Pituitary

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Important for clinical practice

Midline defects and pituitary: awaiting for more genes

Congenital hypopituitarism: clinical, molecular and neuroradiological correlates

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Background: Many studies have recently described mutations in genes encoding transcription factors involved in hypothalamic pituitary (HP) development which result in various degrees of pituitary function impairment (ranging from isolated GH deficiency IGHD, to combined pituitary hormone deficiency, CPHD) and structural HP abnormalities detected by brain MRI. However, none of these previous studies compared subjects affected by hypopituitarism with or without optic nerve hypoplasia (ONH). The aim of the present study was to investigate a large number ($n = 170$) of children with congenital hypopituitarism and/or septo-optic dysplasia (SOD) for their clinical, MRI and genetic findings.

Methods: This is a retrospective analysis of clinical, hormonal, MRI and genetic data relative to 170 children with congenital hypopituitarism, or with ONH, who are at risk of pituitary endocrine defects, in order to determine predictors of hypopituitarism

Results: ONH showed a significant relationship with the absence of septum pellucidum (OR 31.5, $p < 0.001$), anomalies of corpus callosum (OR 10.5, $p < 0.001$) and pituitary stalk (OR 2.3, $p = 0.009$). Markers for a high risk of hypopituitarism were ectopic posterior pituitary (OR 27.2, $p < 0.001$), anterior pituitary hypoplasia (OR 3.1, $p = 0.006$) and pituitary stalk abnormalities (χ^2 20.11, $p < 0.001$), while CPHD was observed to be more frequently associated with abnormal corpus callosum (OR 6.1, $p = 0.008$) and pituitary stalk (OR 2.8, $p = 0.006$). Gender prevalence of hypopituitarism was 3.3:1 for males with normal optic nerve and 1.2:1 for males with ONH. Vasopressin, TSH and ACTH deficiencies showed a higher prevalence in subjects with ONH compared to idiopathic hypopituitarism. Pituitary transcription factor mutations were found in only 5 of 170 subjects, confirming them as a rare condition in patients with sporadic hypopituitarism.

Conclusion: Based on the reported data, ONH is often associated with other neuroradiologic abnormalities and represents a risk factor for the development of pituitary hormone defects, the severity of which is correlated with specific MRI alterations. The high correlation between midline anomalies and pituitary defects is likely compatible with a common genetic origin of both of these disorders, an origin that in most of cases remains largely undefined.

This is a comprehensive study on midline defects, optic nerve involvement and pituitary defects that is worth reading. It shows that gene identifiable mutations are rare in sporadic hypopituitarism in a large cohort from a single center. The study confirms previous reports suggesting that neuroradiological findings of septum pellucidum agenesis in association with optic nerve hypoplasia with or without abnormalities of the corpus callosum and structural hypothalamic-pituitary abnormalities are risk factors for severe pituitary dysfunction. Early identification of such CNS malformations can predict the outcome in terms of the number of pituitary hormone deficiencies and will allow clinicians a better surveillance of evolving pituitary hormone defects including ACTH deficiency.

Endocrine manifestations of the rapid-onset obesity with hypoventilation, hypothalamic, autonomic dysregulation, and neural tumor syndrome in childhood

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Background: ROHHADNET is a new syndrome characterized by early- and rapid-onset obesity with hypoventilation, hypothalamic dysregulation, and neural tumor. It is associated with hypothalamic-pituitary dysfunction, which can be similar to syndromes observed in forms of genetic obesity. The syndrome can cause cardiorespiratory arrest. Early recognition of the syndrome is crucial for the prevention of fatal events. This study reports the clinical and laboratory characteristics of 6 patients with ROHHADNET and discusses differential diagnoses with other endocrine diseases such as pseudo-Cushing syndrome, hypopituitarism, glucocorticoid insufficiency, and adrenal tumors.

Methods: Thorough endocrine investigations were carried out on 6 patients who presented with early-onset obesity associated with growth deceleration as seen in 5 of them. These patients were ultimately diagnosed as having ROHHADNET. Five patients with Cushing disease and a group of obese children served as controls.

Results: All of the patients were apparently normal in their first 2–4 years of life. The initial manifestation of obesity began between 1.5 and 4.3 years, and at the same time growth velocity started to decelerate. Clinical examination was normal in all patients. Investigation of the pituitary adrenal axis revealed a Cushing-like profile in 1 patient, glucocorticoid deficiency with normal ACTH in 2 others and normal findings in the remaining 3. Four patients showed GH deficiency with low IGF-I, 4 had hypogonadotropic hypogonadism, 6 showed hyperprolactinemia, and 5 had various degrees of pituitary/thyroid dysfunction. Pituitary MRI was normal in all but 1 patient who had a Rathke's cleft cyst. CT scan of the adrenals revealed adrenal tumors in 5 patients. Ganglioneuromas were diagnosed 4.5–16 years after the onset of obesity. Hypernatremia was observed in all patients. Three patients manifested cardiorespiratory arrest, and 2 of them died at age 8.5 and 12 years. One patient had symptomatic obstructive sleep apnea. All 6 patients had alveolar hypoventilation. Symptoms of autonomic dysregulation included hyper- or hypothermia, papillary dysfunction, and gastrointestinal dysmotility.

Conclusion: This study shows that the ROHHADNET syndrome can be associated with various hypothalamic pituitary dysfunctions. ROHHADNET syndrome should be considered in all cases of isolated rapid and early-onset obesity, and when ROHHADNET syndrome is suspected, all children with early-onset obesity should be tested for alveolar hypoventilation.

