Pituitary

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This was yet another year with some breakthrough papers in the field of pituitary research. Mutations in the X-linked gene *IGSF1* were found to underlie central hypothyroidism with macro-orchidism, although the function of the gene is still unknown. Another paper showed how Pax7 controls melanotrope lineage differentiation through chromatin modeling, allowing Tpit to bind regulatory regions for melanotrope differentiation. For the first time, inactivating *ACTH* mutations were found as a cause for adrenal insufficiency. Following on from the impressive work on the role of oxytocin in the development of the neurovascular interface of the pituitary is work on FGF3 and FGF10 showing that these growth factors are involved in both axonal guidance and hypophyseal vascularization. There were several clinical papers on Cushing's syndrome – one showing the outcome of surgery in Cushing's disease in an impressive number of patients (n = 200) from a single center, another on the recovery of the adrenal axis after treatment for Cushing disease, and a state-of-the-art review on the diagnosis and management of Cushing's syndrome. Work on SOX2 always continues and this year there are papers on SOX2 and pituitary stem cells, SOX2 and pituitary development and SOX2 and embryonic stem cell differentiation. Lots to choose from!

Mechanism of the year

The selector gene Pax7 dictates alternate pituitary cell fates through its pioneer action on chromatin remodeling

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Background: During organogenesis, selector genes work by defining positional identity so that terminal differentiation within domains or tissue compartments can occur. One example of selector genes in the central nervous system is the Pax family of transcription factors. Thus in the neural tube, Pax3 and Pax6 are expressed dorsally and confer dorsal identity so that dorsal neural tube cell types can terminally differentiate. The molecular mechanisms by which these selector genes control the transcriptional programs remain elusive to date. The pituitary gland is an ideal organ to study the mechanism of terminal differentiation as it contains six secretory cell types. A number of transcription factors important for terminal differentiation of the adult pituitary gland have been identified, and amongst these is Tpit, a T-box-containing transcription factor which is critical for POMC (propiomelanocortin) transcription. POMC is expressed in two lineages: the corticotropes in the anterior pituitary (AP) gland and the melanotropes in the intermediate lobe (IL). Inactivation of *Tpit* leads to absence of POMC-expressing cells of both the melanotrope and the corticotrope lineages and *TPIT* mutations in humans cause isolated ACTH deficiency in children. How Tpit acts to generate two lineages of distinct identity, namely the melanotropes and corticotropes, remains unknown. This article identifies, for the first time, the transcription factor Pax7 as a selector gene switch that promotes melanotrope-specific genes at the expense of corticotropes, hence providing a novel mechanism by which selector genes act during terminal differentiation.

Methods: Expression profiling of differentially expressed genes between the IL and AP. Expression profiling to identify the melanotrope and corticotrope-specific cell lineages. Analyses of transgenic mice

mutant for Pax7 and Tpit to assess the differentiation potential of these transcription factors. ChIP-Seq and FAIRE sequencing to identify chromatin remodeling and transgenic Pax7 gain of function using the Pitx1 promoter.

Results: Pax7 was differentially expressed in the intermediate lobe of the pituitary gland preceding *Tpit* and *POMC* expression. The restricted expression pattern of Pax7 in the IL suggests a role for this factor in melanotrope differentiation. Indeed, *Pax7* null embryos exhibited decreased *POMC* and *PC2* expression specifically in the melanotropes of the IL, whilst expression of the *Tpit* lineage marker remained unchanged. Interestingly, there was a change from melanotrope to corticotrope fate in the absence of Pax7, as seen by the change of the transcriptional signature of IL-expressing genes in the *Pax7*^{-/-} pituitaries. Overexpression of *Pax7* under the control of the *Pitx1* promoter led to exhaustion of the progenitor cell pool by promoting their differentiation. Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) and FAIRE (formaldehyde-assisted isolation of regulatory elements) sequencing revealed regions of accessible DNA; interestingly, Pax7-expressing cells exhibited active chromatin marks, consistent with a role for Pax7 in chromatin remodeling that would allow *Tpit* binding to certain promoter regions that promote melanotrope fate. This chromatin remodeling effect makes Pax7 a pioneer transcription factor with a role in promoting binding of *Tpit* to certain enhancers of the melanotrope lineage differentiation.

Conclusion: Taken together, these findings clearly identify a novel mechanism by which *Tpit*, through the selector gene *Pax7*, acts to generate two distinct lineages within the pituitary gland – the melanotropes and corticotropes. *Pax7* acts as a selector gene that functions as a genetic switch to promote melanotrope lineage. This is accomplished through its pioneering activity by changing chromatin conformation at specific genomic loci, so that *Tpit* can bind and activate melanotrope lineage-specific genes.

In this excellent piece of work from Jacques Drouin's laboratory, a new molecular mechanism that controls the specification of two distinct cell lineages, namely melanotropes and corticotropes, by the same transcription factor *Tpit*, has been established. Melanotropes and corticotropes both express *POMC*, but melanotropes are located in the intermediate pituitary lobe and cleave *POMC* to α -MSH, and corticotropes are located in the anterior lobe. In a series of elegant studies, Pax7 is shown to be a selector gene that confers cell identity towards the melanotrope lineage. Hence, the question of how a single transcription factor *Tpit* governs the differentiation of the two distinct *POMC* lineages has been answered. In this process, Pax7 exerts pioneering activity by chromatin remodeling specific sites so that *Tpit* can transcriptionally activate the melanotrope gene expression program. In the absence of *Pax7*, melanotrope differentiation fails to occur despite *Tpit* expression being unchanged. The mechanism reported in this paper provides us with a novel molecular mechanism by which the pituitary is built. As is the case with all good papers, it opens doors to some important questions: Is there a selector gene for the corticotrope lineages too? What is the specific role of Pax7 in some of the reported Pax7-positive Cushing adenomas?

New gene – but what does it do?

Loss-of-function mutations in IGSF1 cause an X-linked syndrome of central hypothyroidism and testicular enlargement

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Background: Congenital central hypothyroidism (CH) occurs either in isolation or in conjunction with other pituitary hormone deficits. Isolated CH is rare and has been described in association with mutations in thyrotropin-releasing hormone receptor (*TRHR*) or *TSH β* .

Methods: Exome and candidate gene sequencing was used to identify the molecular basis of central hypothyroidism in patients in whom sequencing of known causes of CH had failed to establish the molecular basis.

Results: The authors identified 8 distinct mutations and 2 deletions in *IGSF1* in males from 11 unrelated families with central hypothyroidism, testicular enlargement and variably low prolactin concentrations. *IGSF1* encodes a membrane glycoprotein that is highly expressed in the anterior pituitary gland. Wild-type and mutant forms of *IGSF1* were expressed in heterologous cells, and all of the mutants exhibited immature patterns of glycosylation relative to wild-type, suggestive of their retention in the endoplasmic reticulum. Immunofluorescence and cell-surface biotinylation analyses confirmed that the mutant proteins were either blocked or significantly impaired in their trafficking to the plasma membrane. *Igsf1*-deficient (*Igsf1* Δ^{ex1}) male mice showed diminished pituitary and serum thyroid-stimulating hormone (TSH) concentrations, reduced pituitary thyrotropin-releasing hormone (TRH) receptor expression, decreased triiodothyronine concentrations and increased body mass.

Conclusions: The authors describe a new X-linked disorder, in which loss-of-function mutations in *IGSF1* cause central hypothyroidism, possibly secondary to an associated impairment in pituitary TRH signaling.

