Pituitary

Evelien F. Gevers^{a,b}, Carles Gaston-Massuet^a and Mehul T. Dattani^a

^aDevelopmental Endocrine Research Group, Clinical and Molecular Genetics Unit, Institute for Child Health, London, UK

^bGreat Ormond Street Hospital for Children, Department of Endocrinology, London, UK

Major breakthroughs were made in the field of pituitary research during the last year. A paper by Levkowitz's group shows that oxytocin itself is involved in hypothalamo-pituitary development and functions as a guidance molecule to form the neurovascular interface of the hypothalamo-neurohypophyseal system. Using several fluorescent tags, the vasculature was beautifully visualized in translucent fish so that development could be tracked. The second breakthrough was the accomplishment of the growth from embryonic stem cells in vitro of tissue that was similar to Rathke's pouch and developed into functioning pituitary cells. This development may open the way to future stem cell treatment for a variety of pituitary disorders. Further promising developments regard new treatments for Cushing's disease: the first using a tyrosine kinase inhibitor that targets the EGF receptor, and the second using pasireotide, a somatostatin agonist targeting the somatostatin receptor subtype 5, and further understanding of the pathogenesis of craniopharyngioma. Important for clinical endocrinology is a paper suggesting that permanent hypopituitarism is rare after traumatic brain injury, a paper describing a new association between ACTH deficiency and immune deficiency (DAVID) and the description of the largest cohort so far of isolated ACTH deficiency. Finally, a plethora of papers describing functional cell networks in the pituitary has been published this year.

Mechanism of the year

The hypothalamic neuropeptide oxytocin is required for formation of the neurovascular interface of the pituitary

Gutnick A, Blechman J, Kaslin J, Herwig L, Belting HG, Affolter M, Bonkowsky JL, Levkowitz G
Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel
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Background: The neuropeptides oxytocin and arginine vasopressin are secreted directly into the circulation by the hypothalamus-neurohypophyseal system. These neuropeptides are synthesized by the magnocellular neurons from the hypothalamus and are transported to the neurohypophysis by axonal projections that meet the neurohypophyseal blood vessels. The connection between neurohypophyseal axons and the blood vessels form a congruent neurovascular interface. The development of this congruence and the molecular mechanisms underlying it are poorly understood.

Methods: To dissect the role of hypothalamic neurons in the formation of the neurovascular interface, transgenic zebrafish reporter lines were generated to allow the visualization of these neurons. Visualization of hypothalamic oxytocinergic cell bodies was accomplished by linking GFP to the regulatory region of the zebrafish oxytocin gene *oxtl* creating a *Ox1l:EGFP* transgenic line. Visualization of hypophyseal vasculature was accomplished by using a *vegfr2-Cherry* line. Generation of a double transgenic lines *Ox1l:EGFP;vegfr2-Cherry* allowed visualization of both oxytocinergic axons and blood vessels. Furthermore, conditional genetic ablation of oxytocinergic neurons was accomplished by the creation of a triple transgenic line *otpb:Gal4;UAS:NTRCherry;vegfr2:EGFP*. This transgenic line allows ablation of cells that express nitroreductase (NTR), an enzyme that converts the prodrug metronidazole into a potent cytotoxic agent. Since NTR is only expressed by the hypothalamic oxytocinergic neurons, cell-specific ablation is accomplished.

Results: Visualization of hypothalamic oxytocinergic neurons using the double zebrafish transgenic line *Ox1l:EGFP;vegfr2:Cherry* showed that axonal projections colonize the neurohypophysis before the vessels form. These hypothalamic projections into the neurohypophysis are independent of vessel formation, since *Cloche* mutants, that lack head vasculature, exhibit normal axonal innervation of the neurohypo-

physis. In order to assess if newly arrived neurohypophyseal axons are required for endothelial cells to form vessels, ablation of oxytocin neurons was performed using the triple transgenic line *otpb:Gal4;UAS:NTRCherry;vegfr2:EGFP*. Ablation of these neurons resulted in impaired hypophyseal vasculature indicating that neurohypophyseal axons are required for the formation of the neurovasculature interface.

Conclusion: Oxytocin functions as a guidance cue for endothelial cells to form the neurohypophyseal vasculature.

Although many molecules and pathways have been identified that direct neurovascular development, such as VEGF [1], class 3 semaphorins [2] and Eph:Ephrin signaling [3], the mechanisms that dictate neurovascular function in the secretory endocrine system is largely unknown. In this elegant article, Levkowitz and colleagues show a novel mechanism by which oxytocin-producing neurons signal to endothelial cells to form a congruent neurovascular interface. This neurovascular interface is required for the proper secretion of the hypothalamic neuropeptides into the bloodstream and the anterior pituitary, and therefore it is key to its proper endocrine function. Oxytocin has been reported to stimulate the migration and sprouting of human endothelial cells that express oxytocin receptor [4, 5]. Levkowitz and colleagues identify for the first time a molecular mechanism by which oxytocin functions as a vascular guidance cue to promote blood vessel formation. By using transgenic tools in the optically transparent zebrafish embryo, the authors visualized the formation of the neurovascular interface in the neurohypophysis. Genetic ablation of oxytocin-producing cells results in abnormal vascularization. As is the case for all very good articles, new questions arise from this work: Does oxytocin function as a vascular cue in other organs? Are other hypothalamic releasing factors important in the formation of the portal system that connects the anterior pituitary in mammals? Do other hypothalamic neuropeptides work in a similar fashion to oxytocin in attracting endothelial cells for their contact? Answers to these questions will emerge from future studies, but at present, this work sets the path towards understanding how the intricate congruence between the axonal projections and vessels is established in the pituitary.

New hope

Self-formation of functional adenohypophysis in three-dimensional culture

Suga H, Kadoshima T, Minaguchi M, Ohgushi M, Soen M, Nakano T, Takata N, Wataya T, Muguruma K, Miyoshi H, Yonemura S, Oiso Y, Sasai Y

Neurogenesis and Organogenesis Group, RIKEN Center for Developmental Biology, Kobe, Japan

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Background: No efficient stem cell culture for the generation of anterior pituitary cells is available, due to insufficient knowledge about induction of pituitary primordium (Rathke's pouch) in the embryonic head ectoderm. In this paper, efficient self-formation of three-dimensional anterior pituitary tissue in an aggregate culture of mouse embryonic stem (ES) cells was achieved.

Methods: ES cells were stimulated to differentiate into nonneural head ectoderm and hypothalamic neuroectoderm in adjacent layers within the aggregate, and treated with agonists of hedgehog signaling.

Results: Self-organization of Rathke's pouch-like three-dimensional structures occurred at the interface of these two epithelia. Various endocrine cells were subsequently produced and these cells were able to respond to trophic hormones.

Conclusion: Functional anterior pituitary tissue self-forms in culture after manipulation of ES cells, recapitulating local tissue interactions.