This study focuses on the clinical presentation, laboratory investigation and follow-up of a rarely made diagnosis of a syndrome of early-onset and severe obesity. The syndrome, named ROHHADNET, is associated with hypoventilation, hypothalamic autonomic dysregulation and neural tumor. The syndrome can be distinguished from all other known genetic syndromes with early-onset obesity. ROHHADNET mimics congenital leptin deficiency with undetectable leptin concentrations. Given the possibility of fatal cardiorespiratory arrest in a syndrome with pleiotropic features, it is important to recognize any and all possible symptoms that may indicate such a diagnosis or other types of hypothalamic obesity. It is conceivable that a substantial subgroup of patients with 'simple obesity' might have hypothalamic obesity.

Prenatal MR imaging of the normal pituitary stalk

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Background: Although occasional cases of pituitary stalk (PS) detection by fetal MRI have been reported, well-established data about prenatal MR imaging of the hypothalamic-pituitary area are still lacking. These data would allow an early diagnosis of congenital pituitary disorders. The aim of the present study is the systematic identification and description of PS in healthy fetuses of different gestational ages (GAs) by means of single-shot fast-spin echo T2-weighted images.

Methods: The authors investigated 73 fetuses with GAs between 19 and 37 weeks, normal prenatal MRI and clinical follow-up after birth. Three-plane 4-mm MRI sections with 1.25×1.25 mm in-plane resolution were analyzed in consensus by 2 pediatric neuroradiologists to search for PS, defined as a linear isointense structure connecting the hypothalamic region with the floor of the sella turcica. When it was detectable on sagittal images, the angle between the intersection of the PS and the sellar plane (SP) was measured (PS-SP angle).

Results: The PS was identified at GA between 19 and 25 weeks in at least 1 coronal or sagittal section in 30 of 42 cases (sensitivity 71.4%), while a 100% sensitivity (PS identification in all 31 cases) was observed in the successive gestational ages. The PS-SP angle showed a significant decrease with GA, reaching values below 90° in all fetuses with $GA \geq 25$ weeks.

Conclusion: The authors conclude that MRI, at the current available resolution for clinical prenatal evaluations, is an accurate method for the detection of PS after a GA of 25 weeks. A missing PS after this age could represent an early marker of congenital structural pituitary abnormalities.

Prenatal imaging has benefited from rapid technological progress in the last 10 years. Ultrasound remains the standard screening method for fetal malformations, but it can be hindered by the bony structure of the skull. In particular, it can be difficult to distinguish between white and grey matter, and MRI represents a complementary method to ultrasound for detecting brain malformations. In particular, MRI is needed to detect associated malformations and to obtain a precise diagnosis when ultrasound examination shows ventricular dilation.

Prenatal diagnosis of absent septum pellucidum has been made as early as 22 weeks of gestation and sonographic demonstration of isolated absence of the septum pellucidum with fusion of the frontal horns of the lateral ventricles is a clue for the diagnosis of septo-optic dysplasia [1]. This study reinforces the need for thorough knowledge of normal and abnormal neuroanatomy and shows that fetal MRI can identify the pituitary stalk and that such identification might have an impact on the evaluation of congenital CNS malformations related to pituitary endocrinopathies.

Although a clinical course and development still cannot be predicted based on MRI findings alone, the identification of pituitary stalk agenesis in fetuses with associated brain malformations could be helpful both for the early management of neonatal hypopituitarism and for counseling parents.

Treatment of pituitary-dependent Cushing's disease with the multireceptor ligand somatostatin analog pasireotide (SOM230): a multicenter, phase II trial

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Background: Cushing's disease is caused by a corticotropin-secreting pituitary adenoma. Surgical resection of the adenoma via the transphenoidal route is, so far, the only available effective treatment. Therapeutic trials with different compounds, including somatostatin (SS) analogs have been disappointing. Human corticotroph adenomas express multiple SS receptors (SSR) with a predominance of SSR5. This study reports the results of a phase-II, open-label, multicenter trial in patients with persistent or recurrent Cushing's disease treated with pasireotide, a novel SS analog with high affinity for SSR5.

Methods: Thirty-nine patients with either de novo Cushing's disease or with persistent or recurrent disease participated in the trial. The patients self-administered pasireotide subcutaneously at a dose of 600 µg twice daily for 15 days. The primary efficacy outcome was normalization of mean urinary free cortisol (UFC) after 15 days of treatment. Secondary endpoints were normalization of serum ACTH and cortisol levels.

Results: 22/29 patients (76%) in the primary efficacy analysis showed a reduction in UFC levels after 15 days of treatment. Among them, 5 showed normalization of UFC. These changes were paralleled by a reduction of both serum ACTH and cortisol concentrations.

Conclusion: Treatment with pasireotide produced a reduction of UFC and ACTH in about two thirds of patients with Cushing's disease. This compound opens a new field of investigation for the medical treatment of corticotropin-secreting adenomas.

