalon, both of which are expressed at a markedly reduced level in Gli2 mutants. These data therefore indicate a requirement for Shh/Gli2 at two levels of hypothalamo-pituitary development, namely within Rathke’s pouch to promote proliferation of pituitary progenitors, and secondly within the ventral diencephalon to control early patterning of Rathke’s pouch by regulating Bmp4 and Fgf8 expression.

How can one reconcile the human phenotypes associated with GLI2 mutations with those observed in the mouse? Previous studies [8] have reported the association of hypopituitarism with complex midline abnormalities in the form of holoprosencephaly in patients with GLI2 mutations. Five patients with variable hypopituitarism ranging from isolated GHD to panhypopituitarism were described; of the 3 with panhypopituitarism, all had an absent pituitary although it is unclear whether this included both the anterior and posterior lobes. The endocrine phenotypes were variably penetrant and were not described in detail, and indeed, 2 patients were deceased and no genetic confirmation was possible. Franca et al. describe three families that have variably penetrant hypopituitary phenotypes. Interestingly, apart from a cleft palate in the proband in family 2, none of the other patients had midline defects although polydactyly, previously also reported by Roessler et al. [8], was present in family 1. Four of the 5 patients in this second study had an undescended or ectopic posterior pituitary, and 1 patient had an absent posterior pituitary. None had a completely absent pituitary. This is the first description of a patient with both anterior and posterior pituitary hormone deficiencies in the absence of any midline defects, confirming the findings of Wang et al. that Gli2/GLI2 is required for the formation of both the anterior and posterior pituitary. Interestingly, the parents of the probands in both family 2 and family 3 who transmitted the mutations were unaffected. The authors suggest that the variable penetrance may be due to the impact of environmental factors or possibly a digenic effect whereby mutations in other genes may be contributory to the phenotype.

These two papers add a new player to be considered when screening children with hypopituitarism for genetic mutations. The field of hypothalamo-pituitary development is becoming increasingly complex. Studies such as these help in putting together the jigsaw pieces.
Birth dating studies reshape models for pituitary gland cell specification

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Background: The intermediate and anterior lobes of the pituitary gland are derived from an invagination of oral ectoderm that forms Rathke’s pouch at E9.5. The overlying neural ectoderm then evaginates to form the infundibulum, from which the posterior pituitary and pituitary stalk will derive, which comes into direct contact with Rathke’s pouch. The juxtaposition of Rathke’s pouch and the diencephalon is maintained throughout the early stages of pituitary organogenesis. This close relationship is required for tissue interactions between neural and oral ectoderm which are critical for the initial stages of pituitary specification. During gestation, proliferating progenitor cells are enriched around the pouch lumen, and they appear to delaminate as they exit the cell cycle and differentiate. During late mouse gestation and the postnatal period, anterior lobe progenitors re-enter the cell cycle and expand the populations of specialized, hormone-producing cells. At birth, all cell types are present, and their localization appears stratified based on cell type. Current models of cell specification in the anterior lobe suggest that opposing gradients of FGF and BMP signaling pattern the progenitor cells within Rathke’s pouch before they move on to the anterior lobe where they differentiate.

Methods: The authors conducted a birth dating study of Rathke’s pouch derivatives to determine whether the location of specialized cells at birth is correlated with the timing of cell cycle exit. Pregnant mice were injected with BrdU on each embryonic day of development between E9.5 and E17.5. All embryos were collected at E17.5, and immunohistochemistry performed on pituitary sections.

Results: The authors report that all of the anterior lobe cell types initiate differentiation concurrently with a peak between E11.5 and E13.5. Differentiation of intermediate lobe melanotropes is delayed relative to anterior lobe cell types. Specialized cell types were not grouped together based on birth date, and are dispersed throughout the anterior lobe.

Conclusion: The apparent stratification of specialized cells at birth is not correlated with cell cycle exit, thereby indicating that the currently popular model of cell specification, dependent upon timing of extrinsic, directional gradients of signaling molecules such as FGF8 and BMP4, needs revision. The authors propose that signals intrinsic to Rathke’s pouch are necessary for cell specification between E11.5 and E13.5 and that cell-cell communication likely plays an important role in regulating this process.

This is an intriguing study that sets out to challenge the current dogma of pituitary cell specification, using simple yet elegant experiments. The authors show by using BrdU injections into mice, that the pattern of cell specification that results in the rostral location of gonadotropes, the caudal location for somatotropes and a more intermediate location for corticotropes and thyrotropes, does not appear to be the result of an ordered cell cycle exit. All anterior lobe cell types appear to begin the differentiation process concurrently (E11.5–E14.5), rather than in a temporally discrete manner.