This paper reports a novel syndrome characterized by central hypothyroidism and macro-orchidism. Whole exome sequencing performed independently by two groups led to the identification of loss of function mutations in *IGSF1*, a membrane glycoprotein that is expressed in the pituitary. The function of the gene remains unknown, however a murine model demonstrated a subtle form of hypothyroidism consistent with the human phenotype. Additionally, given the suboptimal FT₄ and TSH responses to TRH, and the reduced expression of *Trhr* in mutant mice, it is likely that *IGSF1* in some way is implicated in TRH signaling. Additionally, *Igsf1* is expressed in human and murine testis, but enlargement of the testes was not identified in the mutant mice. This might reflect a species difference. In humans, 5 of 20 female carriers were affected with mild central hypothyroidism. Three of the patients manifested GH deficiency that later reversed, and 18 of 26 cases were prolactin-deficient, in keeping with the detection of *IGSF1* protein in mouse lactotropes, somatotropes and thyrotropes, but not in gonadotropes. Importantly, identification of the molecular basis of central hypothyroidism in the probands led to the identification of further members of the family in whom central hypothyroidism had previously been undetected, with subsequent commencement of thyroxine.

Further studies will shed light on this unusual phenotype, in particular the GH deficiency. (Is it truly present, is it reversible, is the GH secretory pattern disturbed?), and the macro-orchidism. Additionally, it is likely that the murine mutant used in this study, in which exon 1 was deleted from the *Igsf1* gene, may act as a hypomorph as opposed to a complete loss of function. Hence, conditional transgenesis with a complete knock-out of the gene leading to complete loss of function might shed more light on the role of this gene in mouse and human.

New mechanism

Direct and indirect roles of Fgf3 and Fgf10 in innervation and vascularization of the vertebrate hypothalamic neurohypophysis

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Background: Homeostasis of the adult organism is accomplished by secretion of hormones into the bloodstream by the hypothalamic-pituitary (HP) axis. In this process neurohormones and neurotransmitters are released from axonal terminals into the vascular network that in turn targets the anterior pituitary gland and peripheral organs. To achieve this, the hypothalamo-neurohypophysis neurons

(H-NH) have to establish congruent connections with the blood vessels. This process is genetically regulated and starts during embryogenesis with the innervation of the prospective hypothalamic area, where parvocellular and magnocellular neurons project their axons that will control the secretion of hormones of the anterior pituitary gland. The mechanism that controls the innervation and the vascularization of the neurohypophysis is poorly understood. In this article, 2 members of the fibroblast growth factor family of secreted proteins, Fgf3 and Fgf10, are shown to exert an axonal chemoattractive role of H-NH neurons as well as to promote NH vascularization by promoting endothelial growth.

Methods: To understand the role of *Fgf3* and *Fgf10* during hypothalamic development, the authors used in situ hybridization, complemented with ex vivo embryonic explant culture of the prospective hypothalamic area. Fgf3/Fgf10 blocking antibodies and chemical compounds were used to inhibit Fgf signaling. The authors studied the innervation and vascularization of transgenic mutant *Fgf3* zebrafish, with temporal and conditional ablation of Fgf3 signaling using a dominant negative Gal4-UAS transgenic fish line.

Results: *Fgf3* and *Fgf10* were expressed during embryogenesis in the prospective hypothalamic regions where the H-NH neurons send their axonal projections. This expression pattern is at its peak when axons invade the NH, suggesting a role for these molecules as axonal chemoattractants. In order to further explore this possibility, ex vivo chick explants of prospective NH explant were co-cultured at a distance from the axonal source. This resulted in attraction of axonal projections towards the explant, thus indicating that naive NH secretes chemoattractive molecules. This chemoattraction is mediated through Fgf signaling, as inhibition of Fgf by SU5402, a chemical inhibitor, stopped axonal projections. Moreover, NH explants treated with either Fgf3 or Fgf10 blocking antibodies resulted in abrogation of axonal extension, indicating that innervation of the NH is mediated by Fgf3/Fgf10. However, treatment with high doses of Fgf10 halted axonal projections indicating that, at lower doses, Fgf10 functions as a chemoattracting agent whilst at higher doses it stops axonal projections. Beads soaked with recombinant Fgf10 and Fgf3 elicited the same effect, further supporting a role for these molecules in NH innervation. Interestingly, the prospective NH can elicit endothelial capillary vessel formation in vitro when co-cultured with chorioallantoic membrane. Only explant from early stages can elicit this function, which is abrogated by blocking of Fgf3 and Fgf10 antibodies, indicating that prospective HP can promote vascularization of NH in a time-restricted fashion. In order to assess the role of these molecules in vivo, mutant transgenic fish that lack Fgf3 showed absence of NH innervation accompanied by vascular abnormalities, further demonstrating that Fgf3 is required for innervation of the NH. Moreover, using a cell-specific blockade of Fgf signaling by expressing a dominant negative form of Fgf3 in endothelial cells resulted in vascularization defects, further demonstrating the in vivo role of Fgf3 in the wiring and vascularization of the NH.

Conclusions: Fgf3 and Fgf10 control NH innervation at early stages by acting as a chemoattractant for H-NH axons. Simultaneously they promote hypophyseal vascularization at early stages by affecting growth of endothelial cells.

Little is known about the molecular mechanism governing the formation of the posterior pituitary and hypothalamus. Axons from both the parvocellular and magnocellular neurons have to establish congruent connections with the blood vessels for the proper secretion of hormones into the circulation. In this elegant study, Marisa Plackzec and co-workers identify for the first time a dual novel role for Fgf3/Fgf10 in innervation and vascularization of the prospective hypothalamic region, already at early developmental stages. Using both in vitro assays and chick explant assays, they were able to demonstrate that the prospective hypothalamic region secretes Fgf3 and Fgf10 that act as chemoattractive cues to neurohypothalamic neurons. Moreover, Fgf3 in both chick and zebrafish acts on endothelial cells to initiate vasculogenesis of the posterior pituitary gland.

Last year, we discussed the paper by Gutnick et al. [1], which reported that oxytocin functions as a blood vessel attractant to establish the neurovascular connection of the posterior pituitary. Two new factors, namely Fgf3 and Fgf10, now emerge as main contributors to both wiring and vascularization of the hypothalamus.

Bioinactive ACTH causing glucocorticoid deficiency

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Background: Severe cortisol deficiency associated with elevated concentrations of ACTH is usually due to primary adrenal failure due to a number of causes including developmental disorders, autoimmune disease or infection. Other causes include familial glucocorticoid deficiency (FGD), which can be due to mutations in the genes *MC2R*, *MRAP*, *MCM4*, or *NNT*.

Methods: The authors aimed to identify the molecular basis of the adrenal insufficiency in a 4-year-old girl and a 4-month-old boy who presented with hypoglycemia, normal electrolytes, low cortisol, and high ACTH. Both patients manifested red hair and obesity. A diagnosis of primary adrenal insufficiency was made and initial treatment was commenced with glucocorticoids and mineralocorticoids. The genes known to cause FGD were normal. Following the identification of mutations in the gene encoding *POMC* using whole exome sequencing, wild-type and mutant ACTH and α -MSH peptides were studied in functional assays of receptor binding and cAMP production. Methods included peptide synthesis, ACTH immunoradiometric assay, hormone binding, and activation assays in cells expressing melanocortin receptors.