Cushing's disease is a rare condition in children. It is associated with most of the features of the adult disease and, in addition, it causes growth arrest in virtually all patients. Transphenoidal surgery is a difficult therapeutic procedure, even more so in children. Therefore, a medical approach to treatment of Cushing's disease in the pediatric age would be most desirable. Pasireotide is a novel multireceptor ligand somatostatin analog with high binding affinity for four of the five known somatostatin receptors (1–3 and 5) and has a 40-fold higher affinity for SSR5 than octreotide. Corticotroph adenomas express multiple SS receptors with high abundance of SSR5. Studies in vitro and in experimental animals have shown that pasireotide effectively inhibited ACTH secretion. The results of this study in adult subjects treated with the somatostatin analog pasireotide are promising.

New mechanisms **More than eye involvement**

OTX2 mutation in a patient with anophthalmia, short stature, and partial growth hormone deficiency: functional studies using the IRBP, HESX1, and POU1F1 promoters

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Background: While the role of the transcription factor gene OTX2 for eye development is well known, its relevance in pituitary function regulation is not completely understood. The objective of the present

study was to assess the involvement of OTX2 in pituitary function development by means of functional studies.

Methods: A Japanese girl affected by bilateral anophthalmia and short stature (−3.3 SDS), was diagnosed with isolated GH deficiency (GH peaks of 3.1 and 9.7 µg/l after insulin and arginine administration, respectively, with IGF-I 37 µg/l) at the age of 3 years and 9 months. No significant structural pituitary abnormalities were found on brain MRI. DNA samples from the patient and her parents were analyzed for mutation of OTX2, HESX1, and POU1F1.

Results: A novel OTX2 heterozygous frameshift mutation (c.402insC) was detected, which conserved the homeodomain but lost the transactivation domain. In fact, functional analysis revealed that both the wild-type and the mutant Otx2 proteins showed analogous binding capability to their nuclear target sequence within the interstitial retinoid-binding protein (IRBP), HESX1 and POU1F1 promoters. The wild-type Otx2 protein markedly transactivated the promoters of IRBP (approximately 27-fold), HESX1 (approximately 4.5-fold), and POU1F1 (approximately 19-fold), while the mutant Otx2 protein barely retained transactivation activities, without dominant-negative effects.

Conclusion: This study confirms a significant role for OTX2 in pituitary function regulation, demonstrating that a heterozygous OTX2 loss-of-function mutation can cause GH deficiency and short stature with a mechanism related to decreased HESX1 and POU1F1 transactivation.

A novel dominant negative mutation of OTX2 associated with combined pituitary hormone deficiency

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Background: Mutations in different transcription factors involved in pituitary cell lineage differentiation and regulation of gene expression were described in patients affected by combined pituitary hormone deficiency (CPHD). Mutation of OTX2, a bicoid class homeodomain protein active in forebrain development and transactivation of HESX1 promoter, has not yet been definitively associated with CPHD. The aim of this study was to identify novel mutations of pituitary specific transcription factors in CPHD patients.

Methods: Genomic DNA obtained from 19 patients with hypopituitarism and 50 control subjects was sequenced for HESX1, LHX3, LHX4, OTX2, PITX2, POU1F1, PROP1, and SIX6. Thirty-one additional CPHD patients were studied for mutations in exon 3 of the OTX2 gene. Novel mutations were structurally and functionally characterized.

Results: Two unrelated children, presenting with neonatal hypoglycemia, CPHD and ectopic posterior pituitary with anterior pituitary hypoplasia at MRI, were found to be heterozygous for a novel OTX2 mutation (N233S). Mutant protein did not lose its ability to bind to the bicoid-binding sites, but showed a significant decrease in HESX1 transactivation, suggesting a dominant negative effect of mutant Otx2 on the binding and action of the OTX2 wild-type copy at the HESX1 promoter.

Conclusion: A new heterozygous OTX2 mutation could have a dominant negative inhibitor effect on HESX1 gene expression, leading to both impairment of the correct spatiotemporal sequence required for pituitary cell lineage differentiation and, consequently, to absence or underdevelopment of the anterior pituitary gland with reduced hormonal expression.

OTX2 loss of function mutation causes anophthalmia and combined pituitary hormone deficiency with a small anterior and ectopic posterior pituitary

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Background: The OTX2 (orthodenticle homeobox 2) transcription factor is required for normal ocular and forebrain development. Heterozygous mutations of OTX2 were previously described in patients with severe ocular malformations.

Table 1. Summary of the main characteristics of three novel OTX2 mutations associated with hypopituitarism (molecular consequences, inheritance, clinical, biochemical and neuroradiologic phenotypes)

	Dateki et al. [J Clin Endocrinol Metab 2008;93:3697–3702]	Diazok et al. [J Clin Endocrinol Metab 2008;93:4351–4359]	Tajima et al. [J Clin Endocrinol Metab 2009;94:314–319]
Mutation	c.402insC	A698G (N233S)	c.576-577insCT (S136fsX178)
Type of mutation	Frameshift	Substitution	Frameshift Premature stop codon (codon 178)
Functional consequences	Loss of the transactivation domain Conservation of the homeodomain	Reduced transactivation activity Multiple bicoid binding site promoter	Severely reduced or absent transactivation of POU1F1 and HESX1, respectively Normal nuclear localization
Inheritance	De novo heterozygous mutation No dominant negative effects	De novo heterozygous mutation	De novo heterozygous mutation No dominant negative effects
Subjects	One Japanese female patient	Two unrelated children (case 1 male, case 2 female)	One Japanese male patient
Familial cases	None	None	None
Eye phenotype	Bilateral anophthalmia Optic nerve hypoplasia	Normal	Bilateral anophthalmia Optic nerve hypoplasia
Clinical phenotype	Cleft palate Short stature	Neonatal hypoglycemia hyperbilirubinemia, and poor feeding (case 1) Neonatal respiratory distress, hypoglycemia and seizures (case 2)	Neonatal hypoglycemia, hyperbilirubinemia and failure to thrive Short stature Severe developmental delay
Hormone defects	GH	GH TSH LH, FSH ACTH	GH TSH LH, FSH ACTH
MRI phenotype	Normal	APH EPP Absent or severely hypoplastic PS	APH EPP Absent PS Chiari malformation

APH = Anterior pituitary hypoplasia; EPP = ectopic posterior pituitary; PS = pituitary stalk.