The authors put forward a persuasive argument against the currently held belief that opposing BMP and FGF gradients are responsible for specification and stratification of the various cell types within the anterior pituitary. Both Bmp2 and its intracellular transducers the phosphoSMAD proteins (pSMAD) are expressed throughout Rathke’s pouch by E11.5. This expression pattern persists until E13.5, after which time there is a decrease in pSMAD intensity. Similarly, the phosphorylation status of MAPK (pMAPK), an intracellular mediator of FGF signaling, was used to examine FGF signaling within the pouch. Although pMAPK immunoreactivity was enriched on the dorsal side of the pouch at E10.5, adjacent to the developing infundibulum where Fgf8 and Fgf10 are expressed, by E11.5 pMAPK expression had decreased and was undetectable from E12.5 to E14.5. Hence, although gradients of Bmp and Fgf signaling are present at E10.5, thereafter, during the period when anterior lobe cell types exit the cell cycle between E11.5 and E13.5, BMP signaling is found throughout Rathke’s pouch and FGF signaling cannot be detected.

This study challenges existing beliefs, but also raises the important question of what determines the formation of cell type-specific networks within the pituitary gland, which are increasingly important
The Notch effector gene *Hes1* regulates migration of hypothalamic neurons, neuropeptide content and axon targeting to the pituitary

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**Background:** Proper development of the hypothalamic-pituitary axis requires precise neuronal signaling to establish a network that regulates homeostasis. The developing hypothalamus and pituitary utilize similar signaling pathways for differentiation in embryonic development. Magnocellular neurons located in the paraventricular nuclei (PVN) and the supraoptic nuclei (SON) release arginine vasopressin (AVP) and oxytocin (OT) from their axonal terminals within the posterior lobe of the pituitary. The hypothalamic anterior periventricular (aPV) nucleus contains parvocellular somatostatin (SS)-releasing neurons. Several signaling molecules and transcription factors have been implicated in the formation of hypothalamic neurons. The Notch signaling effector gene *Hes1* is present in the developing hypothalamus and pituitary and is required for proper formation of the pituitary. In this paper, the authors hypothesized that *Hes1* is necessary for the proper formation, migration and projection of AVP and SS neurons to the pituitary.

**Methods:** The hypothalamus and pituitary of *Hes1* null mice were analyzed using immunohistochemistry, quantitative real-time PCR, and mass spectrometry.

**Results:** *Hes1* null mice showed no significant difference in cell proliferation or death in the developing diencephalon during early development. By E16.5, AVP cell bodies were formed in the SON and PVN, but were abnormally placed and by E18.5 also exhibited abnormal axonal projection. GAD67 immunoreactivity was ectopically expressed, which may contribute to cell body misplacement. *Hes1* null pituitaries also had aberrant SS peptide, which correlated with abnormal SS cells in the pituitary and misplaced SS axon tracts. Additionally, POMC and α-MSH were not detected in *Hes1* null mutants, in accordance with previous data suggesting a lack of specification of POMC cells in the intermediate lobe.

**Conclusion:** The data indicate that Notch signaling and *Hes1* facilitate the migration and guidance of hypothalamic neurons, as well as neuropeptide content.

Although much is known about pituitary development in the mouse and human, little is known about the events that lead to normal hypothalamic development. In this elegant study, Aujla et al. have examined the anatomy of the hypothalamus and pituitary in *Hes1* null mice with particular emphasis on AVP neurons. Last year, we commented on the role of *HES1* in maintaining a balance between proliferation and differentiation [11, 12]. In *Hes1* null mutants, AVP cell bodies are abnormally placed, POMC cells are not specified in the intermediate lobe, and SS cells in the pituitary are also abnormal with misplaced SS axon tracts at E18.5. The authors suggest a genetic interaction between the Notch signaling pathway and the extracellular matrix molecule Reelin, that then leads to the guidance of axons to the median eminence and pituitary. In *Hes1* null animals, the median eminence is clearly misplaced within the head, which may contribute to the aberrant cluster of AVP axons and the reduced number of AVP axons within the posterior lobe. The authors suggest that *Hes1* may be important for normal development of support cells such as tanycytes within the median eminence, and that these in turn modulate hypothalamic neurons that travel through it. Disruption of tanycytes indeed results in dysregulated axon guidance. Whatever the underlying mechanism, Notch signaling clearly plays a critical role in hypothalamo-pituitary development. Interestingly, deletion of *Numb*, which also can affect Notch signaling, also results in misplacement of posterior and intermediate lobe cells, as discussed earlier in this chapter. In the future, mutations in this complex...
pathway may well be identified in association with abnormal hypothalamo-pituitary development in humans.

