Pituitary and Neuroendocrinology

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In the pituitary field, stem cells are a hot topic and some outstanding papers have increased our knowledge of pituitary stem cells. This year's chapter also includes several papers with new genes involved in hypothalamic and pituitary syndromes. However, whereas in previous years, the function of genes found to be associated with pituitary and neuroendocrine disease was clear, we now seem to have entered the era where mutations are found in genes whose function is not known, or the mechanism for the disease is not clear.

New gene – mechanism awaited

Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination

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Background: The combination of ataxia and hypogonadism was first described more than a century ago, but its genetic basis has remained elusive.

Methods: Whole-exome sequencing was performed in a patient with ataxia and hypogonadotropic hypogonadism, followed by targeted sequencing of candidate genes in similarly affected patients in a total of 12 patients in 8 families. The functional effects of sequence variants were tested in a zebrafish model.

Results: Digenic homozygous mutations in *RNF216* and *OTUD4*, which encode a ubiquitin E3 ligase and a deubiquitinase, respectively, were found in 3 affected siblings in a consanguineous family. In an unrelated patient, a compound heterozygous truncating mutation in *RNF216* was identified and 4 other patients had a single heterozygous deleterious mutation in *RNF216*. All patients had progressive ataxia and dementia. Neuronal loss was seen in cerebellum and the hippocampus; surviving hippocampal neurons contained ubiquitin-immunoreactive intranuclear inclusions. Defects were detected at the hypothalamic and pituitary levels of the reproductive endocrine axis. Morpholinos were used to knock-down *rnf216* or *otud4* in zebrafish embryos and this resulted in defects in the eye, optic tectum, and cerebellum; defects were more severe when both genes were suppressed and abnormalities were rescued by nonmutant human *RNF216* or *OTUD4* messenger RNA.

Conclusions: The syndrome of hypogonadotropic hypogonadism, ataxia, and dementia can be caused by inactivating mutations in *RNF216* or can have a digenic cause involving sequence variants in *RNF216* and *OTUD4*. These findings link disordered ubiquitination to neurodegeneration and reproductive dysfunction.

Patients with Gordon Holmes syndrome present in adolescence or early adulthood with hypogonadotropic hypogonadism, but no other pituitary abnormalities, and subsequent dysarthria, ataxia and dementia, leading to wheelchair dependency, mutism, and abnormal movements. This extensive paper shows that this intriguing syndrome can be caused by mutations in *RNF216* either singly or in combination with mutations in *OTUD4*, respectively an E3 ubiquitin ligase and a deubiquitinase. Ubiquitination is a fundamental cellular process; ubiquitin ligase attaches ubiquitin to protein sub-

strates, marking them for proteasome-mediated degradation. Deubiquitinases allow target proteins and ubiquitin itself to be recycled and often function in partnership with specific E3 ligases. E3 ubiquitin ligase is structurally similar to parkin, that is mutated in a recessive form of Parkinson's disease and therefore the link with neurodegenerative disease has been made before. But why is only the gonadotrope axis affected, and do other endocrine axes function normally? The LH/FSH deficiency is not complete, and some of the patients described have gone through puberty normally. The neural phenotype suggests it is a hypothalamic disorder, but GnRH testing shows that the response to GnRH wanes with time, suggesting there is also pituitary dysfunction.

A subsequent paper identified mutations in *RNF216* and *STUB1* [1], encoding the protein CHIP, in another family with the same syndrome. CHIP can act in conjunction with E3 ligase and is involved in protein quality control. CHIP is expressed throughout the brain and loss of CHIP function is associated with protein misfolding and aggregates in mouse models of neurodegenerative disease but not in human disease before. Indeed, *Chip*^{-/-} mice have reduced LH/FSH concentrations. This mutation in a human pedigree confirms that ubiquitination is required for normal LH and FSH production. The question why disordered ubiquitination affects the reproductive axis more than other hypothalamic-pituitary axes, remains unanswered.

New gene – important for hypothalamic development

ARNT2 mutation causes hypopituitarism, post-natal microcephaly, visual and renal anomalies

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Background: In the last decade, many genes have been identified that are essential for hypothalamic-pituitary axis development in mice. Identification of these murine genes has allowed identification of mutations in their human orthologues as causes of congenital forms of hypopituitarism and brain abnormalities, e.g. *Hesx1*, *BMP4*, *Otx2* and *Sox2* [2, 3]. This study aimed to identify mutations involved in hypothalamic development in a large consanguineous family with a previously unreported syndrome consisting of secondary microcephaly, fronto-temporal lobe hypoplasia, severe visual impairment, kidney abnormalities and multiple pituitary hormone deficiencies.

Methods: Homozygosity mapping and exome sequencing of the highly consanguineous family was performed. The mutation was confirmed by Sanger sequencing and functional analysis was performed using quantitative reverse transcription PCR in total mRNA from patients' fibroblasts. In situ hybridization of mRNA transcripts in the developing human embryo was used to assess the expression pattern of *ARNT2*.