Results: Exome sequencing identified compound heterozygosity for *POMC* mutations in the girl: one previously described null allele and one novel p.R8C mutation in the sequence encoding ACTH and α -MSH. The boy was homozygous for the p.R8C mutation. ACTH-R8C was immunoreactive, but failed to bind and activate cAMP production in melanocortin-2 receptor (*MC2R*)-expressing cells, and α -MSH-R8C failed to bind and stimulate cAMP production in *MC1R*- and *MC4R*-expressing cells.

Conclusions: This is the first report of glucocorticoid deficiency due to the secretion of an ACTH molecule that lacks biological bioactivity but conserves immunoreactivity, i.e. a bioinactive form of ACTH. *POMC* mutations should thus be considered in patients presenting with apparent ACTH resistance. The findings underline the limitations of immunoassay-based diagnostics and demonstrate the value of genetic analysis. This study also emphasizes the importance of establishing the molecular etiology of the adrenal insufficiency in these 2 individuals, which then allowed cessation of unnecessary mineralocorticoid therapy. Finally, discovery of this mutation indicates that in humans, the amino acid sequence His⁶Phe⁷Arg⁸Trp⁹ is important not only for cAMP activation but also for ACTH binding to *MC2R*.

The authors show that mutations in *POMC* can be associated with a phenotype characterized by low cortisol concentrations and elevated ACTH concentrations. To date, mutations in *POMC* have been identified in 10 patients, all of whom have low or undetectable plasma ACTH concentrations. It is now clear that the phenotypic spectrum can be expanded to include patients with a high ACTH concentration and preservation of mineralocorticoid secretion. Hence the phenotype is similar to that of familial glucocorticoid deficiency, except that, in this study, both patients had red hair and obesity, features which are indicative of *POMC* mutations. The p.R145C missense *POMC* mutation was identified in both probands, and was included in a sequence common to ACTH and α -MSH. The mutation led to a reduction in both cAMP production and binding affinity to *MC2R* (ACTH) and *MC1R* and *MC4R* (ACTH, α -MSH), showing that the amino acid sequence HFRW is essential for ACTH and α -MSH binding to their respective receptors and for cAMP generation as well; in contrast, previous animal studies had suggested that binding of ACTH was dependent on the KKRRP amino acid sequence only [2]. The study shows the importance of keeping an open mind when patients present with unusual phenotypes that are not consistent with known genetic mutations. Ideally, a combination of clinical, biochemical, and genetic data are necessary to understand such unusual clinical syndromes.

Variations in PROKR2, but not PROK2, are associated with hypopituitarism and septo-optic dysplasia

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Background: Loss-of-function mutations in *PROK2* and *PROKR2* have been implicated in Kallmann syndrome (KS), characterized by hypogonadotropic hypogonadism and anosmia. Recent data suggest overlapping phenotypes/genotypes between KS and congenital hypopituitarism (CH), including septo-optic dysplasia (SOD). Mutations in *FGF8* and its receptor *FGFR1* have recently been implicated in the etiology of defects of midline brain development as well as KS/isolated hypogonadotropic hypogonadism (IHH), as have *PROKR2* variants.

Methods: To further characterize the role of *PROKR2* in hypothalamo-pituitary development, the authors screened a cohort of patients with complex forms of CH (n = 422) for mutations in *PROK2* and *PROKR2*, using direct sequencing. Subsequently, a number of functional studies were performed to investigate the cell surface expression of *PROKR2*. *Prokr2*^{-/-} null mutant mice were analyzed using immunocytochemistry against a number of pituitary terminal differentiation markers.

Results: No mutations were identified in *PROK2*. Five *PROKR2* variants were identified in 11 patients with SOD/CH: the novel p.G371R and previously reported p.A51T, p.R85L, p.L173R, and p.R268C. The latter three mutations have previously been associated with impaired function of the protein. Functional studies of the p.G371R substitution revealed no compromise in function compared to the wild-type protein. Surprisingly, a patient with SOD was heterozygous for the p.L173R variant, whereas his phenotypically unaffected mother was homozygous for the variant. Analysis of *Prokr2*^{-/-} mice revealed predominantly normal hypothalamo-pituitary development and terminal cell differentiation, with the exception of reduced LH; this was inconsistent with patient phenotypes and more analogous to the healthy mother. Importantly, the mother did not have KS, unlike the *Prokr2*^{-/-} mice.

Conclusions: The role of *PROKR2* in the etiology of CH, SOD, and KS is unclear, as demonstrated by the lack of a phenotype-genotype correlation, and the association of clinical phenotypes with loss-of-function variants in both heterozygosity or homozygosity. The authors report a phenotypically normal parent, homozygous for the functionally deleterious p.L173R substitution. These data suggest that the variants identified here are unlikely to be implicated in isolation in these disorders; other genetic or environmental modifiers may also impact on the etiology. Given the phenotypic variability, genetic counseling may presently be inappropriate.

PROKR2 variants in multiple hypopituitarism with pituitary stalk interruption

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Background: Pituitary stalk interruption syndrome (PSIS) represents a frequent feature of congenital hypopituitarism, but only rarely has a genetic cause been identified. To date, mutations in *HESX1*, *LHX4*, *SOX3* and *OTX2* have been implicated in children with PSIS. The authors hypothesized that an ectopic posterior pituitary may be a consequence of defective neuronal axon projections along the pituitary stalk or defective angiogenesis of the hypophyseal portal circulation, due to defects in the prokineticin 2 pathway.

Methods: *PROK2* and *PROKR2* and all genes previously known to be involved in hypopituitarism with pituitary stalk interruption (*LHX4*, *HESX1*, *OTX2*, and *SOX3*) were screened for mutations in 72 index cases with pituitary stalk interruption syndrome from the GENHYPOPIT database. In vitro studies were performed to assess the functional consequences of allelic variants, using intracellular calcium

mobilization assays and cell surface expression studies for *PROKR2* and DNA binding studies and transcriptional repression assays for *HESX1*.

Results: The authors identified two heterozygous *PROKR2* mutations (p.Leu173Arg and p.Arg85His), previously reported in Kallmann syndrome or isolated hypogonadotropic hypogonadism, and a novel *PROKR2* variant (p.Ala51Thr). The p.Ala51Thr variant did not have a deleterious effect on the protein, whereas the p.Leu173Arg substitution led to impaired cell surface targeting of the receptor and the p.Arg85His substitution affected G protein coupling of the receptor. Three allelic variants of *HESX1* were identified: the heterozygous p.Phe156Ser and the homozygous p.Arg109X mutations were functionally deleterious, whereas p.Ser67Thr was found as a rare allelic variant in association with the p.Arg85His *PROKR2* mutation in the same patient.

Conclusions: The authors reported *PROKR2* variants in patients with congenital hypopituitarism with pituitary stalk interruption, suggesting a potential role of the prokineticin pathway in pituitary development.