Growing pituitary spheres with hormone-producing cells from adult pituitary progenitors has been achieved [6] but rebuilding the pituitary from ES cells is a more complicated task. Relatively little is known about the formation of Rathke's pouch, knowledge needed to form a pituitary in a dish. The head ectoderm forms the outer parts of the head including the ears and eyes, and part of the ectoderm develops into Rathke's pouch. Interaction with the adjacent neuroepithelial cell layers that will form the hypothalamus is necessary for the formation of Rathke's pouch. In this paper, the authors succeeded in growing hormone-producing cells from ES cells within 3 weeks. They used specific con-

ditions for ES cells to develop into hypothalamic like neuroepithelium and developed conditions in which ES cells formed rostral head ectoderm simultaneously. These aggregates expressed markers of neuroepithelium and head ectoderm adjacently. Hedgehog/BMPs and FGFs (FGF8, FGF10) play a vital role in midline brain development, and Rathke's pouch formation is disrupted in the absence of these factors [7]. The authors found that Sonic hedgehog agonist treatment of the cultures increased the Rathke's pouch marker Lhx3. The resulting 3D structures had a central cavity and the resemblance to Rathke's pouch was striking, as was the topographical location between the neuroepithelium and the rostral head-like ectoderm. The juxtaposition of the two tissues, mimicking the spatial organization in embryonic development, was indeed critical as Rathke's pouch-like vesicles did not develop when neuroepithelial tissue was not present.

One wonders whether this is the first step towards stem cell treatment for pituitary disorders. ACTH-expressing cells developed from the vesicle-like structures and activation of Wnt signaling led to expression of Pit1, GH and prolactin. LH/FSH/TSH expression was also achieved albeit after more intense manipulation of the culture conditions. When ES-derived cell aggregates were implanted under the kidney capsule in hypophysectomized mice, corticosterone was produced. The first step towards stem cell treatment may therefore indeed have been taken.

Another important aspect of this work is that it shows that processes taking place in the embryo with complex movements of structures, can be recapitulated in a dish and that induction of a tissue by adjacent tissue is possible in vitro. This is promising for the formation of other complex tissues in vitro, as noted by others [8].

It is amazing how small a tool kit of growth factors was required for the development of a complex structure like the pituitary from ES cells. The next paper shows that it takes even less to disrupt normal pituitary development and morphogenesis.

New mechanism – and new hope!

Increased wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans

Gaston-Massuet C*, Andoniadou CL*, Signore M, Jayakody SA, Charolidi N, Kyeyune R, Vernay B, Jacques TS, Taketo MM, Le Tissier P, Dattani MT, Martinez-Barbera JP
Neural Development Unit, University College London Institute of Child Health, London, UK
Proc Natl Acad Sci USA 2011;108:11482–11487

Background: Adamantinomatous craniopharyngiomas are pediatric tumors located within the sellar/suprasellar region that often invade nearby structures in the brain and optic nerves. Craniopharyngiomas arise from the pituitary gland and account for 5–10% of all pediatric nonneural intracranial tumors. Mutations in the Wnt signaling effector β -catenin (*CTNNB1*, gene) have been identified in human craniopharyngioma, however neither a causative role for mutations in β -catenin, the cellular origin nor the pathogenesis of these tumors has been firmly established.

Methods: This work used transgenic mice to conditionally ablate exon3 of the β -catenin gene (*Ctnnb1-lox(ex3)*) in *Hesx1*-expressing Rathke's pouch progenitors. Deletion of exon3 (*Hesx1^{Cre/+};Ctnnb1^{+lox(ex3)}*) renders a degradation-resistant form of β -catenin, which results in the constitutive activation of the Wnt pathway in undifferentiated precursors of the pituitary gland.

Results: Activation of the Wnt/ β -catenin pathway in the developing pituitary gland leads to craniopharyngioma-like pituitary tumors that express diagnostic markers of craniopharyngioma, such as β -catenin in cell clusters, cytokeratin 8 and 18, and fail to express hormones. Although all cells of the developing pituitary have mutated β -catenin, only a proportion of precursor cells were responsive to β -catenin mutations and formed tumor lesions. When mutated β -catenin was expressed in differentiated cells, no tumors developed indicating an undifferentiated pituitary precursor origin for craniopharyngioma. The cells responsive to mutated β -catenin were Sox2^{+ve}, p27Kip2^{+ve} Sox9^{-ve} and were quiescent in vivo, all characteristics of pituitary stem cells. Furthermore, the craniopharyngioma-mouse model contains an increased number of pituitary progenitor/stem cells, with a double rate of proliferation that underlies the formation of these tumors.

Conclusion: These results show a causative role for activating mutations in β -catenin in the genesis of craniopharyngioma. Furthermore, the cellular origin of these tumors are pituitary progenitors/stem cells, indicating that, like many other tumors, Wnt/ β -catenin can influence the pituitary progenitor/stem cell pool and that activation of this pathway leads to tumors in mouse and humans.

In this article, Martinez-Barbera and co-workers identify for the first time a causative role of activating β -catenin mutations in the formation of adamantinomatous craniopharyngioma tumors. Although an association between mutations in β -catenin and craniopharyngioma had been reported previously [9, 10], a causative role had been elusive. Moreover, little was known about the etiology and cell origin of the tumors, and further research into these areas was hampered by the lack of an appropriate animal model. In this work, the authors have generated a valuable craniopharyngioma-mouse model that enabled them to perform important experiments indicating that only undifferentiated pituitary progenitors/stem cells are responsive to mutated β -catenin. Thus a previously unknown embryonic and stem cell origin of this tumor is established in this work. Moreover, this mouse model offers the possibility to identify pathways important in progression of craniopharyngioma. In fact, a recent paper by Andoniadou et al. [11] uses this mouse model to show differences in expression profiles of purified β -catenin-accumulating cells, suggesting that deregulation of pathways such as SHH, BMP and FGF are implicated in both mouse and human ACPs. Furthermore, this novel mouse model will be a very useful tool to test chemical compounds for their therapeutic effect in order to identify much needed medical treatment for craniopharyngiomas.

FGF-dependent midline-derived progenitor cells in hypothalamic infundibular development

Pearson CA, Ohyama K, Manning L, Aghamohammadzadeh S, Sang H, Placzek M
MRC Centre for Developmental and Biomedical Genetics and Department of Biomedical Science, University of Sheffield, Sheffield, UK
Development 2011;138:2613–2624

Background: Control of body homeostasis is accomplished by the hypothalamic-pituitary axis. The infundibulum, derived from the ventral diencephalon, is devoid of hormone-producing cells but contains the hypothalamic axonal projection that links the nervous and endocrine systems. Signals from the infundibulum are critical for the development and patterning of the underlying pituitary tissue. Despite its importance, very little is known about the development and cell origin of this critical embryonic structure.

Methods: This paper uses Dil/DiO labeling techniques in chick embryos to fate map cells to establish the origin of cells of the infundibulum. It uses a range of transplantation, immunostaining and in-situ hybridization techniques to identify different molecular profiles of the FP subpopulations.

