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# Neurotransmitter Modulation of the GHRH-GH Axis

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**Abstract**

The role of dopaminergic receptors in the control of GH release remains controversial. The dopamine receptor 2 (D2R) knockout mouse represents a useful model to study the participation of the D2R on growth and GHRH-GH regulation. These knockout mice have hyperprolactinemia and lactotrope hyperplasia, but unexpectedly, they are also growth retarded. In D2R knockout mice there is a significant decrease in somatotrope population, which is paralleled by decreased GH content and output from pituitary cells. The sensitivity of GHRH-induced GH and cAMP release is similar between genotypes, even though the response amplitude is lower in knockouts. We point to an involvement of D2R signaling at the hypothalamic level as dopamine did not release GH acting at the pituitary level, and both somatostatin and GHRH mRNA expression are altered in knockout mice. The similarity of the pituitary defect in the D2R knockout mouse to that of GHRH deficient models suggests a probable mechanism. Loss of dopamine signaling via hypothalamic D2Rs at a critical age may cause inadequate GHRH secretion subsequently leading to inappropriate somatotrope lineage development. Furthermore, GH pulsatility, which depends on a regulated temporal balance between GHRH and somatostatin output might be compromised in D2R knockout mice, leading to lower IGF-I, and growth retardation.


Dopamine is the most abundant catecholamine in the brain. Its involvement and importance as a neurotransmitter and neuromodulator in the regulation of different physiological functions in the central nervous system is well known, and deregulation of the dopaminergic system has been linked with Parkinson's disease, Tourette's

syndrome, schizophrenia, attention deficit hyperactive disorder, drug addiction, obesity and generation of pituitary tumors.

Dopamine exerts its action by binding to specific membrane receptors, which belong to the family of seven transmembrane domain G-protein-coupled receptors. Five distinct dopamine receptors have been isolated, characterized and subdivided into two subfamilies, D1- and D2-like, on the basis of their biochemical and pharmacological properties. The D1-like subfamily comprises D1R and D5R, while the D2-like includes D2R, D3R and D4R. D1 receptors are coupled to stimulatory G proteins, and the D2 subtype to inhibitory Gi/Go proteins. The best-described effects mediated by dopamine acting on D2Rs are the inhibition of the cAMP pathway and modulation of $Ca^{2+}$ signaling [1].

In brain tissues the D2R is expressed predominantly in the caudate putamen, olfactory tubercle and nucleus accumbens. It is also expressed in the substantia nigra pars compacta and in the ventral tegmental area. These are the anatomical regions that give rise to long dopaminergic fibers (A10 and A9), indicating that the D2Rs have a presynaptic location. In contrast, D1-like receptors are exclusively postsynaptic. The D2R is expressed in two isoforms (long and short), and mRNA analysis of the two isoforms has shown that D2R-L is the most abundantly expressed. Outside the brain the D2R is also localized in the retina, kidney, vascular system and pituitary gland.

At the pituitary level, dopamine acting on D2Rs inhibits prolactin secretion from lactotropes, and αMSH secretion from melanotropes. On the other hand, the role of dopaminergic receptors in the control of GH release remains controversial in several respects, i.e. the direction of action (stimulatory or inhibitory) and the species differences encountered.

## Dopamine and GHRH-GH Regulation

Inhibitory as well as stimulatory effects of the amine have been reported on plasma levels of GH in vivo depending upon the experimental conditions used [2]. This may be explained by the ability of dopamine to release both GHRH and somatostatin from the rat hypothalamus [3]. In particular, it has been suggested that dopamine receptors can mediate the stimulation by dopamine of GH, provided other neural inhibitory inputs to the pituitary are removed. L-DOPA stimulates GH secretion in vivo [2], and apomorphine a central dopamine receptor agonist stimulates GH secretion. However, the GH stimulatory action of L-DOPA does not appear to be mediated via dopamine receptors as specific blockade of these receptors with antidopaminergic drugs does not alter the GH response [4, 5]. Instead, L-DOPA's effects appear to depend on conversion to noradrenaline or adrenaline, as alpha-adrenoceptor blockade with phentolamine disrupts the GH response to L-DOPA [6].

In vitro, positive as well as negative GH responses to the catecholamine have been described in pituitary cells [2, 7].

## Dopamine in Acromegaly Treatment

On the other hand, dopamine agonists have been largely used for the treatment of pituitary tumors, particularly prolactinomas but also in acromegaly, and the responsiveness seems to depend on the expression of D2Rs on tumor cells [8].