The above pair of papers shed interesting and novel insights into the etiology of congenital hypopituitarism and the role of *PROKR2* therein. Given the role of other genes implicated in the etiology of Kallmann syndrome (KS) in complex midline abnormalities of the brain and hypothalamo-pituitary region [3, 4], it made sense to look for mutations in *PROKR2* and its ligand *PROK2*. Surprisingly, both groups reported a relatively high incidence of genetic variants in *PROKR2* (2% of a large complex cohort of 422 patients with hypopituitarism/SOD in the study by McCabe et al., 2.8% in a study of 72 patients with pituitary stalk interruption in the paper by Reynaud et al.). Importantly, many of the variants had been shown previously to be implicated in KS, and to lead to a loss or impaired function. As observed previously, mutations may be homozygous or heterozygous, and variably penetrant. In the article by Reynaud et al., the unaffected mother and sister of case A.II.1 both harbored the same genetic change, namely the functionally significant p.Leu173Arg, as did the asymptomatic father of cases B.II.1 and B.II.3 who harbored the same change as his 2 daughters (p.Ala51Thr, possibly a polymorphic variant). Most surprisingly, the unaffected mother of patient VI in the article by McCabe et al. was homozygous for the p.Leu173Arg variant, yet had no evidence of KS, IHH or hypothalamic amenorrhea. However, patient VI had evidence of SOD. These data suggest possible digenic/oligogenic inheritance. Recently, Avbelj Stefania et al. [5] reported that the *PROKR2* L173R represents a founder mutation whose age is estimated at approximately 9,000 years. Inheritance of *PROKR2* L173R-associated GnRH deficiency is complex with highly variable penetrance among carriers, influenced by additional mutations in the other *PROKR2* allele (recessive inheritance) or another gene (digenicity). Indeed, Reynaud et al. reported that a further child born to consanguineous parents (case C.II.1) harbored the previously described p.Arg85His variant in *PROKR2* in association with the p.Ser67Thr variant in *HESX1*. The authors suggest an oligogenic inheritance, however they found no deleterious effect of the *HESX1* variant, and so this conclusion must remain speculative.

McCabe et al. also examined the hypothalamus and pituitary of *Prokr2*^{-/-} null mutant mice, and reported no reduction in expression of terminal differentiation markers apart from LH, as described previously [6]. The pituitary glands were small, in keeping with a reduction in the overall size of the animals. Together with the data from human patients with *PROKR2* mutations, these findings suggest that *mProkr2* is dispensable for proper formation of the hypothalamic-pituitary axis. This is in keeping with data in humans that also suggest that *PROKR2* mutations are not, in isolation, causative of KS, IHH, hypothalamic amenorrhea and SOD/CPHD. They are likely, however, to contribute to the various phenotypes in combination with other genetic mutations and/or environmental factors, a rich area for future research.

The adult pituitary shows stem/progenitor cell activation in response to injury and is capable of regeneration

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Background: In this paper, a transgenic mouse model was constructed to conditionally ablate somatotropes to investigate the regenerative capacity of the adult pituitary and the response of its stem/progenitor cell compartment to damage.

Methods: GHCre/iDTR mice express diphtheria toxin (DT) receptor only after transcriptional activation by GH promoter-driven Cre recombinase. Treatment with DT for 3 days indeed resulted in gradual somatotrope ablation with a final loss of 80–90% 1 week later.

Results: The stem/progenitor cell-clustering side population expanded after injury, concordant with an increase in Sox2⁺ stem/progenitor cells. In addition, folliculo-stellate cells, previously designated as pituitary stem/progenitor cells and significantly overlapping with Sox2⁺ cells, also increased in number. Remarkable was the appearance of Sox2⁺ cells that contained GH. GH⁺ cells considerably regenerated during the months after the toxin. Double Sox2⁺/GH⁺ cells are observed throughout the regenerative period, which could support the recovery of somatotropes from stem/progenitor cells, further supported by 5-ethynyl-2'-deoxyuridine (EdU) pulse-chase lineage tracing.

Conclusion: This study shows that the adult pituitary gland is capable of regeneration and suggests that the stem/progenitor cells are involved in the repopulation of the gland.

In various adult organs, stem cells play a significant role in tissue repair after injury. The pituitary gland has enormous plasticity in terms of regulation of hormone-producing cells, for example the rise in lactotrope number during pregnancy and lactation. Whether the pituitary gland is capable of cell regeneration after cell loss at adult age is not clear.

Sox2⁺ cells localized in the marginal zone around the pituitary cleft, which is a remnant of Rathke's pouch, are believed to be pituitary progenitors. They do not normally express genes encoding pituitary hormones. The authors used a neat system where diphtheria toxin (DT) receptor is expressed in somatotropes only, so that DT treatment will ablate somatotropes only. Three days after DT-induced somatotrope ablation, the 'stem cell – side population', artificially defined as 'non-Sca-1-immunoreactive cells of the Hoechst dye effluxing side population', expanded and the number of Sox2⁺ cells increased. In contrast to the situation before ablation, these Sox2⁺ cells were proliferating. In addition, folliculo-stellate cells were proliferating and SOX2⁺/GH⁺ cells started to appear. The authors suggest therefore that GH-positive cells arise from the increased number of stem/progenitor cells in the pituitary after injury.

There are some pitfalls, however. Variation of Cre expression commonly exists and will result in variable DT-receptor expression and variable ablation of somatotropes, and also somatolactotropes. Indeed, only 80% of somatotropes and 50% of lactotropes were ablated. In this context, we do not know whether the SOX2⁺/GH⁺ cells reflect trans-differentiated hormone-producing cells that started to express SOX2 or really reflect newborn GH-expressing cells developing from SOX2⁺/GH⁺ progenitors. The authors conclude that the evidence supports a role for stem/progenitor cells in the regeneration process of somatotropes at adult age. Indeed, it is not proven but only provides some evidence towards this conclusion. However, it is a good start. Whether we will ever use pituitary stem cells for treatment, for example in *POU1F1* deficiency, remains to be seen. Most forms of multiple pituitary hormone deficiency would require allogenic donor cells and normal hypothalamic function for such treatments to work.

MEN1 gene replacement therapy reduces proliferation rates in a mouse model of pituitary adenomas

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Background: Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by multiple endocrine tumors, such as adenomas of the parathyroids, anterior pituitary and adrenals, and neuroendocrine tumors of the pancreas, duodenum, stomach and sometimes lungs or thymus, and is caused by haploinsufficiency of the tumor suppressor gene *MEN1*, encoding the menin protein. Treatment of these neuroendocrine tumors (NET) is more difficult than of sporadic NETs since tumors of MEN1 patients tend to metastasize more frequently and have a more invasive behavior. Therefore, there is clinical need for better treatment. In vitro studies have shown that expression of *Men1* in tumor cell lines partially re-establishes the wild-type phenotype by reducing proliferation and increasing apoptosis. This is the first study to use *Men1* gene replacement in mice, and this resulted in a reduction of proliferation and tumor growth.

Methods: Replication-deficient adenovirus serotype 5 was used to generate the construct expressing the murine *Men1* cDNA. *Men1* heterozygous mice that develop NET were used. Delivery of adenoviral vectors was achieved by transauricular intratumoral injection of *Men1*.rAd5 and *GFP*.rAd5 (5×10^{10} viral particles of both). Efficiency of transauricular intratumoral injection was assessed by MRI scanning and visualization of the GFP.

Results: *Men1* cDNA was introduced in the replication-deficient adenovirus serotype 5 (rAd5) to create *Men1*.rAd5. In vitro studies showed that the menin protein was produced in *Men1*.rAd5 infected *Men1*^{-/-} murine embryonic fibroblasts (MEFs) and that pituitary tumor cell lines were infected with increasing concentration of *Men1*.rAd5 viral particles. Tumors of animals treated with *Men1*.rAd5 showed decreased proliferation without changes in apoptosis. However, tumor size did not change.