Methods: This study offers a functional characterization of an OTX2 mutation associated with structural hypothalamic-pituitary abnormalities, combined pituitary hormone deficiency (CPHD) and anophthalmia. This mutation was found in a Japanese patient with short stature, anophthalmia, severe developmental delay and CPHD (GH, TSH, ACTH and gonadotropin deficiency). Brain MRI showed ectopic posterior pituitary, anterior pituitary hypoplasia, pituitary stalk agenesis and Chiari malformation.

Results: The patient carried a heterozygous two-base insertion [S136fsX178(c.576–577insCT)] in exon 3 of OTX2 (leading to a premature stop codone introduction), not detected in the patient's parents or in 50 Japanese healthy controls. This mutation determined a loss of function of Otx2 protein, which correctly localized to the nucleus but did not activate transcription of HESX1 and POU1F1 genes. A dominant negative effect was not involved in the pathogenesis of pituitary damage, because co-transfection of cultured cells with vectors containing both mutant and wild-type Otx2 lead to a normal HESX1 and POU1F1 expression.

Conclusion: OTX2 mutation is responsible in this patient for CPHD and structural pituitary abnormalities. Further studies of more patients with OTX2 defects could likely shed new light on the pathophysiology of ocular and pituitary diseases related to OTX2 mutations.

These three studies describe four children with heterozygote mutations of OTX2 associated with isolated or multiple pituitary hormone deficiencies (MPHD), as well as brain abnormalities. The stud-

ies focus on the role of OTX2, not only in eye development, but also in pituitary organogenesis and function (table 1).

The Otx-family of homeobox genes are vertebrate orthologs to the *Drosophila orthodenticle* homeobox gene and include the *Otx1* and *Otx2* genes. After gastrulation, *Otx2* is expressed in the prosencephalon, mesencephalon and cerebellum of the developing central nervous system. In addition, OTX2 mRNA has been found in the retinal pigment epithelium of human fetal retina, and to a lesser extent in the neural retina. Studies on heterozygous *Otx2*-deficient mice (*Otx2*^{+/-}) report various phenotypes including eyes lacking a lens, cornea, and iris, as well as anophthalmia or microphthalmia [2]. Indeed, mice homozygous for a null allele of *Otx2* are embryonic lethal due to severe brain abnormalities [2].

At this point, OTX2 represents an additional gene to be added to the long list of genes (LHX3, LHX4, HESX1, Prop1 and POU1F1, SOX2 and SOX3) that have previously been characterized and whose loss of function causes MPHD, either associated or not with extra-endocrine abnormalities. It is worth pointing out that in the presence of eye abnormalities and pituitary dysfunction, a differential diagnosis between OTX2 and SOX2 mutations and deletion of chromosome 14 del (14) (q22.1q23.1) should be ruled out [3, 4]. Whether OTX2 mutation might be associated with isolated GH deficiency, as reported in one of the studies' 4 patients, remains to be determined during follow-up for evolving pituitary hormone deficiencies.

Overhydration is harmful

Osmotic and nonosmotic regulation of arginine vasopressin during prolonged endurance exercise

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Background: Exercise-associated hyponatremia (EAH) is a relatively frequent condition caused by excessive fluid intake in relation to the kidneys' ability to eliminate excess fluid. The mechanisms involved in the regulation of maximal renal excretory ability during exercise are still not completely known. This study evaluated first the spontaneous secretion of pituitary, natriuretic and adrenal steroid hormones, and cytokines immediately before and after running an ultramarathon. In addition, the authors studied the mechanisms of osmotic and nonosmotic stimulation of arginine vasopressin (AVP) secretion in order to better understand hormonal control of fluid homeostasis during prolonged endurance exercise.

Methods: This was an observational study performed on 82 runners participating in a 56-km ultramarathon. They subjects underwent plasma sodium (Na⁺), plasma volume (estimated by a plasmatic protein based method) and plasma AVP [(AVP)p] measurements before and after the race, without any limitations on fluid intake.

Results: Mean marathon duration was 356 ± 4 min. During this time no significant variations were seen in plasma Na⁺ (139.3 ± 0.3 mmol/l before and 138.1 ± 0.4 mmol/l after the race), though there was a significant reduction in plasma volume (-8.5 ± 0.1%, p < 0.01) and significant increases (all p < 0.0001) in (AVP)p (3.9-fold), oxytocin (1.9-fold), brain natriuretic peptide (4.5-fold), and IL-6 (12.5-fold). Brain natriuretic peptide, oxytocin, and corticosterone variations accounted for 47% of the variance registered in (AVP)p and 13% of the variance in plasma Na⁺ in pathway analyses.