Results: A frameshift mutation (*c.1373_1374dupTC*) in the transcription factor gene *ARNT2* was found that resulted in a premature termination codon. All affected individuals were homozygous for this mutation, which was not identified in 200 ethnically-matched controls. The mutation leads to non-sense-mediated decay, as shown by a 50- to 150-fold reduction in *ARNT2* mRNA expression in affected patients' fibroblast compared to control fibroblasts. *ARNT2* mRNA expression was detected in the developing human brain and pituitary gland at 8 weeks' gestation. Expression was especially high in the hypothalamus, posterior brain lobe, anterior pituitary gland, inner ear, trigeminal ganglion, neural retina, dorsal root ganglia and the developing kidney and lung. This pattern of expression, together with the early perinatal lethality of the *Arnt2* null mouse, indicates that this transcription factor is crucial for the development of the HP axis, eyes, kidneys, and brain.

Conclusions: Loss of function of *ARNT2* causes a previously uncharacterized syndrome. *ARNT2* is critical for the development of the CNS, affecting particularly the hypothalamus-pituitary axis, visual pathways and post-natal growth of the brain in humans as well as kidneys and urinary tract.

Although the genetic control of pituitary development is starting to be elucidated, much less is known about the molecular mechanisms that control the development of the hypothalamus and the relevance to the pathophysiology of human disease. This paper identifies a new regulatory gene required for normal hypothalamic-pituitary function and development of CNS, eyes and kidneys. A mutation in the gene *ARNT2*, coding for a helix-loop-helix transcription factor, leads to nonsense-mediated decay and results in a previously unrecognized congenital syndrome consisting of secondary (post-natal) microcephaly with fronto-temporal lobe hypoplasia, multiple pituitary hormone deficiency, seizures, severe visual impairment and abnormalities of the kidneys and urinary tract. Since the murine *Arnt2* null mice die perinatally, a tissue-specific conditional *Arnt2* knockout mouse model may help to further understand the mechanisms by which this transcription factor affects neurogenesis, pituitary and eye development. The authors discuss the interaction between *ARNT2* and *SIM1*, a transcription factor that is a key player in hypothalamic development. The genetic regulation of hypothalamic development is still poorly understood and this paper adds valuable information on the identification of genes that regulate hypothalamic-pituitary axis development and their role in complex human conditions.

New paradigm

Mutant prolactin receptor and familial hyperprolactinemia

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N Engl J Med 2013;369:2012–2020

Background: Hyperprolactinemia, if not associated with gestation or breastfeeding, is usually pathological. It can be caused by tumours in the anterior pituitary gland or hereditary multiple endocrine neoplasia (MEN) syndromes. This study aimed to identify whether prolactin (*PRL*) or *PRL* receptor (*PRLR*) gene sequence variants are a further cause of hyperprolactinaemia.

Methods: The authors sequenced *PRL* and *PRLR* in 3 sisters with hyperprolactinemia, without pituitary tumour or MEN, 2 of whom presented with oligomenorrhoea and 1 with infertility.

Results: A heterozygous mutation in *PRLR* was found, which resulted in a histidine to arginine change at codon 188 (His188Arg). This substitution disrupted the high-affinity ligand-binding interface of the prolactin receptor, resulting in loss of downstream signaling by Janus kinase 2 (*JAK2*) and signal transducer and activator of transcription 5 (*STAT5*). The variant was not present in 110 controls or in 13,000 alleles in multiple databases.

Conclusion: Familial hyperprolactinemia appears to be due to a germline, loss-of-function mutation in *PRLR*, resulting in prolactin insensitivity.

The authors hypothesized that prolactin receptor gene (*PRLR*) mutations or bioinactive *PRL* would cause hyperprolactinaemia, analogous to *GHR* mutations resulting in *GH* insensitivity and increased *GH* production, a very valid hypothesis. They sequenced *PRL* and *PRLR* in 3 sisters from a non-consanguineous family that were affected by hyperprolactinaemia, galactorrhoea and reduced fertility without a known cause. No *PRL* mutations were found, but a heterozygous change in the *PRLR* was found at a vital location in *PRLR* that is conserved in all mammals and marsupials and is part of the high-affinity (first) binding site of *PRLR*. The mutation segregated with the phenotype, although mutations in males and prepubertal children mean that segregation cannot be established with 100% certainty. His188 has been shown previously to have a major role in binding of *PRL* and the very significant His188-to-Ala188 change reduced binding *in vitro*. A major question however is whether the heterozygous His to Arg change is sufficient to reduce signaling *in vivo*. The authors performed multiple experiments to show that the His-to-Arg change indeed leads to a loss-of-function and acts in a dominant negative way; transfection of 188Ala *PRLR* compared to wild-type *PRLR* in HEK293 cells reduced *JAK2* and *STAT5* phosphorylation and CISH reporter expression, as well as other signaling pathways, and they also found reduced activity of *PRL* on T cells from the patients compared to controls.