Conclusions: This study establishes the proof of concept that *Men1* gene replacement in vivo reduces cell proliferation of anterior pituitary adenomas that develop in *Men1*^{+/-} mice.

This elegant paper by Walls et al. is an important advancement for the development of *MEN1* gene therapy in pituitary adenomas. This work may open the way for treatment by adenovirus gene-mediated delivery of other MEN1-related tumors, for example the pancreas or other pituitary tumors.

New knowledge – SOX2

SOX2 regulates the hypothalamic-pituitary axis at multiple levels

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J Clin Invest 2012;122:3635–3646

Background: Sex-determining region Y (SRY) box 2 (SOX2) haploinsufficiency causes hypogonadotrophic hypogonadism. Associated features include developmental abnormalities of the eye such as anophthalmia, brain abnormalities such as hypoplasia of the corpus callosum and hypothalamic hamartoma, sensorineural hearing loss and esophageal atresia. Rarely, GH deficiency may be a feature. To date, the presence of gonadotrophin deficiency in the face of the normal secretion of other pituitary hormones remains unexplained. *Sox2* is expressed in both the hypothalamus as well as Rathke's pouch during development, but not in the infundibulum or posterior lobe. The authors aimed to investigate the pathogenesis of hypogonadotrophic hypogonadism in SOX2-haploinsufficient patients.

Methods: Given that *Sox2* ablation leads to early embryonic lethality soon after implantation, preventing the study of its function during organogenesis, the authors conditionally deleted *Sox2* in mice, using a strategy whereby the *Hesx1^{Cre/+}* mouse line was crossed with *Sox2^{fl/fl}* animals; this strategy led to ablation of *Sox2* within the pituitary.

Results: First, absence of SOX2 in the developing Rathke's pouch of conditional embryos led to severe anterior lobe hypoplasia with drastically reduced expression of the pituitary-specific transcription factor POU class 1 homeobox 1 (*POU1F1*) as well as severe disruption of somatotrope and thyrotrope differentiation. In contrast, corticotropes, rostral-tip *POU1F1*-independent thyrotropes, and, interestingly, lactotropes and gonadotropes were less affected. Although there was no statistically significant variation from the expected mendelian ratios during embryogenesis (9.5–18.5 dpc), genotyping of post-natal mice from birth to 3 weeks failed to identify any viable *Hesx1^{Cre/+};Sox2^{fl/fl}* mice, showing that deletion of *Sox2* using the *Hesx1-Cre* line leads to neonatal/perinatal death. Second, the authors identified a requirement for *Sox2* in normal proliferation of periluminal progenitors; in its absence, insufficient precursors were available to produce all cell lineages of the anterior pituitary. Differentiated cells derived from precursors exiting cell cycle at early stages, including corticotropes, rostral-tip thyrotropes, and gonadotropes, were generated, while hormone-producing cells originating from late-born precursors, such as somatotropes and *POU1F1*-dependent thyrotropes, were severely reduced. Finally, the authors reported a lack of GnRH immunoreactivity in the median eminence of *Hesx1^{Cre/+};Sox2^{fl/fl}* mice as compared with controls at 18.5 dpc, whereas the expression of *Ghrh*, *Nr5a1*, *Avp* and *Oxt* was similar to controls. Additionally, there was a significant reduction of GnRH neurons in the *Hesx1^{Cre/+};Sox2^{fl/fl}* mice at all stages of development (12.5–18.5 dpc), as GnRH neurons migrate from the olfactory epithelium to the brain, suggesting a possible reduction in neurogenesis of GnRH neurons. In keeping with the murine data, 2 previously characterized patients with SOX2 haploinsufficiency and associated hypogonadotrophic hypogonadism had a measurable response to gonadotropin-releasing hormone (GnRH) stimulation, suggesting that it is not the absence of gonadotrope differentiation, but rather the deficient hypothalamic stimulation of gonadotropes, that underlies typical hypogonadotrophic hypogonadism.

Conclusion: *Sox2* is required for proliferation of Rathke's pouch progenitors and expansion of the anterior pituitary during embryogenesis. Additionally, whilst *Sox2* ablation does not affect the development of the neuroendocrine hypothalamus, it appears to be essential for the generation of GnRH neurons, accounting for the rather specific hypogonadotrophic hypogonadism observed in individuals with haploinsufficiency of SOX2.

Previous data using hypomorphic and *Sox2* conditional alleles had revealed that severe reduction or complete removal of *Sox2* within the developing neural tube does not cause gross morphological defects in the brain or spinal cord, possibly due to redundancy with *Sox1* and *Sox3*. These genetic approaches have, however, revealed an essential role for SOX2 in the maintenance of neurons in specific brain regions, proliferation and/or maintenance of neural stem cells, and neurogenesis.

In this paper, Jayakody et al. used a conditional deletion of *Sox2* in the hypothalamo-pituitary region to show that, although at 8.5–10.5 dpc there were no gross morphological defects in the Rathke's pouch (RP) of the mutants, by 18.5 dpc there was a fully penetrant phenotype of severe anterior pituitary hypoplasia in all embryos analyzed, often with ectopically located anterior pituitary tissue within the oropharyngeal ectoderm. These data suggest that in the absence of *Sox2*, normal early induction of RP occurs, but there is failure of subsequent AP development and expansion, leading to a very small and partially ectopic anterior lobe. Additionally, while terminal differentiation of somatotropes and mature thyrotropes was severely disrupted in the mutants, corticotropes/melanotropes, thyrotropes/gonadotropes and lactotropes were less affected. In keeping with these data, there was a dramatic reduction in *Pou1f1*⁺ cells from 12.5–18.5 dpc in the *Hesx1^{Cre/+};Sox2^{fl/fl}* mutant animals. *Prop1* expression was reduced in the mutant pituitaries at 12.5 dpc. Rostral-tip *Pou1f1*-independent thyrotropes were normal, as were corticotropes. These are the first two differentiated cell types to be specified in the early developing anterior lobe. The relative sparing of lactotropes in the mutant animals remains puzzling, given that lactotrope specification is dependent on *Pou1f1*. The data also suggest that the mutant phenotype is related to the timing of cell-cycle exit; rostral-tip thyrotropes, corticotropes and gonadotropes, whose precursors become post-mitotic from 11.5 dpc, are least affected, whereas the later-differentiating thyrotropes and somatotropes are almost absent.

The surprising finding of this study is that whilst the hypoplastic pituitary is a consequence of loss of Sox2 within the developing RP, pituitary stimulation with high-dose GnRH resulted in LH and FSH production, suggesting that the hypogonadotrophic hypogonadism observed in human patients is due to hypothalamic GnRH deficiency. This study underlines the importance of murine transgenesis in understanding human phenotypes, and also demonstrates the complexity of hypothalamo-pituitary development, whereby the same gene can act at multiple levels within the HP axis during different stages of development. However, the study also emphasizes the differences that are apparent between the murine and human phenotypes, for example, thyrotropes are severely reduced in the mutant mice whereas hypothyroidism has not been documented to date in humans. Additionally, humans with SOX2 haploinsufficiency have slow-growing pituitary tumors, whereas this has not been observed in the murine model [7]. Rather, the next paper shows that Sox2 haploinsufficiency may protect mice from developing pituitary tumors.

p27^{Kip1} directly represses Sox2 during embryonic stem cell differentiation

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Background: Three main factors, called the Yamanaka factors, Oct4, Klf4 and Sox2, are required for reprogramming of cells to induce pluripotent stem cells. Interestingly, mouse embryonic fibroblast (MEFs) that lack the tumor suppressor *p27^{Kip1}* do not require Sox2 to be reprogrammed into pluripotent stem cells. *p27^{Kip1}* is a tumor suppressor gene that when mutated leads to a multiple endocrine neoplasia (MEN) syndrome, characterized by pituitary tumors. The observation that absence of *p27^{Kip1}* leads to reprogramming without the key stem cell reprogramming factor Sox2, made the authors investigate the relationship between *p27^{Kip1}* and Sox2. They found that *p27^{Kip1}* negatively regulates expression of Sox2, and so may induce proliferation and promote tumorigenesis.