Somatostatin Sst2A receptors and D2Rs are frequently co-expressed in adenomas from acromegalic patients. The additive effect of dopamine and somatostatin agonists in lowering GH suggests that the combination of somatostatin and dopamine analogues might be useful in selected patients. Chimeric molecules that are able to bind to both somatostatin and dopamine receptors are being developed for the treatment of acromegaly [9, 10]. The mechanism(s) by which such ligands may act are still unknown. One possible explanation of their increased potency could be through their ability to induce oligodimerization of the receptors at the cell membrane level, and modify, in a ligand-specific manner, the subsequent trafficking and recycling of the receptors [11].

In vitro experiments demonstrated that D2R immunoreactivity in adenomas from acromegalic patients positively correlated with the in vitro GH and PRL suppression by quinagolide in primary cultures from the pituitary adenomas [12]. However, D2R expression was not correlated with the in vivo GH response to quinagolide, suggesting that the in vivo sensitivity of acromegalic patients to dopamine might be affected by other mechanisms, for example antiangiogenesis.

## Dopamine and Growth

With regard to a possible role of the dopaminergic system in growth, it has been shown that GH deficient children increase their growth velocity after 6 months of levodopa treatment, even though the possible intervention of the adrenergic system was not tested [13]. On the other hand it has been described that a group of children with idiopathic short stature, had high frequencies of the A1 allele of the D2R, indicating a polymorphism of the receptor [14].

The D2R knockout mouse represents a useful model to study the participation of the D2R on growth and GHRH-GH regulation. As pituitary D2Rs are mandatory for dopamine inhibition of prolactin synthesis and release, as well as lactotrope proliferation, knockout mice have chronic hyperprolactinemia, lactotrope hyperplasia [15, 16], and after 16 months of age highly vascularized adenomas develop, especially in females, but also in males [17]. Unexpectedly, these mice were also growth retarded evidencing an alteration in the GH-IGF-I axis [18].

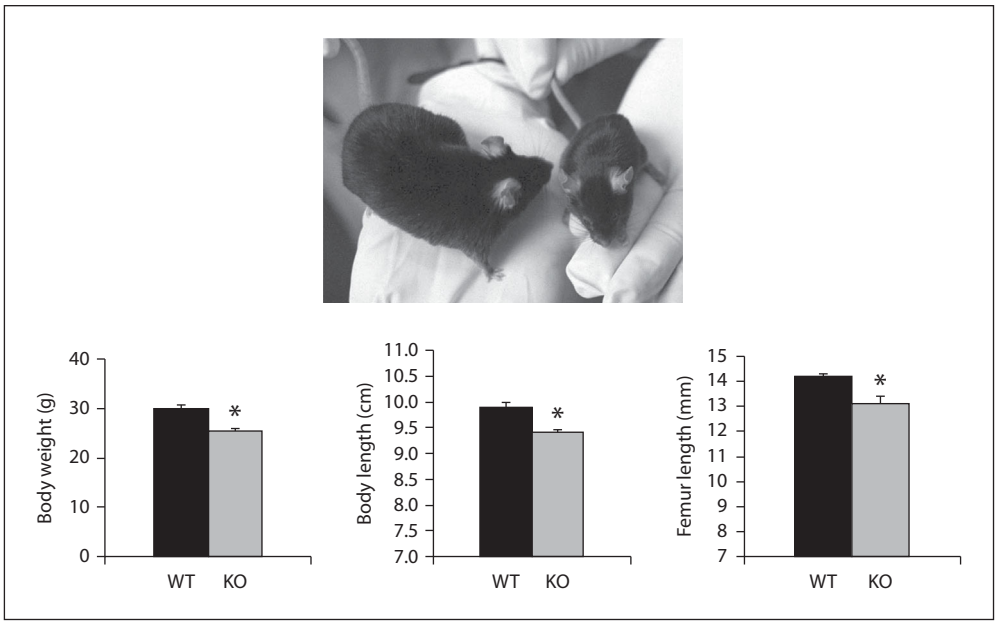


**Fig. 1.** Below: Body weight, body and femur length in wild-type (WT) and D2R knockout (KO) male mice at 6 months of age. Average ± SEM. Modified from Diaz-Torga et al. [18]. On the right, representative photograph. * $p < 0.05$ vs. respective wild-type.

### The D2R Knockout Mouse, a Dwarf Mouse

In wild-type and D2R knockout mice body weight at birth was similar, but growth retardation was evidenced starting on the second month of life. Growth retardation was especially evident in male mice, females were smaller in the first months and there was a growth catch up in the 3rd or 4th month. In males there was an overall body weight decrease of 15%.