The clinical phenotype is similar to that of the *Prlr*^{-/-} mouse (more severe hyperprolactinaemia in females than in males, reduced fertility) but some features remain difficult to explain. The reasons for the galactorrhoea, oligomenorrhoea and infertility are a matter of debate that request further experiments and additional patients.

New hope – stem cells

Mobilized adult pituitary stem cells contribute to endocrine regeneration in response to physiological demand

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Background: This study aimed to answer whether the population of Sox2- and Sox9-double⁺ cells found in the adult pituitary are endocrine progenitors that have the potential to form pituispheres and differentiate into endocrine terminal differentiated cells.

Methods: To assess if Sox2 and Sox9⁺ cells are pituitary progenitors/stem cells, a mouse transgenic approach was taken. Firstly, two knock-in alleles, *Sox2*^{GFP} and *Sox9*:*ires*^{GFP}, were used to isolate pure populations of Sox2 and Sox9 expressing cells by FACS. The potential of these cells to form pituispheres or spheres of undifferentiated precursors with stem cell features was then established. To perform specifically cell lineage tracing of Sox2 and Sox9⁺ cells, two different tamoxifen (TAM) Cre-inducible transgenic lines were used, *Sox2:CreERT2* line and *Sox9:iresCreERT2*, which were then crossed into *Rosa*^{LoxpSTOPLoxpYFP} genetic backgrounds. Administration of TAM activates Cre and results in expression of YFP in the Sox2⁺ or Sox9⁺ expressing cells and all their descendants allowing ‘tracing’ of the daughter cells. By using double immunostaining of GFP and terminally differentiated hormones, cells can be identified that have been derived from either a Sox2 or Sox9 cell.

Results: The pituitary gland contains Sox2⁺ and Sox9⁺ cells that are sphere-forming in stem cell-promoting growth medium. In differentiating medium conditions these cells are able to terminally differentiate into endocrine hormone-producing cells. Cell lineage tracing shows for the first time that the Sox2-Sox9⁺ cells give rise to endocrine adult cells and that they contribute to the novo endocrine cells that appear in adulthood upon endocrine challenges such as ablation of gonads, adrenalectomy or treatment with oestrogen.

Conclusions: The adult pituitary contains Sox2- and Sox9-expressing cells that function as adult pituitary stem/progenitors cells and that can differentiate in hormone-producing cells. These cells can adapt to physiological demand after gonadectomy and adrenalectomy and contribute to development of terminally differentiated endocrine cells.

The pituitary gland cells show slow but dynamic turnover to meet the changing physiological demands, for example during lactation, pregnancy or accelerated growth. It has been proposed that adult pituitary progenitor/stem cells are involved in this process and that differentiation of these precursors allows increased numbers of specific hormone-producing cells in response to such physiological demands. A few laboratories have now identified embryonic pituitary progenitors/stem cells that express the SRY-HMG domain transcription factors Sox2 and Sox9 [4, 5]. Sox2 is expressed very early in pituitary development from embryonic day E9 in the oral ectoderm or Rathke's pouch, which gives rise to the intermediate and anterior lobes of the adult pituitary. Sox2 has a fundamental role in pituitary organogenesis, controlling the proliferation of pituitary progenitors and also cell lineage differentiation [6, 7]. In contrast, Sox9 is expressed in later development, after the first wave of progenitor proliferation has finished at embryonic day (E)14. Expression of both transcription factors is downregulated during the differentiation of endocrine cells and completely disappears once terminal differentiation has occurred [8]. However, in the adult pituitary, a subset of Sox2⁺ and Sox9⁺ cells remain in the epithelium lining of the pituitary cleft, and in some scattered parenchymal cells within the anterior pituitary lobe. These regions have been proposed to be the stem cell niche [4, 9].

Previous studies have shown that Sox2⁺ve cells can be isolated embryonically from the Rathke's pouch and can form pituispheres that can differentiate into pituitary endocrine cell lineages [4, 5]. In this very elegant study, Rizzoti et al. show for the first time that there is a population of pituitary progenitors/stem cells in the adult pituitary gland that have the capacity to differentiate into hormone-producing cells. These stem/progenitor cells are both Sox2⁺ve and Sox9⁺ve and they contribute to the generation of different hormone-producing cell populations upon changing physiological demands. Pituitary hormone deficiencies, either acquired or congenital, are currently treated by hormone replacement. However, physiological pulsatile patterns of secretion are not mimicked by such treatment and lifelong hormone treatment is required. The finding that adult pituitary stem cells can respond to pharmacological treatment, and that the population of Sox2-Sox9⁺ve cells can give rise to hormone-producing cells, opens new avenues for future cellular therapeutic approaches. One day we may be able to use regenerative medicine to treat pituitary disease, but before then we will need to investigate how to harvest these stem cells, how to differentiate them into specific hormone-producing cell networks and how to replace them to allow for endogenous hormone production.