Results: The infundibulum has a dual cell origin and comes from two different cell populations that reside in the ventral floor plate region of the forebrain, a region known to be involved in formation of the infundibulum. One population of cells forms the definitive infundibulum whilst the second population forms the so-called ‘collar zone’ that surrounds the infundibulum. These two distinct cell populations have differential gene expression. Collar zone cells can colonize the infundibulum over time and contain undifferentiated precursors/stem cells that can differentiate into multiple hypothalamic infundibular cell types.

Conclusion: The infundibulum is formed by two separate populations of cells that are derived from different forebrain floor plate cells. One distinct population, the collar cells, which are Fgf3^+ Sox3^+ are required for growth of the infundibulum.

The role of the infundibulum as a signaling center that controls the underlying Rathke’s pouch development has been firmly studied. For instance, absence of infundibulum in the *Nkx2.1* null embryos results in failure of Rathke’s pouch and anterior pituitary gland formation [12]. However, how the infundibulum develops into this separate anatomical structure remains poorly understood. Placzek and colleagues nicely described by using fate mapping and classical embryology techniques that infundibulum cells come from two forebrain floor plate cell populations. These two populations form two distinct structures, the infundibulum proper and the collar zone surrounding the

infundibulum. This newly identified structure differentially expresses *Fgf3* and *Sox3*, and it promotes the maintenance and proliferation of the infundibulum through expression of FGF signals. This adds valuable information to our understanding of the infundibulum structure and raises questions about the requirement or sufficiency of the collar zone cells in infundibulum development. Future ablation experiments of the collar zone cells will provide further information on the exact specific requirement of these cells in infundibulum development.

Old genes – new knowledge

Phenotypic homogeneity and genotypic variability in a large series of congenital isolated ACTH-deficiency patients with *TPIT* gene mutations

Couture C, Saveanu A, Barlier A, Carel JC, Fassnacht M, Fluck CE, Houang M, Maes M, Phan-Hug F, Enjalbert A, Drouin J, Brue T, Vallette S

Institut de Recherches Cliniques de Montreal, Montreal, Que., Canada

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Background: *TPIT*, a T-box transcription factor, is restricted to POMC-expressing cells in the pituitary and essential for both POMC gene transcription and terminal differentiation of POMC-expressing cells. Congenital isolated ACTH deficiency was poorly defined before *TPIT* mutations were identified as its principal molecular cause. The authors enlarged their series of patients with isolated ACTH deficiency to better characterize the phenotype and the genotype of this rare disorder.

Methods: *TPIT* gene exons and exon/intron boundaries were sequenced in these patients. A functional analysis of each new *TPIT* mutation was performed. Clinical information of all 91 patients was collected.

Results: Three distinct groups were identified in the cohort: neonatal-onset complete or partial isolated ACTH deficiency or late-onset isolated ACTH deficiency. No *TPIT* mutations were detected in patients with partial or late-onset ACTH deficiency but *TPIT* mutations were found in 65% of patients with neonatal-onset complete ACTH deficiency. Nine new mutations were detected: four missense, one single nucleotide deletion, three splice-site mutations, and one large deletion. Different mechanisms lead to loss of function of *TPIT*, such as nonsense-mediated mRNA decay, abnormal mRNA splicing, loss of *TPIT* DNA binding or protein-protein interaction defects.

Conclusion: Two thirds of patients with neonatal-onset complete isolated ACTH deficiency have *TPIT* mutations but none of the patients with partial- or late-onset isolated ACTH deficiency had mutations in the gene.

This paper adds to our knowledge of adrenal insufficiency and also emphasizes the need to recognize the condition early. These authors have shown in the past that *TPIT* mutations are responsible for 60% of neonatal isolated ACTH deficiency. They now describe the largest cohort so far, consisting of 91 patients with isolated ACTH deficiency. This gives valuable information about this rare disorder that still results in neonatal deaths, in 25% of the families in this and the previous cohort [13]. *TPIT* mutations were found in 65% of patients with severe neonatal adrenal insufficiency, and in none of those with partial- or late-onset adrenal insufficiency. All patients with identified mutations had extremely severe adrenal insufficiency with very low cortisol concentrations (24 ± 6 nmol/l) from birth and absent or a weak response to intravenous CRH. All presented with severe hypoglycemia, 53% had associated seizures and 64% had cholestatic jaundice, which still is often unrecognized as a sign of hypocortisolism. An impressive 64% of the cases with *TPIT* mutations were familial and consanguinity was observed in 42%. Most mutations (35/37) were homozygous or compound heterozygous; heterozygous parents were unaffected. Mutations were distributed throughout the *TPIT* gene, but mainly in the T box, affecting DNA binding.

Of interest is the fact that, consistent with previous reports, *TPIT* mutations were not responsible for partial- or late-onset isolated ACTH deficiency. Mutations in regulatory sequences of *TPIT* could be responsible for less severe adrenal insufficiency, but these have yet to be identified.

Related pituitary cell lineages develop into interdigitated 3D cell networks

Budry L, Lafont C, El Yandouzi T, Chauvet N, Conejero G, Drouin J, Mollard P

Laboratory of Molecular Genetics, Institut de Recherches Cliniques de Montreal, Montreal, Que., Canada

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Background: Terminally differentiated secreting cells are not distributed randomly in a patchwork-like fashion throughout the pituitary gland. Instead, data suggest that these cells organize themselves in homotypic networks. This was first shown by Bonnefont et al. when growth hormone (GH)-secreting cells were visualized in pituitary slices using high-resolution imaging [14]. The connectivity between the cells of this network is important for the delivery of coordinated secretory pulses of hormones to their target tissues. The current article addresses the question of whether the two least abundant pituitary cell types, corticotrophs and gonadotrophs, are also organized in homotypic cell networks and if the interaction between these cells within the networks is important for their development and function.

Methods: High-resolution multiphoton imaging and confocal reconstruction was used to visualize corticotrophs and gonadotrophs in 3D throughout pituitary development. POMC-GFP and LH-Cer (cerulean fluorescence) transgenic mice were used to specifically detect corticotrophs and gonadotrophs throughout their ontogeny in the pituitary gland.

Results: Like the GH cells, corticotrophs and gonadotrophs also form homotypic cell networks during development. The Pit1-independent cell lineage networks may function in a similar way to the Pit1-dependent somatotroph network to better integrate and propagate cell responses. Structural differences between corticotroph and gonadotroph cell networks exist at the cellular level. Gonadotrophs are in close proximity to microvasculature, whilst the corticotroph network is established away from microvasculature and hence, corticotrophs extend processes or cytonemes. Furthermore, heterotypic interactions occur during development, such that the gonadotroph network depends on differentiated corticotrophs that act as a scaffold.

Conclusion: Pituitary scale-3D high-resolution imaging has identified cell networks of corticotrophs and gonadotrophs. These networks develop specific cell-cell interactions that lead to ordered positioning of pituitary cells.