Conclusion: An important increase in (AVP)p during ultramarathon was found despite unchanged plasma Na⁺, suggesting a non-osmotic limitation of AVP suppression (i.e. osmotic independent AVP stimulation) during exercise, in which some hormones or cytokines may play a significant role. This mechanism may explain the frequent onset of EAH in athletes ingesting fluids in excess in relation to fluid output during prolonged endurance exercise.

Exercise-associated hyponatremia (EAH) has recently emerged as the most common life-threatening complication of endurance exercise. Although the primary cause of EAH is a relative overconsump-

tion of fluids beyond the ability of the kidneys to excrete excess fluid, the mechanisms that limit maximum renal excretory ability during exercise remain unknown. Detailed balance studies performed during recovery from an ultramarathon race show that runners with EAH excreted a large volume of dilute urine in contrast to finishers with normonatremia who excreted a small volume of highly concentrated urine; both groups had equivalent sodium losses as reflected by positive sodium balances during recovery. The change in serum sodium concentration after endurance exercise was inversely proportional to the change in body weight, and the athletes with EAH tended to gain weight during exercise. In fact, most runners with EAH are overhydrated as a result of excessive and perhaps ill-advised water ingestion over an extended race during which water excretion is limited by osmotically stimulated AVP secretion [5]. In the past, several athletes died from mismanagement of EAH until the use of a 3% hypertonic saline was established as the standard treatment [6]. This study explains the mechanism by which osmotic and nonosmotic regulation of arginine vasopressin during prolonged endurance exercise are associated with alterations in water homeostasis leading to EAH. Understanding the pathophysiology of water imbalance during exercise is of great importance for differentiating between dehydrated and overhydrated athletes and for managing those with symptomatic EAH so as to avoid unnecessary deaths.

The role of neurotransmitters in somatotroph proliferation

Neuronal M3 muscarinic acetylcholine receptors are essential for somatotroph proliferation and normal somatic growth

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Background: The molecular pathways involved in the proliferation and maintenance of pituitary cell lineages are not yet well understood. Pharmacological studies suggest that muscarinic cholinergic pathways play an important role in the control of GH secretion in both animals and humans. However, due to the presence of 5 distinct muscarinic receptor subtypes and their spreading distribution in the CNS, it has been difficult to elucidate their specific contribution by means of classic pharmacological tools. To better understand the role of the cholinergic system in the control of somatotroph function and GH secretion, the authors generated a set of mutant mice lacking the muscarinic type 3 receptor (Br-M3-KO).

Methods: Br-M3-KO mice were generated using Cre/loxP technology. These mice lack M3 receptors only in the brain as shown by combined radiological and binding/immunoprecipitation studies.

Results: Br-M3-KO mice did not display any significant changes in body composition or in glucose metabolism. They are dwarfs and show reduced serum GH and IGF-I levels, normal thyroid hormone and sex steroid concentrations and increased corticosterone and ACTH levels. They show pronounced hypoplasia of the anterior pituitary with normal brain weight. The pituitary content of GH and prolactin was markedly reduced with normal LH, FSH and TSH content. Stimulation with GHRH caused a markedly reduced GH response compared to control animals. M3 receptor expression was found in GHRH neurons, and hypothalamic GHRH and somatostatin levels were significantly reduced in Br-M3-KO mice. Treatment with a GHRH analog was effective in preventing the various hormonal and morphological characteristics of Br-M3-KO mice.

Conclusion: Mice lacking M3 muscarinic receptors have dramatic anterior pituitary hypoplasia associated with reduced pituitary GH and prolactin content. As a consequence, serum GH and IGF-I were markedly reduced leading to greatly reduced postnatal growth. These results revealed that M3 receptors play an important role in somatotroph proliferation and function and represent a potential pharmacologic target.

GH secretion is under the dual control of hypothalamic GHRH and somatostatin release. The release of these two hypothalamic hormones is, in turn, regulated by a complex network of neurotransmit-

ters and neuropeptides. GHRH is also a trophic hormone for the somatotrophs. Animals and humans lacking GHRH or its receptors show GH deficiency associated with marked pituitary hypoplasia and reduced pituitary GH content. The cholinergic system plays an important role in the control of GH secretion in humans. Several pharmacological studies have documented that central muscarinic cholinergic pathways play a role in stimulating GH release in humans. However, due to the presence of 5 diverse muscarinic receptors subtypes and to their overlapping distribution throughout the CNS, it was previously impossible to establish their exact role and relative contribution by means of classical pharmacological tools. This study elegantly shows that mice selectively lacking muscarinic receptor type 3 in the brain show profound anterior pituitary hypoplasia associated with reduced pituitary GH and prolactin content and reduced serum GH and IGF-I concentration. As a result, these animals show reduced longitudinal growth in postnatal life. Treatment with a GHRH analog is capable of preventing such alterations. These results are important in elucidating the role of the muscarinic receptor pathways in the control of GH secretion, and open a new window for a potential pharmacologic target.

New paradigms New weapons for gene hunting

Identification of candidate genes for human pituitary development by EST analysis

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Background: During embryonic development, the 6 specific pituitary cell types originate and differentiate under the control of various mechanisms. It is suggested that a large number of signal molecules and transcription factors essential for pituitary development are still unknown, and that there are important differences in gene expression profiles between mice and humans. This study reports differentially expressed genes from expressed sequence tag (EST) in fetal and adult pituitaries.

Methods: Total RNA was extracted from 2 human male fetal pituitaries. A cDNA library was constructed from a 16-week-old male fetal pituitary. All the ESTs were grouped into clusters of sequences thought to encode for one gene. The gene and ESTs were localized on chromosomes by searching the UniGene database.