Sox2⁺ stem/progenitor cells in the adult mouse pituitary support organ homeostasis and have tumor-inducing potential

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Background: The embryonic pituitary gland contains Sox2-positive cells (Sox2⁺ve) that form undifferentiated spheres and are able to self-renew and differentiate into hormone-producing cells. Therefore, this population of cells is considered to be progenitor/stem cells. This paper aimed to show, firstly, whether this population of cells is maintained in adulthood and contributes to hormone-producing cells, and secondly, whether they can give rise to pituitary tumors when harboring somatic mutations in oncogenes.

Methods: The knock-in allele Sox2^{GFP} was used to localize Sox2⁺ve cells in the adult pituitary to mark the stem cell compartment and undifferentiated precursors including S100B⁺ve cells that mark folliculostellate cells. To specifically cell lineage trace Sox2⁺ve cells in adult pituitaries, a tamoxifen Cre-inducible transgenic line was used. To achieve this Sox2:CreERT2 was crossed to two different transgenic lines: (1) Rosa^{LoxpSTOPLoxpYFP} to cell lineage trace and (2) β-catenin^{LoxpEXON3Loxp} to activate oncogenic β-catenin in the progenitor/stem Sox2⁺ve cells compartment. Upon administration of tamoxifen, Cre is activated only in Sox2⁺ve cells and in turn floxes out the silencing STOP cassette to allow expression of YFP (yellow fluorescence protein) in all their descendants allowing 'tracing' of future daughter cells to assess their contribution to differentiated endocrine cells. Activation of oncogenic form of β-catenin in the stem cell population combined with cell lineage tracing allows assessment of Sox2⁺ve cells in tumour formation.

Results: The population of Sox2⁺ve cells in the adult pituitary gland can self-renew and give rise to all terminally-differentiated endocrine cells. In addition, these cells have tumour-inducing potential; expression of oncogenic β-catenin in these cells triggers formation of tumours that are similar to adamantinomatous craniopharyngioma. Unexpectedly, tracing of the tumour-initiating cells using Rosa^{LoxpSTOPLoxpYFP}; Sox2:Cre ERT2; β-catenin^{LoxpEXON3Loxp} shows that the tumour is composed of non-derived Sox2⁺ve cells, indicating that Sox2⁺ve surrounding cells transform to become tumorigenic.

Conclusions: The adult pituitary gland contains Sox2⁺ve progenitor/stem cells that have the ability to self-renew and to terminally differentiate into all hormone-producing cells. These adult pituitary Sox2⁺ve progenitor/stem cells also have the potential to form tumours if an oncogenic mutation is acquired. Moreover, the Sox2⁺ve cells form the initial tumour lesions but act in a paracrine fashion and result in transformation of surrounding cells to into tumorigenic cells.

This paper, published back-to-back with Rizzoti et al. (see above), shows that the adult pituitary gland contains Sox2⁺ve progenitor/stem cells. These cells are able to contribute to all pituitary cell lineages, and also have tumor-initiating potential if a mutant oncogene is expressed. Importantly, using cell lineage tracing, the authors show that the tumor is not formed from the originating Sox2⁺ve

cells, but instead is formed by the surrounding cells that have been transformed. This observation establishes a novel mechanism in which a mutation in the stem cell population leads to transformation of neighboring cells (due to stem cell-secreted factors). How these cells act in a paracrine fashion to modify the neighboring cells is not known. Future molecular experiments are required to elucidate this and to understand and intervene in pituitary tumor formation.

Notch signaling in postnatal pituitary expansion: proliferation, progenitors, and cell specification

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Background: Mutations in *PROP1* account for up to half of the cases of combined pituitary hormone deficiency. However, few signaling pathways have been identified that regulate *PROP1* expression. Notch signaling has been linked to *Prop1* expression, but the developmental periods during which Notch signaling influences *Prop1* and overall pituitary development remain unclear.

Methods: Mice with a conditional knock-down of *Notch2* in pituitary progenitors were created to establish the requirement for Notch in pituitary hormone producing cells. The authors also examined the consequences of chemical Notch inhibition during early postnatal pituitary maturation.

Results: Loss of *Notch2* has little influence on early embryonic pituitary proliferation but is crucial for postnatal progenitor maintenance and proliferation. Notch signaling is necessary embryonically and postnatally for *Prop1* expression and *Pit1* lineage hormone cell expansion, as well as repression of the corticotrope lineage.

Conclusion: The authors identified temporal and cell type-specific roles for Notch signaling throughout pituitary development including postnatal development.

Prenatal Notch signalling is critical for the development of many organs, but evidence is emerging that Notch also plays a role in postnatal life, especially in the maintenance of stem cells and progenitors. Indeed, Notch2 and Notch3 continue to be expressed in the adult pituitary and Notch3 overexpression is related to non-functioning pituitary adenomas. Notch2 is expressed in the pituitary cleft lining cells, where Sox2⁺ stem cells/progenitors reside. In some of these cells, the Notch target *Hes1* as well as *Prop1* continue to be expressed postnatally.