Methods: The authors performed cell reprogramming experiments in primary *p27*-null mouse embryo fibroblasts. ChIP, RNA quantification and protein assays were used to identify the binding of *p27^{Kip1}* regulatory regions in the Sox2 enhancer and to assess transcriptional regulation of Sox2. *p27^{Kip1}* null mice were crossed with *Sox2^{+/-}* mice to assess rescue of the phenotype of the *p27^{Kip1}* null mice.

Results: Cells obtained from *p27^{Kip1}* null mice could be converted into induced pluripotent stem cells (iPSC) without the reprogramming factor Sox2. Sox2 protein was increased in cells lacking *p27^{Kip1}*, suggesting that *p27^{Kip1}* negatively regulates Sox2 expression. The authors demonstrated by ChIP that *p27^{Kip1}* was able to bind to a 4Kb Sox2-distal enhancer, together with a repressive complex p130-E2F4-SIN3A. Moreover, overexpression of *p27^{Kip1}* resulted in a reduction of Sox2 expression. Importantly, in vivo, haploinsufficiency of Sox2 in the *p27^{Kip1}* null mice resulted in normalization of some of the features, notably the gigantism, pituitary hyperplasia, pituitary tumor formation and retinal dysplasia.

Conclusions: These experiments provide genetic and biochemical support for a direct regulation of the developmental transcription factor Sox2 by *p27^{Kip1}*.

In this elegant piece of work, Han Li and colleagues identify regulation of the progenitor/stem cell factor Sox2 by the tumor suppressor protein *p27^{Kip1}*. Germline mutations of the *p27^{Kip1}* gene, also known as *CDNK1B*, are responsible for a subset of MEN syndromes, notably characterized by pituitary tumors [8]. *p27^{Kip1}* null mice have increased body size, organ hyperplasia, pituitary tumors and retinal dysplasia. This study establishes a previously unknown relationship between *p27^{Kip1}* and Sox2 and shows that absence of *p27^{Kip1}* leads to de-repression of Sox2. The authors show that haploinsufficiency of Sox2 is able to rescue the MEN phenotype of *p27^{Kip1}* null mice. Sox2 is a crucial gene in the development of multiple tissues, and is also required for maintaining the pituitary progenitor/stem cell population and normal hypothalamic-pituitary development, as outlined in some of the other papers in this and the previous year's chapters. Mutations in SOX2 cause a spectrum of hypothalamic-pituitary abnormalities ranging from hypogonadotrophic hypogonadism to septo-optic dysplasia. This paper presents the first genetic and molecular evidence for a relationship between Sox2 and *p27^{Kip1}*. There may be a 'ying-yang' balance of effects, where too little SOX2 results in pituitary hormone deficiency and too much SOX2 results in pituitary tumors.

Outcome of surgical treatment of 200 children with Cushing's disease

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Background: Factors influencing the outcome of surgical treatment of pediatric Cushing's disease (CD) have not been fully established.

Methods: The authors assessed the outcome of surgery for pediatric CD and factors influencing the outcome in 200 consecutive patients with CD treated at a single tertiary center at the National Institutes of Health (NIH) from 1982 through 2010.

Results: Mean age at symptom development was 10.6 ± 3.6 years (range 4.0–19.0) and at surgery was 13.7 ± 3.7 years. 27 patients (13%) had prior surgery at another institution. MRI identified adenomas in 97 patients (50%) and accurately defined localization (in 99%), which was more accurate than inferior petrosal sinus sampling (accurate in 72%). 195/200 patients (98%) achieved remission after surgery (189 (97%) were hypocortisolemic; 6 (3%) were eucortisolemic postoperatively). Identification of an adenoma at surgery, immunohistochemical ACTH-producing adenoma, and noninvasive ACTH adenoma were associated with initial remission ($p < 0.05$). Younger age, smaller adenoma, and absence of cavernous sinus wall or other dural invasion were associated with long-term remission ($p < 0.05$). A 9 a.m. serum cortisol $<1 \mu\text{g/dl}$ after surgery had a 96% positive predictive value for lasting remission.

Conclusion: Pituitary surgery for CD in children can be safe, effective, and durable. Undetectable cortisol concentration postsurgery predicts lasting remission. Lasting remission is associated with younger age at surgery, smaller adenomas, and lack of dural invasion.

This is a beautiful report of prospective data collection and evaluation of pre- and postoperative management of pediatric Cushing's disease in a single center. 200 patients seen between 1982 and 2010 were included. In line with previous reports, there was predominance of males (F:M 0.8:1) in patients <15 years, but more females among older patients (3.8:1) [9]. An astonishing 98% of patients reached remission immediately after surgery and 90% remained in remission for at least 10 years, the highest success rate reported so far [10, 11]. Rapid weight gain and poor growth were the most frequent symptoms, followed by the presence of dorsal cervical or supraclavicular fat pads. MRI with SGPR (standard spin echo sequences and spoiled gradient recalled) acquisition sequences improved the poor detection of adenomas from 50 to 65%. IPPS correctly predicted the site of the adenoma in 70%, similar to reports of other series [9, 12].

Adenoma size was on average 7.9 mm and invasion occurred in 20%. In all patients, trans-sphenoidal adenomectomy, or partial hypophysectomy if no adenoma was identified, was performed. In 13%, no adenoma was identified and an ACTH-producing tumor was only found on histology in 30% of these cases. Not surprisingly, these children had the worst outcome. As reported before, a postoperative morning cortisol concentration $<1 \mu\text{g/dl}$ predicted lasting remission with an accuracy of 96%. Interestingly, only 5% developed DI and only 3% symptomatic hyponatremia. Unfortunately, the data did not include postoperative assessment of pituitary function.

This paper shows that outcome is better for younger children and for non-invading tumors, so our next task is to reduce the time between the first symptoms and the diagnosis. This study also highlights the value of prospective databases to audit the management and outcome of patients with rare diseases, both at institutional and national levels. Other examples are the UK National Diabetes Audit and the European DSD database.

Recovery of the hypothalamic-pituitary-adrenal axis in children and adolescents after surgical cure of Cushing's disease

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Background: The hypothalamic-pituitary-adrenal axis (HPAA) recovers slowly after trans-sphenoidal surgery (TSS) for Cushing's disease (CD), but data on recovery times are not available.

Methods: The authors aimed to assess time to recovery of the HPAA after TSS in children with CD. 57 patients with CD (6–18 years, mean 13.0 ± 3.1 years) were given a standard regimen of glucocorticoid tapering after TSS. Synacthen stimulation tests were performed 6-monthly for up to 36 months. Age, sex, pubertal status, body mass index, length of disease, midnight cortisol, and urinary free cortisol at diagnosis were analyzed for effects on recovery. Primary outcome was complete recovery of the HPAA, defined by a cortisol concentration $>18 \mu\text{g/dl}$ in response to $250 \mu\text{g}$ ACTH.