Results: The transcription maps derived from the EST analysis revealed that developmentally relevant genes (Sox4, ST13 and ZNF185) were dominant in the cDNA library of the fetal pituitary, while hormones and hormone-associated genes (GH1, GH2, POMC, LH, CHGA, and CHGB) were dominant in the adult pituitary. Forty novel ESTs were identified.

Conclusions: The results of this study suggest a distinct pattern of gene expression between fetal and adult pituitaries. The transcriptome approach was proven to be an effective way of identifying novel genes involved in pituitary development.

Pituitary development, differentiation, and function are under the regulation of a complex network of transcription factors and, ultimately, depend on the secretion and function of hypothalamic releasing and inhibiting hormones. A number of transcription factors are expressed at different times in embryogenesis and affect the development of multiple cell lineages. It is likely that a large number of factors essential for pituitary development and cell differentiation and function have yet to be completely understood. In this study, a transcriptome approach using EST analysis revealed differences in gene expression between fetal and adult pituitaries but, most importantly, pituitary tissue was shown to express cytokines and their receptors, transcription factors, and those involved in the cell cycle, DNA replication and signal transduction, as well as 40 novel ESTs. These ESTs most probably reflect the expression of some yet unknown genes and microRNAs, particularly in fetal pituitary cells, which are involved in pituitary development and function.

p21(Cip1) restrains pituitary tumor growth

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Background: Pituitary tumorigenesis can be caused by disruption of the mechanisms controlling normal cell cycle. A number of genetic factors have been implicated in the formation of pituitary adenomas in humans and animals. Pituitary tumor-transforming gene (Pttg) is a mammalian securin that facilitates sister-chromatid separation during metaphase, and its overexpression causes cell transformation and promotes tumor formation. P21^{Cip1/Kip} is a transcriptional target of p53 and acts to constrain cell cycle, and may mediate either suppression or promotion of cell proliferation.

Methods: Triple mutant Rb^{+/-}Pttg^{-/-}p21^{-/-} mice were generated. Seventy-nine human pituitary adenomas of various phenotypes were analyzed for PTTG and p21 expression.

Results: p21 deletion restores pituitary tumor development in Rb^{+/-}Pttg^{-/-} mice and enhances pituitary cell proliferation. It also increases growth and transformation of Rb^{+/-}Pttg^{-/-} mouse embryonic fibroblasts. P21 is induced in GH3 cells overexpressing PTTG and in human pituitary tumors. GH-secreting pituitary adenomas also expressed markers of senescence.

Conclusions: This study shows that a proto-oncogene switches between oncogenic and tumor-suppressive modes depending on the genetic context. PTTG is abundantly expressed in human pituitary tumors, and behaves as either a tumor suppressor or oncogene depending on p21 status which controls cell cycle stability. Either deletion or overexpression of Pttg promotes pituitary cell aneuploidy and senescence. High levels of PTTG cause defective cell cycle progression and chromosomal instability, and promote pituitary tumor formation. Activation of pituitary DNA damage pathways triggers p21, which may restrain further growth and carcinogenesis.

Cell cycle control of pituitary development and disease

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This is a comprehensive review on the mechanisms controlling pituitary development. One of the key issues in tumorigenesis is loss of control of cell cycle. This article clearly describes how pituitary cells develop and differentiate as controlled by a number of factors, and how disruption of some of these pathways results in genomic instability leading to disease or tumor formation.

Understanding how cell cycle regulation controls pituitary cell homeostasis may help us to find new therapeutic strategies against pituitary diseases.

Regulation of the cell cycle is controlled by a number of factors, some of which are tissue- and cell-specific. These studies show that factors which play an important role in the control of cell cycle are involved in pituitary disease and tumorigenesis. Although pituitary adenomas are rare in children, it is believed that they are somehow more aggressive than in adult subjects. Prolactinomas, corticotropinomas or somatotroph adenomas may have serious adverse consequences on growth, puberty, bone formation, and cardiovascular and reproductive function. The diagnosis is often delayed. Understanding how these factors control pituitary development and how cell cycle regulation controls pituitary biology may help us to find new diagnostic and therapeutic tools.

Pituitary autoimmunity: 30 years later

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This is a review addressing the complex issues related to pituitary autoimmunity, a heterogeneous condition ranging from histologically confirmed lymphocytic hypophysitis to the incidental detection of pituitary autoantibodies in otherwise healthy subjects. The most frequent clinical presentation of this increasingly recognized condition is a sellar mass at pituitary MRI, which requires differential diagnosis from other nonfunctioning pituitary masses, primary among them pituitary adenoma. Noteworthy is the temporal association between hypophysitis and pregnancy or immunotherapies blocking CTLA-4. Many efforts have been made to identify pituitary autoantigens that could shed light on the pathophysiology of autoimmune hypophysitis, as well as be used for its diagnosis; it emerges from this review that even with these efforts, many challenges still remain. The authors of the review describe both the steps forward taken so far for comprising this condition and the several questions that remain unanswered as yet.

Novel autoantigens in autoimmune hypophysitis

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Background: Pituitary autoantibodies are associated with autoimmune hypophysitis and various other conditions. They have no extensive clinical application, principally because of a current lack of accurate laboratory methods for their detection. Such methods need to be based on the specific pituitary autoantigens against which autoimmune response is raised. Subsequently, the identification of pituitary autoantigens will lead to the construction of antigen-specific immunoassays.