When body growth gain was determined, it became evident that in D2R knockout male and female mice maximal growth retardation compared with that in wild-type mice, occurred during the first half of the second month of life, and thereafter animals grew normally.

Body length and the rate of skeletal maturation recapitulated the genotypic dimorphic pattern demonstrated for body weight (fig. 1). These results suggested that the D2R was involved, albeit indirectly, in body growth. A chronic treatment with recombinant GH in the first month of life reversed the body weight decrease, indicating that peripheral sensitivity to GH was maintained [unpubl. results].

Average serum GH levels in wild-type male and female mice were high during the first month of life and decreased to adult levels by 3 months of age. The distribution profile of these random GH measurements between 1 and 2 months of age revealed

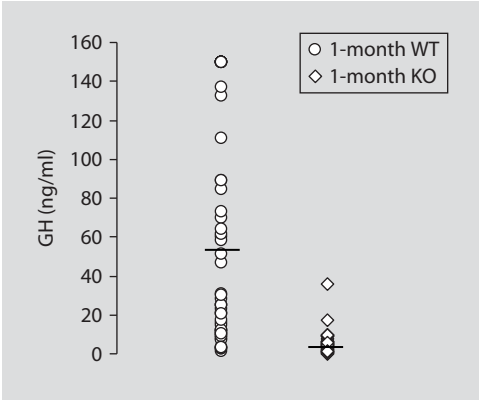


**Fig. 2.** Individual GH measurements in wild-type (WT) and D2R male knockout mice (KO), at 1 month of age. n = 54 and 43.

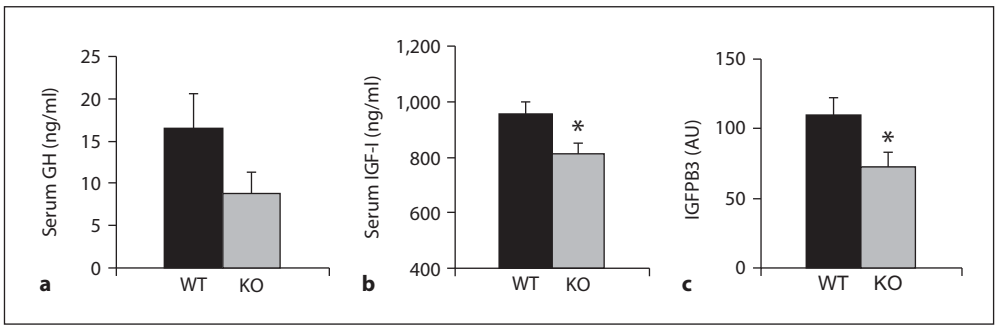


**Fig. 3.** Serum GH (**a**), IGF-I (**b**) and IGF-binding protein 3 (IGFBP3) (**c**). Adapted from Diaz-Torga et al. [18]. * $p < 0.05$ versus respective wild-type mice.

a high variability, probably reflecting an exaggerated pulsatility compared with adult values (fig. 2). In contrast, in female and male knockout mice GH levels were not increased during the first months of age.

This developmental period is characterized by a low effect of the somatostatin inhibitory control at the pituitary level, as well as low expression of hypothalamic somatostatin [19, 20]. The lack of increased GH levels in the first month of life had long-lasting consequences, as IGF-I levels as well as IGFBP-3 were low in adult knockout mice, even though serum GH levels were not different between genotypes in adult mice (fig. 3). This was the first evidence indicating that the D2R is involved in GH release in the first months of life [18].

The assertion was further supported by the acute GH-lowering effect of a D2R antagonist (sulpiride) in 1-month-old wild-type mice [18]. A D1R antagonist was ineffective. The effect of sulpiride was lost as the animal matured, emphasizing the importance of a low somatostatin tone to permit the unfettered effect of dopamine on GH release.