Results: HPAA recovered in 75% of patients, with 67 and 95% recovering by 12 and 18 months, respectively. The mean time to recovery was 12.6 ± 3.3 months. Survival analyses estimated a 75% chance of recovering within 14 months. A cut-off of at least $10\text{--}11 \mu\text{g/dl}$ of cortisol as the peak of ACTH stimulation testing at 6 months after TSS yielded the highest sensitivity (70–80%) and specificity (64–73%) to predict full recovery of the HPAA at 12 months. Importantly, 2 of the 4 patients who recovered fully within 6 months had recurrent CD.

Conclusions: The standardized tapering regimen used for glucocorticoid replacement after TSS led to recovery of the HPAA in most patients within the first postoperative year. Multiple factors may affect this process, but an early recovery may indicate disease recurrence.

Cortisol overproduction in Cushing's disease results in negative feedback and suppression of hypothalamic CRH production, resulting in adrenal insufficiency after removal of the ACTH-producing tumor. Adrenal function needs to be followed up not only to assess the appropriate time to discontinue HC treatment, but also to detect patients with recurrent disease. Although this study was not prospective, has missing data, and children with early recurrence were excluded, it still gives valuable data on the postsurgical HPAA in a large number of patients with Cushing's disease. Good HPA function was defined as a cortisol response of $18 \mu\text{g/dl}$ (500 nmol/l) after the non-physiological high-dose ($250 \mu\text{g}$ ACTH) Synacthen test. 10% recovered within 6 months, and 60% recovered within a year. A peak cortisol cut-off of $>10\text{--}11 \mu\text{g/ml}$ predicted for a normal response 6 months later with a reasonable specificity and sensitivity. This time scale is similar to reports of recovery of pituitary GH and TSH axes. An important message of this paper is that HPA recovery within 6 months may predict relapse of Cushing's disease.

Interestingly, hydrocortisone replacement schedule consisted of only 2 doses per day, starting at a dose of $8\text{--}12 \text{ mg/m}^2/\text{day}$, followed by a rapid reduction after 4 months of 2.5 mg/day every 4–6 weeks and omission of the afternoon dose as soon as possible. After passing the ACTH test, emergency hydrocortisone treatment was advised for a further 6 months. This tapering regimen required adjustment of dose in only 6% of cases (patients were warned to expect symptoms of adrenal insufficiency during the first 3–4 months after surgery) and no hospitalization for adrenal insufficiency was required in any patient, suggesting that this tapering regimen is safe. Many centers use three times daily dosing and stop further dose reduction when a physiological dose is reached. As the authors point out, it is not clear how their empirical tapering regimen affected adrenal recovery.

Xanthogranuloma, Rathke's cyst, and childhood craniopharyngioma: results of prospective multinational studies of children and adolescents with rare sellar malformations

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Background: No consensus guidelines exist for the management of craniopharyngioma (CP), Rathke's cyst (RC), and xanthogranuloma (XG) which are closely related, rare, sellar masses. This study assessed clinical manifestations, treatment and outcome of patients with such masses.

Methods: The authors performed a multicenter surveillance trial of patients <18 years with a histological diagnosis of CP, XG, or RC. 117 patients with CP, 14 with XG, and 14 with RC were included.

Results: The 5-year OS (overall survival) rates were 1.00 ± 0.00 in RC and XG and 0.97 ± 0.02 in CP. The 5-year EFS (event-free survival) rates were 0.85 ± 0.10 in RC, 1.00 ± 0.00 in XG, and 0.50 ± 0.05 in CP. Surgical resection of XG resulted in complete remission without recurrence. Recurrences occurred in RC (14%) and CP (59%). CP leads most often to severe hypothalamic sequelae such as obesity and others affecting QoL, mostly due to presurgical involvement (59%) and postsurgical lesions (44%) of posterior hypothalamic structures. Centers with lower neurosurgery patient loads used more radical surgical approaches to treat CP, resulting in higher rates of obesity and reduced QoL. Despite 46% anterior hypothalamic involvement, severe obesity is not encountered in XG.

Conclusion: The treatment of choice in XG and RC is radical surgery. In CP, less radical surgical approaches that preserve hypothalamic structures are recommended. Regular imaging during follow-up is recommended for CP and RC because of frequent relapses. Treatment of patients with sellar masses should be confined to experienced multidisciplinary teams.

Craniopharyngioma (CP), Rathke's cyst (RC), and sellar xanthogramuloma (XP) are closely related lesions. CPs arise from neoplastic transformation of ectodermal cell remnants of Rathke's pouch and the craniopharyngeal duct. RCs are also Rathke's pouch-derived epithelial lesions. XG of the sellar region are composed of cholesterol clefts, macrophages, chronic inflammatory cellular reaction, and hemosiderin deposits. Outcomes of various treatment modalities for CP are emerging, allowing for development of clinical guidelines, but very little data of long-term follow-up exist for RC and XP, hence the importance of this prospective study. CPs and RCs are frequently seen in clinical practice, but XG is rare.

Unexpected findings were that hydrocephalus was seen in 15% of patients with RC, that duration of symptoms was longest in XG, and that XG commonly invaded the hypothalamus (in 48% of cases) although not as deeply as CP. Surgical outcomes were excellent for RC and XG but, as expected, were less favorable for CP. Despite frequent hypothalamic invasion, obesity was not a common complication of XG, even after surgical removal, in contrast to CP.

In this study, only patients with a histological diagnosis and surgical removal of RC, CP or XG were included. RC are commonly found as incidentalomas and small RC are believed not to require treatment [13, 14]. In the present study, the causal relationship between symptoms and the sellar masses could not be assessed, nor the outcome of conservative treatment and the question whether RC requires treatment, and if so, the optimal timing of this. Further prospective studies of the management of patients with sellar masses are needed to address these questions and to enable the development of clinical guidelines for the management of XG and RC.

A novel mutation in the upstream open reading frame of the CDKN1B gene causes a MEN4 phenotype

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Background: Multiple endocrine neoplasia syndrome type 4 is an autosomal dominant syndrome characterized by a varying combination of tumors affecting at least two endocrine organs. It resembles MEN1, but patients have no mutation in *MEN1*. Recently p27^{Kip1}, a cyclin-dependent kinase 1, has been shown to have tumor suppressor activity because it inhibits multiple cyclin-dependent kinases, stopping cells progressing from G1 to S phase. Moreover, germline mutations in the gene *CDKN1B* that encodes p27^{Kip1} are responsible for multiple endocrine neoplasia (MEN) type 4 syndromes. This study identifies for the first time a 4 base-pair deletion in the upstream open reading frame sequence (uORF) of the *CDKN1B* gene that affects transcriptional levels of p27^{Kip1} in a patient with pituitary and adrenal neoplasia.

Methods: 25 patients with MEN1-related symptoms were screened for mutations in *CDKN1B*. Sequencing of the open reading frame, exon-intron boundaries and upstream sequences identified no mutations, apart from 1 patient with a 4 base-pair deletion in the uORF. Functional studies (dual luciferase assay, site-directed mutagenesis, and polysome profiling) showed an effect of the mutation on transcriptional levels of p27^{Kip1}.