Here, the authors tested the role of Notch in the postnatal mouse pituitary by conditionally deleting *Notch2* or chemically inhibiting Notch signaling. Important in this paper, and differentiating these experiments from others, this work was done in vivo. Cell-cell contact is necessary for Notch signaling, one cell expressing the ligand, and the other cell expressing the receptor, and in vitro experiments do not represent in vivo environment.

In the mouse, the pituitary expands considerably in the first 3 weeks postnatally and this period was therefore chosen to reduce *Notch2*. In *Foxg1*-expressing cells, postnatal reduction of *Notch2* reduced the number of progenitor cells, reduced pituitary cell proliferation and anterior lobe volume, and altered pituitary cell populations (fewer *Pit1* cells, more *POMC* cells), concomitantly with a reduction of *Prop1* expression. This not only suggests that Notch signaling is important postnatally but also that *Prop1* is a proper Notch target, at least in the mouse. Whether Notch signaling is important in human postnatal pituitary development cannot yet be concluded. However, *PROP1* mutations are the most common genetic cause of combined pituitary hormone deficiency in humans and it is well known that hormone deficiencies can develop postnatally in patients with *PROP1* mutations. The future will tell whether mutations in genes of the Notch signaling pathway will be linked to postnatally-acquired hypopituitarism in humans.

miR-200b and miR-429 function in mouse ovulation and are essential for female fertility

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Background: microRNAs are small non-coding RNAs involved in the post-transcriptional regulation of gene expression. This paper investigated their roles in reproduction.

Methods: mir-200b and miR-429 knockout (DKO) mouse lines were produced and the reproductive phenotypes were analyzed.

Results: The authors observed anovulation and infertility in female mice lacking the microRNAs miR-200b and miR-429. Both miRNAs are strongly expressed in the pituitary gland, where they suppress expression of the transcriptional repressor ZEB1. Eliminating these miRNAs, in turn, inhibits luteinizing hormone (LH) synthesis by repressing transcription of its β -subunit gene, which leads to lowered serum LH concentration, an impaired LH surge, and failure to ovulate.

Conclusion: These results reveal roles for miR-200b and miR-429, and their target the *Zeb1* gene, in the regulation of the LH surge by the pituitary.

This paper is interesting for several reasons. The first aim was to analyze the role of micro-RNAs in reproduction. The authors choose miR-200b as an interesting candidate as it is highly expressed in testis. For that purpose, they produced a mouse line with a complete deletion of miR-200b in association with a non-functional mutant of miR-429. These two microRNAs belong to the same miRNA-cluster, and they have the same seed sequence suggesting they share similar target genes. The homologous recombination of this construct led to the normal synthesis of miR-200a, the third member of this miRNA-cluster. The consequence of these two knockouts was different in male and female mice. Males were fertile and had a normal development. Female mice showed almost complete infertility due to an absence of the LH surge in the afternoon of the proestrus. The finding that a deletion of two micro-RNAs leads to an anovulation by a defect of LH surge was completely unexpected. In the second part of the paper, the authors demonstrated that the absent LH surge was due to a pituitary defect and not a hypothalamic defect. Moreover, they characterized *Zeb1* as a target gene of miR-200b in the pituitary and showed that *Zeb1* inhibits LH β -expression. The absence of expression of both miR-200b and miR-429 in the hypothalamus (by Northern blot) indicates a specific pituitary effect of these microRNAs in the regulation of the gonadotropic axis. This study defines *Zeb1* as a new pharmacological target to modulate the activity of the gonadotropic axis.

New mechanism: GHRH neurons and hypoglycemia

Profiling of glucose-sensing neurons reveals that GHRH neurons are activated by hypoglycemia

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Background: The brain plays a major role in the control of the glucose homeostasis. Different types of neurons are able to sense glucose and their activity may alter the peripheral use of glucose. It is therefore fundamental to develop mouse lines to have a comprehensive view of these regulations.

Methods: The authors generated and validated transgenic mice with a neuronal/endocrine-specific *Gck* promoter driving cre expression and mated them to mice with cre-dependent expression of an EGFP-tagged ribosomal protein construct (EEF1A1-LSL.EGFP.L10) that can be used to map and profile cells expressing the glucokinase.

Results: The authors observed significant *Gck* expression in hypothalamic and limbic regions in cells that are activated following administration of glucose or 2-deoxyglucose. Transcriptional profiling from *Gck-cre/EEF1A1-LSL.EGFP*L10 mice enriched known and previously unknown glucose-sensing populations including neurons expressing growth hormone-releasing hormone (GHRH). Electrophysiological recordings showed that hypoglycemia activates GHRH neurons.

Conclusion: These studies provide a means for mapping glucose-sensitive neurons. In addition, these mice allowed generation of transcriptional profiles from cell types expressing cre in a cell-specific manner.