Existence of long-lasting experience-dependent plasticity in endocrine cell networks

Hodson DJ, Schaeffer M, Romano N, Fontanaud P, Lafont C, Birkenstock J, Molino F, Christian H, Lockey J, Carmignac D, Fernandez-Fuente M, Le Tissier P, Mollard P

Centre National de la Recherche Scientifique, Institut de Génomique Fonctionnelle, Montpellier, France

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Background: Hormone-producing cells in the pituitary are organized in homotypic cell networks rather than randomly placed through the pituitary gland. In some biological systems that are organized into networks, an experience-dependent plasticity exists that confers adaptive advantage to physiological changes. For instance in the nervous system, continuous stimulation leads to changes in the wiring and number of synaptic contacts. This article explores a potential role for experience-dependent plasticity in the organized cell networks of the pituitary gland.

Methods: Transgenic mice expressing DsRed under the *Prl* promoter were used throughout this work. Moreover, a multibeam two-photon high-resolution calcium imaging system was utilized to analyze changes in cytosolic Ca^{2+} as well as visualization of prolactin cells in vivo.

Results: By using lactation to repeatedly stimulate the lactotroph cell network, the authors show that the lactotroph cell network undergoes functional plasticity in response to stimuli. Upon lactation, this cell population establishes a functional connectivity template. This is mediated through cell morphological changes and gap junctions and establishes a long-lasting pattern that results in a long-term experience-dependent plasticity.

Conclusion: The pituitary lactotrophs arrange themselves in homotypic cellular network that have long-term experience-dependent responses to physiological changes. This allows for better functional adaptation to physiological changes to mount homeostatic responses.

Influence of estrogens on GH-cell network dynamics in females: a live in-situ imaging approach

Schaeffer M, Hodson DJ, Meunier AC, Lafont C, Birkenstock J, Carmignac D, Murray JF, Gavois E, Robinson IC, Le Tissier P, Mollard P

Centre National de la Recherche Scientifique, Institute of Functional Genomics, Institut National de la Sante et de la Recherche Médicale, Montpellier, France
Endocrinology 2011;152:4789–4799

Background: Pituitary hormone production is tightly regulated and adapts to changing physiological status and environmental stimuli. The pituitary gland must therefore undergo marked structural and functional plasticity. This is thought to primarily rely on changes in cell proliferation and size. This study investigated whether cell motility, important for organ development during embryogenesis, represents an additional mechanism to promote plasticity within the adult pituitary gland.

Methods: Multiphoton time-lapse imaging methods were used to track cell dynamics over a period of 12 h in GH-eGFP transgenic mice to assess adaptation of the GH axis to varying gonadal steroid environments.

Results: Ovariectomy induced a dramatic increase in cell motility associated with GH-cell network remodeling. Estradiol treatment after ovariectomy prevented these changes. Estradiol also increased coordinated GH-cell activity during multicellular calcium recordings, suggesting enhanced network connectivity. Male castration did not result in similar alterations.

Conclusion: GH-cell motility is involved in the structural and functional pituitary plasticity in response to changing estradiol concentrations in the female.

These three articles, by Chauvet, Hodson and Schaeffer from Mollard's laboratory elegantly show that terminally differentiated hormone-producing cells of the pituitary are organized in homotypic cell networks and that these cellular networks generate experience-dependent responses. Previous work by Bennefont et al. refuted the previous dogma of pituitary cells being organized in a random patchwork fashion, and showed that GH cells form a three-dimensional network of cadherin-linked cells [14]. This somatotroph network exhibits a large-scale coordinated increase in intracellular calcium associated with hormone pulses. This distribution of cells into networks facilitates the coordinated physiological responses to stimuli. This article raised the question of whether other pituitary cell types exhibited a specific cell network distribution. Making use of powerful 3D two photon high-resolution imaging techniques combined with mouse transgenics, Chauvet and colleagues now demonstrate that two other pituitary cell types, corticotrophs (POMC-eGFP) and gonadotrophs (LH-Cer), are organized in distinct homotypic cell networks that are intertwined. Interestingly, heterotypic interactions between the corticotroph and the gonadotroph networks exist, as shown by hyperplasia of the gonadotroph network in the *Tpit* null mice that lack POMC cells. Moreover, the corticotroph network acts as a scaffold to gonadotrophs during development, further indicating the interdependence of these two cell networks. Impressive work from Hodson et al., delves deeper into the function of these networks, and identifies for the first time the important phenomenon of experience-dependent plasticity within the lactotroph network. Hodson and co-workers utilize system biology with high-resolution imaging and physiology to understand the impact on the lactotroph network upon continuous stimulation (lactation). Interestingly, a pattern/template is established by cell-cell communication during lactation which may improve cell population responses to future lactation. These exciting studies not only offer novel insights into our understanding of pituitary physiology, but also suggest new tools and methods to further characterize pituitary phenotypes in mutant mouse strains. Previous work in the pituitary development area has mostly centered on a two-dimensional analysis of the pituitary gland, and by doing so, important information on how genes affect cell network formation or function, and thus pituitary function, has been overlooked.

Pulsatile patterns of pituitary hormone gene expression change during development

Featherstone K, Harper CV, McNamara A, Semprini S, Spiller DG, McNeilly J, McNeilly AS, Mullins JJ, White MR, Davis JR

Developmental Biomedicine Research Group, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK

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Background: Recent research has attempted to investigate the important issue of the timing of transcription in living cells. Studies on clonal cell lines have shown that transcription is often pulsatile and stochastic, with implications for cellular differentiation. However, few studies have investigated changes in transcriptional activity during development at cellular resolution within a physiological context. In this study, the authors aimed to study the transcription of the gene encoding prolactin at various developmental stages.

Methods: To investigate single-cell transcriptional activity in real-time in living tissue, the authors used bioluminescence imaging of pituitary tissue from transgenic rats in which luciferase gene expression is driven by the prolactin (*Prl*) promoter. The authors studied both fetal and neonatal pituitary tissue to assess whether dynamic patterns of transcription change during tissue development.

Results: The authors report that gene expression in single cells is highly pulsatile and rapidly turns on and off at the time lactotrophs first appear during murine fetal development (E16.5); later on *Prl* gene expression levels increase but become stabilized as the tissue develops in early neonatal life. Since isolated cells, generated from enzymatic dispersion of pituitary tissue, display pulsatile luminescence, the stabilized transcription pattern might depend upon tissue architecture or paracrine signaling. Nascent cells in embryonic tissue also showed coordinated transcription activity over short distances, further indicating that cellular context is important for transcription activity.

Conclusion: These data show that transcriptional activity is a dynamic process, and cells alter their patterns of gene expression according to their context and developmental stage, with important implications for cellular differentiation.