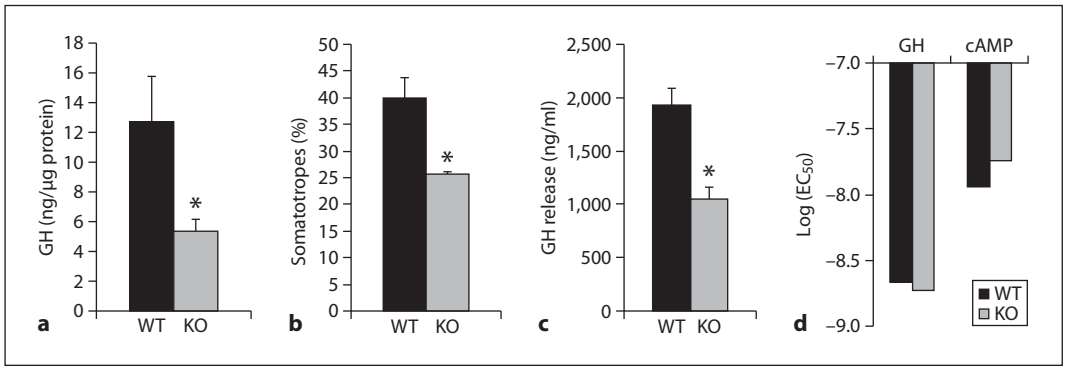


**Fig. 4.** Pituitary GH concentration (**a**), percentage of somatotrope cells in relation to total pituitary cells (**b**), and GH secretion from primary pituitary cultures of wild-type (WT) and D2R knockout (KO) mice (**c**). **d** Effective GHRH concentration (log M) which produces 50% increase in GH or cAMP in primary pituitary cultures of wild-type (WT) and D2R knockout (KO) mice. * $p < 0.05$ vs. respective wild-type. Adapted from Garcia-Tornadu et al. [21].

Even though GH levels in adult animals were not different between genotypes, there was a marked reduction in somatotrope number in knockouts, indicating a decreased somatotrope population size (fig. 4b) [21]. This result was paralleled by decreased pituitary GH concentration and GH secretion from pituitary cells cultured in vitro (fig. 4a, c).

In spite of the reduction in somatotrope cell number in knockouts, the functional capacity of somatotropes was not impaired, as similar dose response curve of GHRH induced GH release was observed in both genotypes (fig. 4d), even though GH net secretion was lower, in general, proportional to the low pituitary GH cell number [21]. Therefore, total GH response per pituitary was reduced, and this could account for lower IGF-I and IGFBP-3 observed in adult knockout mice [18].

This suggested that the mitotic capacity of somatotropes is very sensitive to alterations in neonatal GHRH action while the maintenance of the GH biosynthetic and secretory processes has less sensitivity to such changes.

GHRH-R protein in pituitary membranes from knockout mice was reduced to 46% of the level found in wild-type mice [21], a percentage which was higher than the reduction of somatotropes (35 %). In accordance, GHRH- induced cAMP generation was also decreased in knockouts, but the dose sensitivity was similar (fig. 4d). Somatostatin control of basal and GHRH- or ghrelin-stimulated GH release was similar between genotypes, even though D2Rs and the somatostatin receptor SSTR5 interact physically through hetero-oligomerization in neurons to create a novel receptor with enhanced functional activity [11].

In order to determine if pituitary D2Rs were involved in GH release, the effect of dopamine was tested in cultured pituitary cells. Dopamine did not modify GH acting at the pituitary level either in 1-month-old or adult mice [21]. To this regard, it has

been documented that dopamine D1Rs and not D2Rs participate in GH release at the pituitary level [7], and that D2Rs are found mainly in lactotropes. Therefore, an involvement of D2R signaling at the hypothalamic level was inferred.

GH pulsatility originates at the hypothalamic level. In rodents, males exhibit narrow GH pulses with a frequency of about one pulse every 3–4 h, and prolonged nadir values below 1–2 ng/ml. Female rats exhibit relatively broader pulses with an irregular frequency and nadir values of 5–20 ng/ml. This sexually differentiated pattern is determined by the complex interplay between GHRH and somatostatin [22].

As periodical sampling is very difficult in mice, an indirect parameter of GH pulsatility has been commonly used: measurement of the major urinary proteins (MUP). MUPs (20 kDa) represent the major protein component of mouse urine. MUP expression requires pulsatile occupancy of liver GH receptors, and adult males secrete more than 3 times as much MUP as do females [23].

These proteins are synthesized in the liver, secreted through the kidneys, and excreted in urine in milligram quantities per milliliter. This abundant protein excretion is thought to play a role in chemo-signaling between animals to coordinate social behavior [24].

Knockout mice had lower MUPs, and the difference could be caused by a different pattern of GHRH or somatostatin release and action. For example, in the lit/lit mouse, which has a point mutation in the GHRH-R gene, MUPs are also decreased [25].

Further evidence of a central participation of D2Rs was the decrease in hypothalamic GHRH mRNA found in knockout compared to wild-type mice. This decrease was not simply secondary to GH deficiency or dwarfism because other transgenic lines with dwarfism and pituitary GH deficiency, caused by a primary somatotrope defect, showed the expected increase in GHRH expression [26].