This paper has three main findings. Firstly, it describes an innovative *in vivo* tool to analyze the transcriptome in cells expressing a gene of interest at low levels. This new mouse line amplifies the biological marker used to purify mRNA-associated polyosomes from cells expressing the cre-recombinase under the control of a specific promoter. This approach is clearly innovative as other systems were applicable only to cells expressing high levels of the gene of interest. Secondly, this approach was used to analyze the distribution of neurons expressing glucokinase, which were found widely distributed in the hypothalamus, in nuclei known to be involved in the control of the glucose metabolism and energy homeostasis. By dual labeling, the authors showed the expression of the reporter gene in neurons responding to peripheral signals like leptin. These neurons therefore act as glucose-sensor neurons (rapid response) as well as metabolic-sensor neurons (long response). Thirdly, although the association of GH synthesis with glucose level was well known, the mechanism of the neuroendocrine pathway governing this association was unknown. Here, the authors show that glucokinase-expressing neurons also express high levels of GHRH. This result confirms that GHRH neurons are probably the 'primary neurons' behind the GH secretory response to hypoglycemia.

New paradigm: Endoplasmic reticulum-mitochondria interactions

Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance

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Background: Hypothalamic endoplasmic reticulum (ER) stress has emerged as a causative factor in leptin resistance, but the specific mechanisms are largely unknown. Mitofusin 2 (MFN2) plays critical roles in both mitochondrial fusion and the establishment of mitochondria-ER interactions. It was therefore interesting to test the function of MFN2 in the ER stress induced by obesity and leptin resistance.

Results: The authors showed that mitochondria-ER contacts in anorexigenic pro-opiomelanocortin (POMC) neurons in the hypothalamus are decreased in diet-induced obesity. POMC-specific ablation of *Mfn2* resulted in loss of mitochondria-ER contacts, defective POMC processing, ER stress-induced leptin resistance, hyperphagia, reduced energy expenditure, and obesity. Pharmacological relief of hypothalamic ER stress reversed these metabolic alterations.

Conclusion: MFN2 in POMC neurons acts as an essential regulator of systemic energy balance by fine-tuning the mitochondrial-ER axis homeostasis and function. These results argue for a crucial involvement in mediating ER stress-induced leptin resistance.

The central control of food intake is complex. It involves orexigenic neurons (AgRP, NPY) along with anorexigenic neurons (POMC, CART). Leptin resistance is a hallmark of common obesity. Studies in rodents have revealed that POMC neurons are the primary targets of leptin and these neurons are resistant to leptin in obesity models. ER stress is defined by a condition in which there is a disruption of the proper folding and secretory capacity of the ER and an increased load of unfolded proteins in its lumen. This stress initially aims to restore the normal function of the ER. However, the inability to

restore these normal functions induces cell death. ER and mitochondria form contacts and mitofusin 2 is known to establish such mitochondria-ER interactions.

Here, the authors showed that *Mfn2* deletion in POMC neurons causes a primary ER stress, defective α -MSH processing, leptin resistance, and obesity. It is interesting to note that diet-induced obesity causes a downregulation of *Mfn2* in the hypothalamus. The authors therefore propose that the disruption of mitochondria-ER interaction under high fat diet is associated with *Mfn2* downregulation, which contributes to obesity and leptin resistance. Why and how ER stress or modification of mitochondria-ER interactions induce leptin resistance remains unclear. The authors propose several hypotheses which need further experiments.

New mechanism: Brain-blood barrier plasticity

Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access of metabolic signals to the arcuate nucleus in response to fasting

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Background: The delivery of blood-borne molecules conveying information on the metabolic status to the brain is restricted by the blood-brain barrier (BBB). In the median eminence, the fenestrated microvessels are permeable to these molecules. The metabolic diffusion of the permeability of the BBB in the median eminence is poorly understood.

Methods: The authors analyzed the BBB in the median eminence after food deprivation in mice.

Results: The decrease in blood glucose levels during fasting alters the structural organization of the blood-hypothalamus barrier, resulting in the improved access of metabolic substrates to the arcuate nucleus. These changes are mimicked by 2-deoxyglucose-induced glucoprivation and are reversed by raising blood glucose levels after fasting. Furthermore, VEGF signaling blocks fasting-induced barrier remodeling and impairs the physiological response to refeeding.

Conclusion: These results implicate glucose in the control of blood-hypothalamus exchanges through a VEGF-dependent mechanism and demonstrate a hitherto unappreciated role for tanycytes and the permeable microvessels associated with them in the adaptive metabolic response to fasting.

A complex network of both peripheral and central signals continuously informs the CNS about the nutritional status, thus enabling a response adapted to the energy demand. In the hypothalamus, the arcuate nucleus (ARC) plays a fundamental role in integrating circulating stimuli of hunger and satiety that cannot cross the blood-brain barrier such as leptin, insulin, and ghrelin. In the median eminence of the hypothalamus, microvessels with fenestrated endothelium allow entry into the brain of glucose and leptin, which then activate ARC neurons whose axonal extremities project into the median eminence. The presence of tight junctions between tanycytes, the cells which line the third ventricle, impedes the diffusion of these molecules into the rest of the brain. The median eminence is therefore a unique region of the brain which receives and sends information from the periphery.