This is an important study using a combination of transgenesis and single cell transcriptional assays. The authors show that prolactin gene expression is dynamic from an early stage. During embryogenesis, from E16.5 in the rat embryo, lactotrophs show pulsatile transcriptional responses of prolactin gene expression. As gestation progresses, the transcriptional activity is more stable. Hence, lactotrophs alter their transcription pattern depending on their developmental context. These data suggest the importance of transcriptional patterns on cell differentiation. The authors suggest that pulsatile gene expression early in tissue development may occur before lineage commitment, when numerous genes might exist in a poised state and can be transcribed at low levels. Commitment to a cell lineage leads to higher expression of the required genes and silencing of other genes. This hypothesis is supported by observations that chromatin structure exists in a globally open and plastic conformation in embryonic stem cells and adopts a more closed structure upon cell differentiation. The authors also show that the stable transcriptional activity of neonatal lactotroph cells switches to a pulsatile pattern when the pituitary tissue is enzymatically dispersed. Hence, the architecture of the tissue in which the cells are located is also critical in determining patterns of gene expression. These studies have wider applications, and suggest that gene transcription may vary according to developmental stage and the cellular context. Paracrine cell signaling may be critical for normal gene expression, and it is important to note that transcriptional activity may differ in isolated cells *in vitro* and *in vivo*.

Disruption of SoxB1-dependent Sonic hedgehog expression in the hypothalamus causes septo-optic dysplasia

Zhao L, Zevallos SE, Rizzoti K, Jeong Y, Lovell-Badge R, Epstein DJ

Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

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Background: The congenital birth defect septo-optic dysplasia (SOD) is characterized by optic nerve, mid-line forebrain and/or pituitary gland abnormalities. Only a few genes that cause SOD have been identified, amongst others *Hesx1*, *Sox2* and *Sox3* are implicated. The precise role of other genes in the etiology of SOD is poorly understood. *Sonic hedgehog* (*Shh*) is an important gene that regulates early formation and patterning of the central nervous system. Its haploinsufficiency in humans causes holoprosencephaly (HPE). *SHH* is crucial for the formation of the hypothalamus and HPE patients can exhibit a compromised hypothalamic-pituitary axis. However, studies into the role of *Shh* in murine hypothalamic development have been hampered by the severe phenotype of the *Shh* null embryos. In this paper, using a conditional gene targeting approach, the requirement of the hypothalamus for *Shh* is unraveled.

Methods: Use of a transgenic approach to achieve specific ventral-diencephalic (VD) deletion of *Shh* by crossing a *Shh^{loxP/loxP}* to the *SBE:Cre* line (where Cre is expressed under the *Shh*-brain enhancer-2 *SBE2*). This transgenic line (*Shh^{Δhyp}*) results in deletion of *Shh* in the VD whilst expression of *Shh* in other parts of the brain remains uncompromised, allowing the study of the role of *Shh* in the hypothalamus and in the phenotype of SOD.

Results: Expression of *Shh* in the VD is required for proper patterning of the prospective hypothalamic region. Absence of *Shh* in *Shh^{Δhyp}* embryos leads to hypoplasia of the pituitary gland and abnormal early patterning and positioning of the hypothalamus. Moreover, *Shh* morphogen activity from the VD is required for the proper patterning of the retina and for optic disc formation. The *SBE2* enhancer that directs expression of *Shh* in the hypothalamus contains highly conserved SOXB1-binding sites and *Sox2* and *Sox3* directly regulate *Shh* expression by binding to the *SBE2* enhancer.

Conclusion: Ventral diencephalic expression of *Shh* is regulated by *Sox2* and *Sox3*, and the absence of *Shh* signaling causes SOD-like features in the mouse.

This article describes, for the first time, the requirement of *Shh* signaling from the embryonic prospective hypothalamic region in the formation of the hypothalamic-pituitary axis and eye development. A role for oral ectoderm-*Shh*-derived signaling has been described to be important for pituitary formation, and the lack of *Shh* in the oral ectoderm results in the absence of ventral pituitary cell types [3, 7]. However, the role of *SHH* emanating from other sources such as the ventral diencephalon in the formation of the hypothalamus was unknown. Absence of *Shh* from the prechordal plate causes HPE; the telencephalic vesicles fail to divide, resulting in cyclopic embryos. Due to this severe phenotype the study of *Shh*-associated phenotypes was hampered. Epstein and colleagues ablated *Shh* from the ventral diencephalon (the prospective hypothalamic region) by using a neat conditional transgenic line that expresses Cre under the *Shh*-ventral diencephalon-specific enhancer (*SEB2:Cre*) [15]. This method allows expression of *Shh* to be normal in the prechordal plate, thereby bypassing the severe *Shh*-HPE phenotype. Interestingly, *Shh* expression from the ventral diencephalon is required for both eye disc formation and hypothalamic-pituitary development. In the absence of *Shh* signaling the pituitary is hypoplastic and the hypothalamic region displays patterning defects, reminiscent of SOD. The authors go one step further and identify SoxB1-binding sites in the *Shh*-ventral diencephalon enhancer and show with biochemical assays that *SOX2* and *SOX3* regulate *Shh* expression in the VD. As *SOX2* and *SOX3* have been shown to be involved in SOD, these phenotypes could be linked to the lack of early *Shh* expression from the prospective hypothalamus. Thus, understanding how these genes regulate transcriptional cascades will provide a further explanation for a congenital malformation such as SOD.

EGFR as a therapeutic target for human, canine, and mouse ACTH-secreting pituitary adenomas

Fukuoka H, Cooper O, Ben-Shlomo A, Mamelak A, Ren SG, Bruyette D, Melmed S
Pituitary Center, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA
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Background: Cushing's disease is a condition in which the pituitary gland releases excessive adrenocorticotrophic hormone (ACTH) as a result of an adenoma arising from ACTH-secreting cells. Optimal therapy currently entails surgical adenoma resection, with initial remission rates of 65–90% for microadenomas and <65% for macroadenomas. However, 10-year recurrence rates are 10–20% for microadenomas and up to 45% for macroadenomas. Pituitary-directed medical therapies are mostly ineffective, and new treatment options are needed. As these tumors express EGFR, the authors tested whether EGFR might provide a therapeutic target for Cushing's disease.

Methods: The effect of gefitinib, a tyrosine kinase inhibitor (TKI) that targets the EGFR, was tested on human corticotroph adenoma cells that express EGFR, and on ACTH-producing canine pituitary tumor cells. Additionally, the drug was tested on a murine corticotroph adenoma, investigating both cell proliferation and apoptosis.

Results: The authors show that in surgically resected human and canine corticotroph cultured tumors, blocking EGFR with gefitinib suppressed expression of proopiomelanocortin (POMC) as well as ACTH secretion. In mouse corticotroph EGFR transfectants, ACTH secretion was enhanced, and EGFR increased Pomc promoter activity, an effect that was MAPK-dependent. Blocking EGFR activity with gefitinib attenuated Pomc expression, inhibited corticotroph tumor cell proliferation, and induced apoptosis. As predominantly nuclear EGFR expression was observed in canine and human corticotroph tumors, EGFR was preferentially targeted to mouse corticotroph cell nuclei, which resulted in higher Pomc expression and ACTH secretion, both of which were inhibited by gefitinib. In athymic nude mice, EGFR overexpression enhanced the growth of explanted ACTH-secreting tumors and further elevated serum corticosterone concentrations. Gefitinib treatment decreased both tumor size and corticosterone concentrations; it also reversed signs of hypercortisolemia, including elevated glucose concentrations and excess omental fat.