**Reviews – ion channels and pituitary stem cells**

**Ion channels and signaling in the pituitary gland**

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Like neurons, hormone-producing pituitary cells express numerous voltage-gated channels enabling sodium, calcium, potassium, and chloride flux. Pituitary cells also fire action potentials spontaneously, like neurons, accompanied by a rise in intracellular calcium. In some cells, spontaneous electrical activity results in intracellular calcium concentration sufficiently high for hormone secretion or gene transcription to occur. In other cells, the function of such action potentials is to maintain the cells in a responsive state with cytosolic calcium concentration near, but below, the threshold level for hormone secretion. Some pituitary cells also express gap junction channels, which could be used for intercellular Ca signaling. Endocrine cells also express extracellular ligand-gated ion channels. Hypothalamic and intrapituitary hormones can activate ion channels and this results in amplification of the cell’s pacemaking activity and facilitation of calcium influx and hormone release. Pituitary endocrine cells also express numerous G-protein-coupled receptors, and these can stimulate or silence electrical activity and action potential-dependent calcium influx and hormone release. Other members of this receptor family can activate calcium channels in the endoplasmic reticulum, leading to a cell type-specific modulation of electrical activity. This review summarizes the current understanding of the relationship between voltage-gated ion channels, ligand-gated ion channels, gap junction channels, and G-protein-coupled receptors in pituitary cells.

This is an extremely comprehensive review, counting 72 pages and 761 references. Ion channels provide a basic signaling system for individual pituitary cells. This review discusses in great detail how this signaling system works and how it affects hormone release from individual cells. Like neurons, endocrine anterior pituitary cells express numerous voltage-gated ion channels, but also many other channels, like cation-conducting cyclic nucleotide-modulated channels (that translate a change in concentration of cyclic nucleotides to changes in membrane potential) and transient receptor potential channels (of which TRPV5 and 6, that play a role in bone formation, are members). Flux through these channels leads to altered membrane potential. As in neurons, a change in action potential allows for Ca influx through Ca channels. The pattern and frequency of electrical activity and Ca flux determines the coupling to hormone secretion in single cells.

The review covers Na, K, Ca and Cl channels, cyclic nucleotide-modulated channels, voltage-gated and ligand-gated channels, gap junction channels (connexins and pannexins), receptor channels (acetylcholine receptor, 5-HT receptor, GABA receptor, glycine receptor, glutamate receptor, purinergic receptor (for ATP, ADP and adenosine)), transient receptor potential channels, and channels expressed in the endoplasmic reticulum (IP3 receptors, ryanodine receptors). It also discusses the regulation of electrical activity and Ca mobilization by G-protein-coupled receptors in pituitary cells, such as neuropeptide receptors (ghrelin receptors, GnRH receptors) but also other G-protein-coupled receptors such as acetylcholine receptors, endothelin receptors and angiotensin receptors, to name a few.

Ion channels, action potentials and calcium flux may not be on the forefront of the mind of many pediatric endocrinologists. This review may serve as a mini-textbook to remind ourselves how the system works, and how it regulates hormone release, which is on the forefront of our minds on a daily basis.
Pituitary stem cell update and potential implications for treating hypopituitarism

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Stem cells are characterized by the expression of key marker genes for undifferentiated cells, the ability to self-renew, and the ability to regenerate tissue after cell loss. Several recent reports have suggested the presence of pituitary stem cells in the form of either a SOX2+ cell population, GFRα2+ cells, a side population of cells, nestin+ cell population, or folliculostellate cells. In this paper, the authors give a critical review of the field and suggest studies that could resolve points of debate. Recent studies have used SOX2, nestin, GFRα2, and SCA1 to identify pituitary stem cells and progenitors but future studies will be needed to resolve the relationships between cells expressing these markers. The authors hypothesize that there are two critical roles of stem cells: one in establishing the pituitary gland during development, and the other involved in maintenance of the mature pituitary gland in response to physiological challenges and normal cell turnover. The hypothesis of two different populations of stem cells, one involved in embryogenesis and one involved in maintenance function after birth, remains highly controversial. Members of the Sox family of transcription factors are likely involved in the earliest steps of pituitary stem cell proliferation and the earliest transitions to differentiation. The transcription factor PROP1 and the NOTCH signaling pathway may then regulate the transition to differentiation. Identification of the stem cell niche is important for several reasons and the authors suggest that the niche may be the marginal zone around the lumen of Rathke’s pouch, between the anterior and intermediate lobes of mouse pituitary, since cells in this region are able to give birth to all five pituitary hormone cell lineages. Stem cells have been shown to play a role in tumorigenesis in some tissues, and their role in pituitary hyperplasia, pituitary adenomas, and tumors is an important area for future investigation. The ability to cultivate and grow stem cells in a pituitary pre-differentiation state might also be helpful for the long-term treatment of pituitary deficiencies.