In this paper, Langlet et al. report that metabolic changes alter the accessibility of peripheral molecules to the ARC. Fasting and low glucose levels facilitate access of these molecules into the brain by increasing microvessel fenestration and by changing the organization of the tight junctions between tanycytes. These changes are mediated by increased VEGF synthesis in the hypothalamic tuberal region. Altogether, the authors propose a new concept in which blood glucose levels modulate the organization of the tight junctions of tanycytes as well as the permeability of microvessels in the median eminence.

Hormones and diet, but not body weight, control hypothalamic microglial activity

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Background: The arcuate nucleus (ARC) of the hypothalamus plays a key role in sensing metabolic feedback and regulating energy homeostasis. Recent studies revealed activation of microglia in mice with high-fat diet (HFD)-induced obesity (DIO), suggesting a potential pathophysiological role for inflammatory processes within the hypothalamus. This study aimed to further investigate the metabolic causes and molecular underpinnings of such glial activation.

Methods: The authors analyzed microglial activity in wild-type (WT), monogenic obese *ob/ob* (leptin-deficient), *db/db* (leptin-receptor mutation), and type-4 melanocortin receptor knockout (*Mcr4* KO) mice on either a HFD or on standardized chow (SC) diet.

Results: Following HFD exposure, the total number of ARC microglia, immunoreactivity of ionized calcium binding adaptor molecule 1 (iba1-ir), cluster of differentiation 68 (CD68-ir), and ramification of microglial processes were increased. The *ob/ob* mice had less iba1-ir and ramifications. Leptin replacement rescued these phenomena. The *db/db* mice had similar iba1-ir comparable with WT mice but had significantly lower CD68-ir and more ramifications than WT mice. After 2 weeks of HFD, *ob/ob* mice showed an increase of iba1-ir, and *db/db* mice showed increase of CD68-ir. Obese *Mcr4* KO mice fed a SC diet had comparable iba1-ir and CD68-ir with WT mice but had more ramifications than WT mice. Intriguingly, treatment of DIO mice with glucagon-like peptide-1 receptor agonists reduced microglial activation independent of body weight.

Conclusion: Diet type, adipokines, and gut signals, but not body weight, affect activity of hypothalamic microglia in obesity.

Microglia are a type of glial cell that are the resident macrophages in the brain and spinal cord, and thus act as the first and main form of active immune defense in the central nervous system. Activated microglia are usually considered deleterious to neural cells because of their release of toxic molecules such as pro-inflammatory cytokines. Recent data suggest that microglia can also be beneficial to the developing brain. In obesity, microglia adopt a pro-inflammatory state. This activation may thus participate in the disturbed neuronal homeostasis observed in obesity.

This paper shows that microglial activation in the arcuate nucleus is induced by high fat diet but not by obesity per se. It is interesting to note that leptin-deficient mice have a lower microglial activity than wild-type animals. The GLP-1 receptor agonist exendin-4 deactivates microglial activity induced by HFD. This agonist is known to protect neurons in other models of neurodegenerative disorders by decreasing the synthesis of cytokines. This pathway is an interesting new target to protect ARC neurons from the deleterious effect of inflammatory cytokines in obesity. In addition to the understanding of hypothalamic dysfunctions in obesity, these results may be relevant to other conditions with microglial activation such as preterm babies.

The orphan receptor Gpr83 regulates systemic energy metabolism via ghrelin-dependent and ghrelin-independent mechanisms

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Background: The G protein-coupled receptor 83 (Gpr83) is widely expressed in hypothalamic nuclei that regulate energy metabolism.

Methods: To delineate its physiological function, the authors investigated the regulation of Gpr83 expression in the hypothalamus under different conditions, and analyzed the phenotype caused by *Gpr83* gene knockout. They also performed in-vitro experiments to investigate the biochemical function of Gpr83.

Results: The hypothalamic expression of Gpr83 was regulated in response to nutrient availability and was decreased in obese mice compared with lean mice. In the arcuate nucleus, Gpr83 co-localized with the ghrelin receptor (Ghsr1a) and the agouti-related protein. In vitro analyses showed that heterodimerization of Gpr83 with Ghsr1a diminished activation of Ghsr1a by acyl-ghrelin. The orexigenic and adipogenic effect of ghrelin was accordingly potentiated in Gpr83-deficient mice. *Gpr83* knockout mice had normal body weight and glucose tolerance when fed a regular chow diet, but were protected from obesity and glucose intolerance when challenged with a high-fat diet, despite hyperphagia and increased hypothalamic expression of agouti-related protein, Npy, Hcrt and Ghsr1a.