Conclusions: These results suggest that inhibition of EGFR signaling may be a novel strategy for treating Cushing's disease, and gefitinib may be a potential therapeutic agent in Cushing's disease.

A 12-month phase 3 study of pasireotide in Cushing's disease

Colao A, Petersenn S, Newell-Price J, Findling JW, Gu F, Maldonado M, Schoenherr U, Mills D, Salgado LR, Biller BM
Department of Molecular and Clinical Endocrinology and Oncology, Section of Endocrinology, University of Naples Federico II, Naples, Italy
N Engl J Med 2012;366:914–924

Background: Cushing's disease is associated with high morbidity and mortality. Therapy is challenging, with relapse in up to 30% of patients. Corticotroph adenomas express somatostatin receptors, predominantly somatostatin-receptor subtype 5. A novel potential therapy, pasireotide, has a unique, broad somatostatin-receptor-binding profile, with high binding affinity for somatostatin-receptor subtype 5.

Methods: In this double-blind, phase 3 study, 162 adults with Cushing's disease and a urinary-free cortisol level of at least 1.5 times the upper limit of the normal range were randomly assigned to receive subcutaneous pasireotide at a dose of 600 µg (82 patients) or 900 µg (80 patients) twice daily. Patients with urinary-free cortisol not exceeding 2 times the upper limit of the normal range (ULN) and not exceeding the baseline level at month 3 continued to receive their randomly assigned dose; all others received an additional 300 µg twice daily. Primary endpoint: a urinary-free cortisol level at or below the upper limit of the normal range at month 6 without an increased dose. Open-label treatment continued through month 12.

Results: Twelve of 82 patients in the 600-µg group and 21 of 80 patients in the 900-µg group met the primary endpoint. The median urinary-free cortisol level decreased by approximately 50% by month 2

and remained stable in both groups. A normal urinary-free cortisol level was achieved more frequently in patients with baseline levels not exceeding 5 times the ULN range than in patients with higher baseline levels. Serum and salivary cortisol and plasma corticotropin levels decreased, and clinical signs and symptoms of Cushing's disease diminished. Pasireotide was associated with hyperglycemia-related adverse events in 118 of 162 patients; other adverse events were similar to those associated with other somatostatin analogues. Despite reductions in cortisol concentrations, blood glucose and glycated hemoglobin concentrations increased soon after treatment initiation and then stabilized; treatment with a glucose-lowering medication was initiated in 74 of 162 patients.

Conclusion: The significant decrease in cortisol concentrations in patients with Cushing's disease who received pasireotide supports its potential use as a targeted treatment for corticotropin-secreting pituitary adenomas.

These two papers tackle the thorny issue of Cushing's disease, a condition that can be challenging to treat. In children and adolescents with Cushing's disease, 80–85% have surgically identifiable microadenomas; 20% relapse post-surgery with a net cure rate of 70–75%. Radiotherapy is used if surgery is unsuccessful; children respond more rapidly, with a cure rate of 92%. However, GHD is common and other pituitary hormone deficiencies may occur, although with a lower frequency.

Recent advances have revealed a role for novel signaling pathways in the etiology of Cushing's disease, one of which is the EGFR signaling pathway. The paper by Fukuoka et al. has utilized this pathway to identify novel medical therapies; EGFR led to increased *POMC* promoter activity with an increase in ACTH secretion. The authors then used a tyrosine kinase inhibitor, gefitinib, to block EGFR signaling and found that corticotroph tumor cell proliferation was reduced and apoptosis induced. In mice, *in vivo* studies revealed that Gefitinib treatment decreased both tumor size and corticosterone concentrations; it also reversed signs of hypercortisolemia. These data are promising, but whether they will translate to human patients with Cushing's disease remains to be seen. In particular, the interruption of the EGFR signaling pathway in other organs may lead to a number of unwanted effects.

In the second study, Colao and colleagues have used a somatostatin receptor subtype 5 agonist, pasireotide, for the treatment of Cushing's disease. The results are promising, with a reduction in cortisol secretion and improvement in clinical features such as hypertension. However, there is a significant increase in the degree of hyperglycemia, with adverse events due to elevated blood glucose concentrations being documented in 118/162 patients. Currently, the potential indication for this drug would appear to be in resistant cases of Cushing's disease.

Concepts revised

Permanent hypopituitarism is rare after structural traumatic brain injury in early childhood

Heather NL, Jefferies C, Hofman PL, Derraik JG, Brennan C, Kelly P, Hamill JK, Jones RG, Rowe DL, Cutfield WS
Liggins Institute, University of Auckland, Auckland, New Zealand
J Clin Endocrinol Metab 2012;97:599–604

Background: Hypopituitarism or isolated GH deficiency can occur after traumatic brain injury. This study aimed to determine the incidence of permanent hypopituitarism in a group of young children after structural traumatic brain injury (TBI).

Methods: This is a cross-sectional study with longitudinal follow-up. Pituitary function was dynamically tested in all subjects. Diagnosis of GH deficiency was based on assessment of stimulated GH peak (<5 µg/l), IGF-I, and growth pattern. ACTH deficiency was diagnosed if there was a subnormal response to two serial Synacthen tests (peak cortisol <500 nmol/l) and a Metrapone test.

Results: 198 survivors of structural TBI sustained at an early age (1.7 ± 1.5 years) were studied 6.5 ± 3.2 years after injury. Brain injury was inflicted in 33% and accidental in 68%. Precocious puberty occurred in 2 patients, just within the expected rate for the normal population. Peak stimulated GH was <5 µg/l in 16 participants (8%), but these children had normal IGF-I and normal growth. Stimulated peak

cortisol was low in 17 (8%), but all had normal ACTH function on Metyrapone testing. One participant had a transient low serum T₄.

Conclusion: Permanent hypopituitarism is rare after both inflicted and accidental TBI in early childhood. Precocious puberty was the only pituitary hormone abnormality found, but the prevalence did not exceed that of the normal population.

This is an important study that gives insight into the incidence of pituitary dysfunction after brain injury, which may develop probably as a result of injury to portal vessels. Many reports over the last decade have emphasized the occurrence of such pituitary insufficiency, and a recent meta-analysis in adults reported the rate of hypopituitarism in traumatic brain injury being as high as 30%. Studies in children show a highly variable rate of hypopituitarism (5–60%), possibly due to the variable severity of brain injury, small cohort size and nonstandardized methodology. This study by Heather et al. stands out because of its large homogeneous cohort of patients with severe brain injury leading to structural abnormalities (198 patients, 40% intensive care treatment, 66% intracerebral hemorrhage, 66% cerebral injury and 70% skull fractures) and its thorough analysis of pituitary function.