Results: Transcription and translation of some genes can be influenced by sequences termed upstream open reading frame, uORFs. These elements can affect the translational machinery. A small 4 base-pair deletion in the p27^{Kip1} uORF was identified by sequencing, so the question arose whether this deletion can affect translation of the protein. Initial studies demonstrated that the level of mRNA did not change, indicating that transcription occurs normally. However, luciferase assays showed lower luciferase levels for the construct compared to wild type. Moreover, in vitro expression of the mutant uORF driving p27^{Kip1} in HEK 293 cells led to a smaller amount of generated protein. This suggests that the 4 base-pair deletion is sufficient to affect translation of the protein.

Conclusions: The deletion identified in this study in the p27^{Kip1} uORF is a novel example of a uORF-affecting mutation that can lead to endocrine neoplasia. The data clearly show a negative effect of the 4 base-pair deletion on translation of the tumor suppressor p27^{Kip1}.

MEN4 has been described as a new type of multiple endocrine neoplasia [15]. The phenotype resembles MEN1, and the most common phenotypic features are parathyroid and pituitary adenomas. So far, heterozygous mutations at various residues of the tumor suppressor gene p27^{Kip1} have been found in patients with MEN4. As discussed earlier in this chapter, p27^{Kip1} negatively regulates the stem cell factor SOX2 [16]. This paper shows for the first time how mutations in upstream open reading frame regulatory regions of p27^{Kip1} affect translation of the protein, and are likely the cause of MEN4 in these patients. This paper provides novel insights into the role of uORFs in protein translation in general and in the pathogenesis of MEN4 specifically. It sets the road ahead for the investigation of mutations in uORFs of other genes involved in pituitary disease as it is foreseen that mutations in regulatory regions will affect protein production and so influence pituitary development and homeostasis.

Developmental analysis and influence of genetic background on the *Lhx3* W227ter mouse model of combined pituitary hormone deficiency disease

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Background: Although a number of genes have been implicated in the etiology of congenital hypopituitarism, the majority of these disorders remain unexplained. Little is known about their etiology, especially the developmental profiles and the influence of genetic background on disease progression. Animal models for Combined Pituitary Hormone Deficiency (CPHD) provide valuable tools to investigate disease mechanisms and inform diagnostic and treatment protocols. *Lhx3*/LHX3 is a transcription factor that is expressed during the early stages of pituitary and CNS development and plays essential roles in cell differentiation within the anterior pituitary, and for the differentiation of neurons within the brain and spinal cord. Mutations in *LHX3* are associated with severe CPHD in addition to sensorineural hearing loss and a rigid cervical spine resulting in limited head rotation.

Methods: The authors aimed to investigate hormone production during pituitary development and the influence of genetic background on phenotypic severity in the mouse model of an *Lhx3* mutation (*Lhx3*^{W227ter/W227ter}). In humans, this mutation is associated with CPHD without cervical spine rigidity. In previous studies, knock-in of the W227ter mutation in a murine model of mixed background (129/Sv⁺C57BL/6) led to the generation of a mouse model of CPHD without any CNS defects. Through breeding of *Lhx3*^{W227ter/+} mice of mixed background (purity 98.4%); the *Lhx3*^{W227ter/W227ter} genotype was placed onto the 129/Sv and C57BL/6 backgrounds. The mice were then analyzed using immunohistochemistry at different stages. Proteomic analysis of wild-type and mutant pituitaries was performed.

Results: *Lhx3*^{W227ter/W227ter} embryos had deficiencies of ACTH, α -glycoprotein subunit, GH, PRL, TSH β , and LH β during prenatal development. Furthermore, mutant mice had a reduction in the critical pituitary transcriptional activator-1 (PIT1/POU1F1). Intriguingly, the genetic background affected viability: whereas *Lhx3*^{W227ter/W227ter} animals were found in the expected frequencies in the C57BL/6 background, homozygous animals were not viable in the 129/Sv genetic background. The hormone marker and PIT1 reductions observed in *Lhx3*^{W227ter/W227ter} mice on a mixed background were also seen in the separate strains but in some cases were more severe in 129/Sv. Proteomic analysis revealed lower levels of PRL, pro-opiomelanocortin (ACTH), and α -glycoprotein subunit proteins in *Lhx3*^{W227ter/W227ter} mice. A number of protein networks were affected in the mutated animals and included cell cycle, cellular maintenance and protein translation networks.

Conclusion: These data show that hormone deficiencies are manifest in early prenatal stages in this CPHD model system. Furthermore, as is noted in human disease, genetic background impacts the phenotypic outcome of these monogenic endocrine diseases.

This paper reports functional studies of a mutation in *LHX3* that is associated with a pituitary-specific phenotype, with no abnormality of neck rotation, unlike other described *LHX3* mutations. The W227ter mutation is the most carboxy-terminal mutation in *LHX3* [17], and the pituitary-specific phenotype underlines the importance of the carboxy-terminus in pituitary development. Previous studies showed that knock-in of this mutation in a mixed murine background led to a pituitary-specific phenotype [18]. In this study, the authors back-crossed the *Lhx3*^{W227ter/+} heterozygous mice on a mixed background to generate mice with two pure backgrounds (C57BL/6 and 129/Sv). Intriguingly, the phenotypes were different, being more severe in the 129/Sv mice. The latter were not viable, unlike those in the C57BL/6 background, which were born in the expected ratios. This is consistent with data in humans, where phenotypes can vary markedly, even with the same genetic mutation. Importantly, this paper also shows that the mutant animals manifested both structural and hormonal pituitary abnormalities prenatally, with reduced Pit1/Pou1f1 expression.

Proteomic analysis revealed not only changes in hormonal expression, but also in genes implicated in cell cycle progression, cellular maintenance, and protein translation networks. Surprisingly, in view of increased apoptosis and pituitary hypoplasia in *Lhx3*^{-/-} and *Lhx3*^{W227ter/W227ter} mutants, 17 proteins associated with apoptosis were downregulated. It is important to note that proteomic analysis was performed in adult animals, and the results therefore need to be interpreted cautiously.

Cushing syndrome in pediatrics

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Cushing syndrome is characterized by truncal obesity, growth deceleration, skin changes, muscle weakness, and hypertension. Cushing syndrome in childhood usually results from the exogenous administration of glucocorticoids. This article presents the causes and discusses the treatment of endogenous Cushing syndrome. It also discusses the clinical and molecular genetics of inherited forms of this syndrome. Cushing syndrome needs to be diagnosed and treated properly when first recognized; improper treatment can turn this otherwise completely curable disorder into a chronic ailment. Barriers to optimal care of the pediatric patient with Cushing syndrome are discussed.

This is yet another excellent review by Stratakis that discusses in detail symptomatology, underlying genetic causes, diagnostic tests and surgical and medical treatment of Cushing syndrome. Diagnostic testing including specificity and sensitivity of tests, the importance of the correct sequence of tests and the need to avoid imaging before a biochemical diagnosis has been reached, are discussed. This review not only serves as support for the clinician investigating or excluding Cushing syndrome, but can also be used as the basis for teaching.

Food for thought

Kallmann syndrome

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In the era of PREMS and PROMS ('patient reported experience measures' and 'patient reported outcome measures', respectively), this account of an adult who had a delayed diagnosis of Kallmann syndrome at the age of 22 years is well worth reading. It describes the long road before the correct diagnosis was made and the psychological consequences of the delay. Hopefully, increased awareness of Kallmann syndrome among our non-endocrine colleagues has already increased the number of referrals made at the correct age and to the correct specialist, with hopefully improved long-term outcomes.

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