Conclusion: These data suggest that Gpr83 modulates ghrelin action but also indicate that Gpr83 regulates systemic metabolism through other ghrelin-independent pathways.

G protein-coupled receptors (GPCR) form a very large family of cell surface receptors. Their ligands are very heterogeneous and are involved in almost all physiological pathways. These receptors are widely studied as their activities are easily modulated and, to date, only few GPCRs including Gpr83 are 'orphans', i.e. their ligands are yet to be discovered. The findings of this study support the authors' hypothesis that Gpr83 may be involved in the central control of the energy metabolism as it is expressed in the arcuate nucleus, the lateral nucleus and the paraventricular nucleus, and its expression is induced by glucocorticoids.

The authors proposed an original mechanism for Gpr83's physiological function. Rather than characterize the natural ligand of Gpr83, which could be a very long process, they showed that Gpr83 expression in heterologous cells reduced the activation of the ghrelin receptor (Ghsr1a) by its own ligand, ghrelin. This effect seems to be mediated by the heterodimerization of Gpr83 with Ghsr1a. This result is interesting as it adds Gpr83 as a new GPCR able to form heterodimers with Ghsr1a. Indeed, several reports have shown that Ghsr1a may form heterodimers with other GPCR's involved in the central control of energy metabolism. Although, a very large number of GPCR heterodimers have now been reported in vitro, the in vivo confirmation and the physiological consequences of these interactions have been reported for only very few receptors. This paper provides convincing evidence that Gpr83 knockout enhances ghrelin activation of food intake but not on GH synthesis. However, the authors did not prove that Gpr83 interacts with Ghsr1a in the hypothalamus. The results must thus be confirmed by other methods to definitively validate the concept.

Despite displaying hyperphagia, Gpr83 knockout mice were surprisingly lean, resistant to diet-induced obesity and resistant to diet-induced glucose intolerance. This phenotype indicates that the physiological function of Gpr83 is not restricted to the modulation of ghrelin action. It reveals that Gpr83 might be an interesting new pharmacological target to control food intake. The natural ligand of Gpr83, if it exists, must be characterized to further the understanding of the physiological function of this receptor.

The IGSF1 deficiency syndrome: characteristics of male and female patients

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Background: Ig superfamily member 1 (IGSF1) deficiency is a novel X-linked syndrome, characterized by central hypothyroidism and macro-orchidism. Clinical data, as well as features of female carriers, are still scarce. The objective of this study was to characterize the phenotype associated with IGSF1 deficiency in both sexes.

Methods: Ten families (n = 42) with IGSF1 deficiency were examined using an identical protocol in the university clinics of Leiden, Amsterdam, Cambridge, and Milan. Biochemical measurements were performed in a central laboratory.

Results: Male patients (n = 24, age 0–87 years) showed central hypothyroidism (100%), hypoprolactinaemia (n = 16, 67%), and transient partial GH deficiency (n = 3, 13%). There was a delay of pubertal testosterone production, growth spurt and pubic hair development. However, testicular growth started at a normal age and all adult males had developed macro-orchidism. Body mass index, percentage fat, and waist circumference tended to be elevated and the metabolic syndrome occurred in most of the patients aged above 55 years. Heterozygous female carriers (n = 18, age 32–80 years) showed central hypothyroidism in 6 (33%), hypoprolactinaemia in 2 (11%), and GH deficiency in none. Body mass index, percent fat, and waist circumference were relatively high, and the metabolic syndrome was present in 3 cases.

Conclusion: The phenotype of male patients with IGSF1 deficiency consists of central hypothyroidism, hypoprolactinaemia, delayed puberty, macro-orchidism, and increased body weight. A subset of female carriers also exhibits central hypothyroidism.

In *Yearbook 2013* we discussed the initial paper reporting a novel X-linked syndrome caused by loss-of-function mutations or deletions in Immunoglobulin Superfamily member 1 (*IGSF1*, OMIM #300888) [10]. *IGSF1* is a plasma membrane glycoprotein, highly expressed in Rathke's pouch and the adult anterior pituitary gland. Pathogenic *IGSF1* mutations impair protein trafficking to the cell surface. The main features of the IGSF1 deficiency syndrome are congenital central hypothyroidism and macro-orchidism. These observations appear secondary to reduced pituitary TRH receptor expression or TRH signaling although the exact pathophysiological mechanism, especially of the macro-orchidism, remains unclear. A few more cases from Japan have been described since the initial paper, but the current paper is the first to describe a larger cohort of patients. The main message is that not only TSH deficiency, but also prolactin deficiency and variable GH deficiency can occur as well as the metabolic syndrome in older male patients. Female carriers can also have pituitary TSH deficiency and prolactin deficiency. Age at onset of testicle enlargement was normal but testosterone production and action was delayed, and 100% of male patients developed macro-orchidism. This detailed phenotypical characterization will not only help to recognize the IGSF1 deficiency syndrome clinically, but will also help to elucidate the underlying mechanisms as well as the function of IGSF1.

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