In contrast to other studies, none of the patients had permanent hypopituitarism, despite the severe degree of brain injury. Precocious puberty was the only endocrine abnormality found although, despite the large size of the group, the rate was too low to know whether this was due to brain injury. Low GH responses to GH testing were noted (33% had a GH peak <10 µg/l) but, in contrast to other studies and following national guidelines, a diagnosis of GH deficiency was only made if there was a combination of low GH peak (<5 µg/l) on two stimulation tests, low IGF-1 concentration and poor growth. The authors show biochemical and growth details of the patients with GH peaks <5 µg/l, supporting the absence of GHD although growth charts were not shown. Of note is the presence of obesity in all of these children (BMI +2 to 3 SD); the authors suggest that this may be the reason for their low GH peak concentration. Similarly, a diagnosis of ACTH deficiency was only made if both Synacthen and Metyrapone tests were abnormal, but this occurred in none of the patients.

In conclusion, permanent hypopituitarism is uncommon after severe brain injury in young children when stringent clinical criteria for pituitary insufficiency are used. Routine testing of pituitary function, as suggested for adults, may not be necessary in children and will lead to a high number of abnormal test results, the significance of which is unknown.

Concepts (not) revised

Aged PROP1-deficient dwarf mice maintain ACTH production

Nasonkin IO, Ward RD, Bavers DL, Beuschlein F, Mortensen AH, Keegan CE, Hammer GD, Camper SA
Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA
PLoS One 2011;6:e28355

Background: *PROP1* mutations in humans are associated with the phenotype of multiple pituitary hormone deficiencies (MPHD) that typically progress from growth deficiency/insufficiency (GHD/GHI) diagnosed in infancy to include other hormone (GH) deficiencies including TSH, prolactin and gonadotrophin deficiencies. This progressive reduction in other anterior pituitary hormones eventually includes ACTH deficiency. It is therefore critically important to test the hypothalamo-pituitary-adrenal (HPA) axis regularly in order to detect evolving ACTH deficiency at a pre-symptomatic stage. Congenital deficiencies of GH, prolactin, and thyroid stimulating hormone have been reported in the *Prop1*(null) (*Prop1*(-/-)) and the Ames dwarf (*Prop1*(*df/df*)) mouse models, but corticotroph and pituitary adrenal axis function have not been thoroughly investigated in these murine models.

Methods: *Prop1* null mutants were generated on the N4 B6 background. Corticosterone, ACTH and blood glucose concentrations were measured basally and in response to restraint stress. Adrenal glands were weighed.

Results: The N4 B6 background results in a wasting phenotype in the mutant mice that is associated with severe hypoglycemia and lethality in approximately one third of the mice, between weaning and adult-

hood; remaining homozygotes live with no signs of illness. Circulating ACTH and corticosterone levels are elevated in juvenile and aged *Prop1* mutants, indicating activation of the pituitary-adrenal axis. Despite this, young adult *Prop1*-deficient mice are capable of responding to restraint stress with further elevation of ACTH and corticosterone. The elevation in ACTH and corticosterone concentrations, which is present basally, is likely due to low blood glucose concentrations, which in turn is possibly due to GH deficiency. In keeping with the chronically elevated ACTH and corticosterone concentrations, the ratio of adrenal weight to body weight was elevated in *Prop1*^{-/-} males compared to wild type animals.

Conclusion: The *Prop1*-deficient mouse model differs from the human patients who display progressive hormone loss and hypocortisolism. The underlying reason for the ACTH deficiency in humans remains unknown.

Mutations in the gene *PROP1* are not infrequently identified in patients with familial MPPH. The phenotype includes GH, TSH, prolactin and gonadotrophin deficiencies and can be highly variable. In particular, the TSH and gonadotrophin deficiencies can develop later, with puberty commencing spontaneously in a number of cases but then failing to progress. Two clinical features of human *PROP1* deficiency are particularly perplexing; namely the later evolution of ACTH deficiency with hypocortisolemia and an enlarged pituitary gland that then involutes. For neither phenomenon has a satisfactory explanation been advanced to date. This paper has attempted to answer the first of these questions. However, the authors clearly show that murine *Prop1* mutants manifest increased lethality, but this is not related to ACTH or corticosterone deficiency. In fact, the mutant mice have elevated ACTH and corticosterone concentrations, and these chronically elevated measurements are probably related to GH deficiency. In keeping with these data, the adrenals were relatively enlarged in the affected mice. Hence these data suggest important differences between mice and humans in terms of the HPA axis.

New syndrome

Deficit in anterior pituitary function and variable immune deficiency (DAVID) in children presenting with adrenocorticotropin deficiency and severe infections

Quentien MH, Delemer B, Papadimitriou DT, Souchon PF, Jaussaud R, Pagnier A, Munzer M, Jullien N, Reynaud R, Galon-Faure N, Enjalbert A, Barlier A, Brue T
Centre de Recherche en Neurobiologie et Neurophysiologie de Marseille, Aix-Marseille Universite, Marseille, France
J Clin Endocrinol Metab 2012;97:E121–128

Background: The French GENHYPOPIT network contained 21 patients with isolated ACTH deficiency without a TPIT mutation. Three of those (13.6%) displayed common variable immunodeficiency (CVID). The authors present this previously unrecognized disease association.

Methods: The hypothesis of ACTH deficiency being associated with antipituitary autoimmunity or lymphocytic hypophysitis was tested using assays for pituitary auto-antibodies. A candidate gene approach and sequence analysis was used to detect a common underlying gene alteration.

Results: All patients including a pedigree with two affected siblings had ACTH deficit diagnosed from 5 to 15 years, and CVID diagnosed from 2 to 8 years revealed by recurrent infections. Three of the four patients had a hypoplastic pituitary. One patient had low IGF-I and subnormal GH response to stimulation, suggesting that secretion of other pituitary hormones may also be affected. All patients proved negative for pituitary autoantibodies and had no alteration in the genes *LIF*, *IKAROS* or *EOS*.

Conclusions: The remarkable association of two rare disorders affecting two functionally related systems in four patients from three independent pedigrees, including a familial case, provides strong evidence of the existence of a disease association: deficit in anterior pituitary function and variable immune deficiency, or DAVID.

The paper shows the importance of maintaining databases for the detection and understanding of rare diseases. The GENHYPOPIT database is a clinical research network in France that contains approx. 700 index cases with CPHD. The authors recognized that of 31 patients with isolated ACTH deficiency, 4 patients (both male and female) from 3 separate families also had Common Variable Immune Deficiency. Most patients showed anterior pituitary hypoplasia on MRI but no other anatomical abnormalities. CVID is a hugely heterogeneous disorder but all patients suffered from the same type of CVID supporting the notion that this is a previously unrecognized disease association that the authors named 'Deficit in Anterior pituitary function and Variable Immune Deficiency', or DAVID. A candidate gene approach was not sufficient to pinpoint the underlying genetic aetiology, but next generation sequencing is what is really needed to establish the cause of this new disease association.