Methods: The aim of the present study was to search for new pituitary autoantigens using sera as probes in proteomic assays. A comparison was also made between the immunoblotting and immunofluorescence methods to determine which was the most accurate for diagnosis of autoimmune hypophysitis. The authors analyzed 28 sera from subjects with autoimmune hypophysitis (14 histologically confirmed and 14 clinically suspected) and matched them with 98 control sera, obtained from 14 patients with pituitary adenomas, 15 with Graves' disease, 33 with Hashimoto's thyroiditis, and 36 healthy subjects. All sera were incubated with human pituitary cytosolic proteins separated by one-dimensional (1D) gel electrophoresis in order to identify the molecular weight regions which were more frequently recognized by hypophysitis sera. The corresponding proteins were then sequenced by means of immunoblotting and mass spectrometry. Furthermore, an immunofluorescence test was performed to evaluate the binding between sera and *Macaca mulatta* pituitary sections.

Results: Hypophysitis sera recognized a band in the 25- to 27-kDa region more frequently than healthy subjects ($p = 0.004$) or pituitary adenoma patients ($p = 0.044$). The band contains two proteins: chromosome 14 open reading frame 166 (C14orf166) and chorionic somatomammotropin. Immunoblotting positivity for the 25-to 27-kDa region showed better sensitivity (64 vs. 57%) and specificity (86 vs. 76%) than immunofluorescence for identifying subjects with histologically confirmed hypophysitis; its performance, however, was not sufficiently accurate to justify the introduction of immunoblotting into routine clinical practice.

Conclusion: Two candidate pituitary autoantigens were identified in this study, but the demonstration of their possible pathogenetic role in autoimmune hypophysitis and the development of an accurate laboratory assay for their detection both require further investigation.

T regulatory cells distinguish two types of primary hypophysitis

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Background: Primary hypophysitis is a frequently recognized condition, though further insights into its pathophysiology are needed to reach the goal of administering etiology-based treatment. The present study aimed to conduct further research into the pathogenesis of primary hypophysitis.

Methods: The authors used immunohistochemistry to characterize the immune cells detected at histological examination in pituitary biopsies performed for suspected 'lymphocytic' hypophysitis. In particular, they analyzed 2 cases at the clinical, cellular and molecular levels, showing that at least 2 main types of lymphocytic hypophysitis exist. The first case was a 29-year-old female subject presenting with diabetes insipidus, headache, menstrual irregularities, galactorrhea and an enlargement of the anterior pituitary and pituitary stalk at MRI. The second was a 52-year-old man with headache, combined pituitary hormone deficiencies (including suspected diabetes insipidus for polyuria and polydipsia) and a pituitary mass dislocating the optic chiasm. These subjects underwent transsphenoidal pituitary resection and histological examination revealed lymphocytic hypophysitis in both cases.

Results: The first entity is compatible with the classical description of autoimmune lymphocytic hypophysitis associated with an imbalance between T-helper and T-regulatory cell response in favor of T-helper (particularly the so-called 'Th 17 cells', expressing IL-17). On the other hand, the second picture is more complex, with a predominant role of T-regulatory cells that could act in the control of an immune response directed against non-self antigens, suggesting an homeostatic immune process of either infectious or infiltrative etiology.

Conclusion: These data focus attention on two different pathogenetic mechanisms of primary hypophysitis. In light of a possible medical treatment for this condition, the authors showed that in a particular case, active autoimmune process could benefit from immunosuppressive treatment, whereas in the immune homeostatic process, immunosuppression is not indicated. Naturally, to identify the type of autoimmune process involved in a single case and to establish a pathogenetic-driven treatment, pituitary biopsy is needed. Thus, these preliminary data may in the future induce all of us to revise our diagnostic and therapeutic algorithms for suspected hypophysitis.

These 3 papers address comprehensively the role of autoimmunity in determining anterior and posterior pituitary dysfunction in a condition encompassing comparable, albeit less common, pediatric and adult entities. Indeed, the hypothalamic-pituitary stalk and posterior pituitary-anterior pituitary unit have been reported as targets of autoimmune processes, i.e. lymphocytic hypophysitis, lymphocytic infundibulo-hypophysitis or lymphocytic infundibulo-neurohypophysitis, affecting both anterior and posterior pituitary function. Despite histological evidence of tissue lymphocyte infiltration, the role of humoral autoimmunity in lymphocytic hypophysitis has been debated since the first description in the early 1980s of vasopressin cell autoantibodies by Scherbaum and Bottazzo [7]. Moreover, the precise pathogenetic role of these autoantibodies has never been demonstrated, regardless of the high number of clinical studies that report a high prevalence of both anterior pituitary autoantibodies and antibodies against vasopressin cells. One pitfall may be ascribed to the methodology of detecting and measuring these autoantibodies, and the other one to the fact that specific autoantigens have not yet been recognized.