This comprehensive review attempts to describe the current state of knowledge in what is a controversial and rapidly evolving field. A number of groups have described cells that demonstrate some of the characteristics associated with stem cells, namely pluripotency, self-renewal, ability to proliferate, and to differentiate into diverse cell types. However, considerable debate exists as to which of these cell types best reflects a true pituitary stem cell. This review dissects the characteristics of each of these cell types. The review also discusses the potential role of stem cells in the etiology of pituitary tumors, and looks forward to pituitary stem cell therapy for a number of conditions including congenital hypopituitarism, trauma and damage. The field of stem cells progresses rapidly and no doubt will the authors themselves generate new data that will make this review outdated.

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Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor

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Background: Pituitary adenylate cyclase-activating polypeptide (PACAP) is known to broadly regulate the cellular stress response. In contrast, it is unclear if the PACAP-PAC1 receptor pathway has a role in human psychological stress responses, such as post-traumatic stress disorder (PTSD).
**Methods:** Research interviews, SNP analysis of salivary DNA and radioimmunoassays for PACAP38 were used in more than 1,200 highly traumatized subjects with and without PTSD. Fear potentiation was assessed by measuring the ‘acoustic startle reflex’. A Human Methylation Bead Chip was used to assess methylation of the PAC1 receptor. Rats were used to assess estrogen-induced changes in PACAP and ADCYAP1R1 in the bed nucleus of stria terminalis.

**Results:** In heavily traumatized female but not male subjects, an association of PACAP blood concentration with fear physiology, PTSD diagnosis and symptoms was found. 44 single nucleotide polymorphisms (SNPs) spanning the PACAP and PAC1 genes, demonstrated a sex-specific association with PTSD. A single SNP in a putative estrogen response element within the PAC1 gene (ADCYAP1R1), rs2267735, predicted PTSD diagnosis and symptoms in females and was associated with fear discrimination and with ADCYAP1R1 messenger mRNA expression in human brain. Methylation of ADCYAP1R1 in peripheral blood was also associated with PTSD. Complementing these human data, ADCYAP1R1 mRNA is induced with fear conditioning or estrogen replacement in rats and mice.

**Conclusion:** Perturbations in the PACAP-PAC1 pathway are involved in abnormal stress responses underlying PTSD in females. These sex-specific effects may occur via estrogen regulation of ADCYAP1R1. PACAP blood concentration and ADCYAP1R1 SNPs may serve as useful biomarkers to further our understanding of PTSD.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is produced in CNS neurons, especially in the hypothalamus and limbic structures, and peripheral neurons, for example in the gut. PACAP is co-produced with VIP, and PACAP and VIP share two receptors, but PACAP also has its own receptor PAC1 (encoded by ADCYAP1R1). PACAP was named after its ability to stimulate cyclic AMP production in the anterior pituitary. PACAP and PAC1 are known to function in the control of the stress response and PACAP is required for normal activation of the hypothalamo-sympatho-adrenal and hypothalamo-pituitary-adrenal axes in response to stressors. Indeed, mice deficient in PACAP or PAC1 displayed reduced anxiety and have blunted CRH and corticosterone responses to stress [13]. A role for PACAP in schizophrenia and chronic depression has also been suggested.

The group of May et al. hypothesized that PACAP-PAC1 is involved in post-traumatic stress disorder and analyzed PACAP concentration, SNPs in ADCYAP1 and its receptor ADCYAP1R1, and methylation status of CpG island in ADCYAP1R1 in a large cohort of more than 1,200 traumatized patients with and without PTSD. They showed that their hypothesis was correct. SNPs in PACAP, in PAC1 and in a putative estrogen response element in PAC1 gene demonstrated a sex-specific association with PTSD. PACAP38 concentration was higher in females with vs. without PTSD and predicted PTSD symptoms like re-experiencing of trauma, avoidance and hyperarousal (assessed with the acoustic startle response). Female patients with a SNP in the putative estrogen response element within PAC1, had increased dark-enhanced startle, a measure of increased anxiety. Furthermore, methylation of PAC1 was significantly associated with PTSD symptoms, suggesting that environmental and epigenetic mechanism likely affect the long-term response to trauma.

This work suggests that PACAP-PAC1 is involved in both behavioral and hormonal responses to trauma and stress. The sex specificity of the relation between PACAP-PAC1 and PTSD is of interest since females are twice more likely to suffer from PTSD than males. Further work on the PACAP-PAC1 pathway may pave the way for the development of novel biomarkers and potential therapeutic targets for PTSD.

**References**