Important for clinical practice

Symptomatic heterozygotes and prenatal diagnoses in a nonconsanguineous family with syndromic combined pituitary hormone deficiency resulting from two novel LHX3 mutations

Sobrier ML, Brachet C, Vie-Luton MP, Perez C, Copin B, Legendre M, Heinrichs C, Amselem S

Institut National de la Sante et de la Recherche Medicale Unite 933, Universite Pierre et Marie Curie-Paris 6, Hopital Armand Trousseau, Paris, France

marie-laure.sobrier@inserm.fr

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Background: LHX3 is a member of the LIM family of transcription factors that has been shown to be important for the normal development of the anterior pituitary gland and motor neurons. To date, 11 mutations have been reported in *LHX3*; all patients were homozygous from consanguineous pedigrees, with various syndromic forms of combined pituitary hormone deficiency (CPHD), including limited rotation of the neck and sensorineural hearing loss.

Methods: The authors aimed to report the family history and the molecular basis of a nonconsanguineous patient with syndromic CPHD, limited neck rotation, severe scoliosis, but normal intelligence. His father and paternal grandmother displayed limited head rotation. In view of this, the authors sequenced the *LHX3* gene. Novel mutations were then inserted into plasmids and functional studies were performed using transcriptional activation assays with the human PRL promoter.

Results: Two new *LHX3* mutations were identified. The paternally inherited c.252-3C>G mutation, which disrupts an acceptor splice site, led to severely truncated proteins containing a single LIM domain, resembling LIM-only proteins. Co-expression studies revealed a dominant-negative effect of this LIM-only protein over the wild-type LHX3. The maternally inherited p.Cys118Tyr mutation resulted in partial loss of transcriptional activity and synergy with POU1F1. Given the severity of the patient's phenotype, prenatal diagnoses were performed on two occasions: the first led to termination of the pregnancy whilst the second resulted in the birth of a healthy boy.

Conclusion: This study reports the first nonconsanguineous patient with *LHX3* mutations and supports the pleiotropic roles of LHX3 during development and its involvement in a complex disease phenotype. Isolated limitation of head rotation may exist in heterozygous carriers and may result from a dominant-negative effect. These data allowed the first published description of prenatal diagnoses of this severe condition.

Mutations within the early transcription factors and signaling molecules involved in anterior pituitary gland formation are often associated with complex syndromic phenotypes in association with hypopituitarism. Initially, mutations in *LHX3* were identified in patients with hypopituitarism associated with limited neck rotation. It was felt that corticotrophs were spared and ACTH deficiency was not a feature of the syndrome. Subsequently, patients with *LHX3* mutations were noted to be ACTH-deficient, and in patients with mutations in the C-terminus of the protein, neck rotation was normal. Additionally, hearing impairment is now recognized as a consistent feature of the LHX3-deficient phenotype.

In this paper, Sobrier et al. confirm the association of hypopituitarism, hearing impairment and skeletal abnormalities in patients with LHX3 deficiency. However, they also show a dominant negative effect for the splicing mutation identified in this family. Importantly, the proband's father and paternal grandmother, both of whom carry the splicing mutation, have a mild phenotype characterized by limited neck rotation. It would therefore be important to screen heterozygous carriers of *LHX3* mutations for milder phenotypes; the existence of both dominant and recessive mutations has already been identified in the *POU1F1* and *HESX1* genes. These studies further emphasize the importance of functional characterization of genetic mutations.

Food for thought

Dwarfism in mice lacking collagen-binding integrins $\alpha_2\beta_1$ and $\alpha_{11}\beta_1$ is caused by severely diminished IGF-1 levels

Blumbach K, Niehoff A, Belgardt BF, Ehlen HW, Schmitz M, Hallinger R, Schulz JN, Bruning JC, Krieg T, Schubert M, Gullberg D, Eckes B

Department of Dermatology, University of Cologne, Cologne, Germany

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Background: $\alpha_2\beta_1$ and $\alpha_{11}\beta_1$ integrins are the major receptors for collagen I. Collagen I is the major collagen of bone and $\alpha_{11}\beta_1$ integrin is thought to have an important role in bone turnover, in contrast to $\alpha_2\beta_1$.

Methods: Integrin $\alpha_2\beta_1$ -deficient mice and integrin $\alpha_{11}\beta_1$ -deficient mice were bred to generate mice deficient in both integrins.

Results: These mice have a normal size at birth but develop dwarfism within the first 4 weeks of life. They have shorter, less mineralized, and functionally weaker bones but there are no growth plate abnormalities or osteoblast dysfunction. All organs are proportionally smaller, suggesting a systemic cause for the overall size reduction. Serum IGF-1 concentrations of mice lacking either $\alpha_2\beta_1$ or $\alpha_{11}\beta_1$ or both integrins were reduced by 39, 64, or 81% respectively. Growth hormone-releasing hormone expression in the hypothalamus and growth hormone gene expression in the pituitary glands were also reduced in these mice.

Conclusion: Collagen-binding integrin receptors are involved in the control of the growth hormone/IGF-1 axis. Thus, coupling hormone secretion to extracellular matrix signaling via integrins represents a novel concept in the control of endocrine homeostasis.

This is an interesting paper that convincingly shows that mice lacking $\alpha_2\beta_1$ and $\alpha_{11}\beta_1$ integrins, the major receptors for collagen 1, develop dwarfism with a proportional reduction in size of all organs in the first month of life. They have a reduction of serum IGF-1 concentrations, pituitary GH content and hypothalamic GHRH content without evidence of other pituitary hormone deficiencies. Intrinsic bone metabolism and bone development is normal and the authors therefore argue that the dwarfism is due to GH and IGF-1 deficiency. This uncovers a novel mechanism for growth regulation and suggests a feedback loop from extracellular matrix in the bone to the hypothalamus-pituitary involving collagen I. Feedback regulation from bone is not new – feedback from bone to pancreas and brain to control energy metabolism has been shown [16] but this is the first study that would point to integrins as part of a feedback loop.

Alternatively, integrins could play a role in the hypothalamus or pituitary itself; in communication in neuronal networks, differentiation of precursors into hormone-producing cells [17] or in actual hormone production and release. For example, the cell adhesion molecule E-cadherin, which plays a key role in epithelial mesenchymal transition, is involved in the organization and function of the pituitary somatotroph network and in the development of GH adenomas [18]. In addition, integrins are involved in the proliferation of pituitary folliculostellate cells [19]. A role for other cell adhesion molecules like integrins in hypothalamic and pituitary function would therefore be possible [20].

A further point of interest is that the reduction of growth in the mutant mice occurred mostly in the first month of life, and remained constant in the next 2 months, which the authors argued was not

due to reduced food intake. IGF-1 concentration was also most severely affected at 1 month of age, and had almost normalized at 3 months of age. This growth and hormone pattern is reminiscent of that seen in constitutionally delayed growth or 'transient GH deficiency' in children, and may be a mouse model for this variant human growth pattern.

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