In the study by Caturegli's group, the identification of two candidate autoantigens which might play a direct role in autoimmune hypophysitis on the one hand, and the recent experimental induction of lymphocytic hypophysitis in mice on the other hand [8], open the door to the development of accurate assays for the detection of autoantibodies. In addition, drug induction of autoimmune hypophysitis by cytotoxic T-lymphocyte-associated antigen-4 blockage using the human anti-CTLA4 monoclonal antibody CP-675,206 for the treatment of melanoma [9] is a confirmation of the direct role of T-cell immunity. Finally, the identification by Mirocha et al. of two different potential pathogenetic mechanisms, i.e. one potentially directed against self-antigens and the other against non-self antigens (post-infection), both of which induce primary hypophysitis, adds another piece to the puzzle of these intriguing conditions. Thus, patients with an autoimmune-driven hypophysitis would benefit from steroids or other immunosuppression treatment, whereas immunosuppression may exacerbate conditions that are not autoimmune. Pituitary biopsy, although invasive, is currently the

only option we have to determine the exact pathogenesis and may be warranted in some cases in order to provide correct treatment.

Not only for steroidogenesis

Central nervous system-specific knockout of steroidogenic factor 1

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Background: Steroidogenic factor-1 (SF-1) is a member of the nuclear hormone receptor family of transcriptional regulators and is an essential regulator of the enzymes involved in the steroidogenic pathway. SF-1 is also important in the establishment and function of endocrine axes. Analysis of global SF-1 KO mice confirmed its role in endocrine development in the ventromedial hypothalamus (VMH), adrenals, and gonads. The VMH regulates sexual behavior, energy homeostasis, thermogenesis, and cardiovascular function, and is the only region of SF-1 expression in the mouse brain. This review discusses the functional role of SF-1 in the brain.

Methods: To understand the specific role of SF-1 in the brain, CNS-specific SF-1 KO mice were generated using Cre-loxP technology.

Results: SF-1 KO mice have increased anxiety-like behavior, and a number of novel SF-1-responsive genes related to anxiety-like behavior were identified, including corticotrophin-releasing hormone receptor 2 (*crhr2*). This finding indicates that *crhr2* may be a direct target of SF-1 in the VMH. Cannabinoid receptor 1 (*CB1R*) was also found to be a SF-1-responsive gene in the VMH, indicating that SF-1 is implicated in energy homeostasis at the level of the VMH.

Conclusions: This paper has defined specific roles of SF-1 in the function of the VMH in anxiety-like behavior and in energy homeostasis, independent of any effects on steroid hormones.

Genetic modification of experimental animals has been helpful for elucidating diverse aspects of physiology. Generation of animal models that selectively lack the expression of one or more genes, either globally or in selected areas, is extending our understanding of endocrine physiology. Steroidogenic factor-1 (SF-1; officially named NR5A1) was originally identified and characterized as an essential regulator of the enzymes that make steroid hormones in steroidogenic cell lines. Subsequently, several groups have reported that SF-1 is a key regulator in the establishment and function of endocrine organ axis. It is now assigned with a new CNS function. This review reports the results of studies in mice selectively lacking SF-1 in the VMH, showing that this regulator of transcription participates in the control of anxiety-like behavior and energy homeostasis, independent of any effects on steroid hormones.

More on the fetal origin hypothesis

Early postnatal nutrition determines somatotrophic function in mice

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Background: Abundant evidence suggests that early life events have consequences for later disease. A number of diseases seem to have a developmental origin in both humans and animals. Epidemiological studies in humans have shown a correlation between perinatal food deprivation and obesity, type II diabetes, hypertension, alterations in pituitary-adrenal-axis function, and cardiovascular disease. Likewise, pre-

natal and postnatal events in experimental animals can determine adult diseases. A general notion is that the growth-retarded fetus will adapt to the intrauterine environment by modifying endocrine and metabolic pathways, and these modifications may persist postnatally. This study investigated the effects of early postnatal underfeeding or overfeeding on the growth and GHRH/GH/IGF-I axis function in mice.

Methods: Under- or overfeeding was induced in mice by cross-fostering. Endocrine regulation of growth and the development of specific adult diseases were investigated.

Results: The first effects of food restriction or overfeeding were growth retardation and deceleration, respectively. Differences in body weight and length persisted into adulthood despite normal feeding after weaning. Blood glucose was lower in the underfed than in the overfed animals, and serum insulin and leptin were markedly low in food-restricted mice and very high in those that were overfed. Glucose and leptin concentrations normalized with free access to food, while insulin concentrations did not readily normalize. Both overfeeding and underfeeding led to reduced glucose tolerance and hypertension later in life, suggesting long-term modifications in energy metabolism and cardiovascular impairment. The effects of food restriction and overfeeding on growth were paralleled by changes in pituitary GH content, circulating IGF-I and ALS, and hypothalamic GHRH gene expression. These alterations were consistent with the observed phenotypes, and persisted throughout adulthood.

Conclusion: This study shows that early postnatal food restriction or overfeeding induces permanent alterations in growth and the GHRH/GH/IGF-I axis. In addition, metabolic and cardiovascular alterations were also observed, consistent with the hypothesis that early perinatal nutritional events may be linked to adult disease.

This is an elegant study which lends further support to the 'fetal origin of adult health and disease hypothesis'. A number of epidemiological studies in humans have linked fetal malnutrition to postnatal growth and adult disease. This study shows that in mice adverse effects on growth may be mediated by modifications in GHRH/GH/IGF-I axis function, and that these modifications persist up to adulthood. In addition, features of the metabolic syndrome were reproduced in this experimental model of under- or overfeeding. Most children with IUGR catch up, and show no subsequent growth disorder, but it may initiate a process that eventually leads to an increased incidence of the metabolic syndrome. These findings may have clear implications for children with intrauterine growth retardation.

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