Reduced levels of GHRH within the hypothalamus or GHRH action at the pituitary level during a critical developmental window have a long lasting impact on body weight [27–29], and induce an inadequate clonal expansion of the somatotrope population. The requirement of GHRH for the normal development of the somatotrope lineage is evident from studies examining the etiology of growth retardation in the spontaneous mutant mouse *lit/lit*, in which somatotropes fail to proliferate normally, resulting in a mature pituitary containing a limited number of GH cells [27]. Humans with mutations in the GHRH receptor show that defective GHRH receptor signaling results in profound, selective GH deficiency and dwarfism [30].

Furthermore, experimental ablation or inhibition of GHRH by chemical or immunological means [31, 32] provides strong circumstantial support for the notion that both acute and chronic GH release is strongly dependent on the proper functioning of arcuate GHRH neurons.

No inactivating mutations or deletions in the GHRH gene have yet been reported in human subjects, but GHRH deficiency has been described in rodents as part of more complex phenotypes resulting from deletion of other genes, or after the expression of human GH (hGH) transgenes in central nervous system to inhibit GHRH

expression [33]. Recently, a report describing a targeted disruption of the GHRH gene has confirmed directly the requirement for GHRH for normal growth and GH production [34].

At birth and during the first two weeks of life there was no difference in pituitary GH concentration between genotypes. This is consistent with the GHRH-independent somatotrope development described in *lit/lit* mice [27], or the similar growth rate in mice with a partial disruption of the GHRH gene (GHRH-M2) mice during the first weeks [35], and data from animals which overexpress hGH reporter gene driven by a potent promoter, in which the effect of excess GH is only apparent after 3 weeks of age [36]. Furthermore, congenital absence of the human pituitary gland does not result in abnormal birth or newborn weight [37], indicating that fetal and early postnatal development may occur independently of GH. But the requirement of GHRH for the normal development of the somatotrope lineage after birth has been clearly demonstrated as exemplified above.

On the other hand, hypothalamic somatostatin mRNA was increased in knockout mice. As D2Rs are inhibitory, the primary effect of disruption of D2Rs may be the lack of dopamine inhibition of somatostatin neurons. In this regard, it has been described that dopamine neurons in the periventricular nucleus are close to somatostatin neurons [38]. The decrease in hypothalamic GHRH content observed in knockout mice might result from the increase in somatostatin, as it has been shown that somatostatin neurons innervate GHRH neurons, and decrease GHRH expression [39].

In line with our findings, it has been described that neonatal administration of octreotide, a long-lasting somatostatin analogue decreases growth rate, hypothalamic GHRH, and sexual differentiation of GH pulsatilily [40]. Even though the number of arcuate GHRH mRNA-containing neurons was not affected by the octreotide treatment, GHRH mRNA levels per neuron were decreased by 30%, and median eminence GHRH stores by 50%.

### Is There Any Clinical Significance to Our Findings?

With regard to a possible role of the dopaminergic system in growth, it has been shown that a group of children with idiopathic short stature, had high frequencies of the A1 allele of the D2R, indicating a polymorphism of the receptor. The alterations in the dopaminergic system encountered were a low binding capacity for dopamine and reduced dopaminergic function [41]. In these children there was a mild GH deficiency, decreased nocturnal GH secretion, slightly retarded bone maturation, and low blood levels of IGF-I. Therefore D2Rs might participate in some cases of idiopathic short stature.

On the other hand, D2Rs may be involved in altered hormone secretion in chronic treatment with antidopaminergic drugs. In this regard, it has been described that during neuroleptic treatment of schizophrenic patients GH nocturnal rise is blunted, and that this effect is related to the D2R-binding capacity of neuroleptics used [42].

## Conclusions

Neural networks which control GH secretion participate in the fine tuning of the GHRH-GH axis. Hypothalamic D2R modulation of GHRH or somatostatin neurons, whether directly or indirectly, may be important in the regulation of the GH axis. We point to an inhibitory effect of dopamine acting on D2Rs on somatostatin. Lack of D2Rs increases somatostatin expression, and as somatostatin is inhibitory to GHRH, this neuropeptide is decreased. If this alteration takes place early in development, altered secretion of GHRH might lead to an inappropriate somatotrope lineage development. Furthermore, GH pulsatility, which depends on an adequate temporal balance between GHRH and somatostatin output might be compromised in D2R knockout mice, finally leading to lower IGF-I, and growth retardation.

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## References

1 Missale C, Nash SR, Robinson SW, Jaber M, Caron MG: Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.
2 Muller EE, Locatelli V, Cocchi D: Neuroendocrine control of growth hormone secretion. Physiol Rev 1999;79:511–607.
3 Kitajima N, Chihara K, Hiromi H, Okimura Y, Fujii Y, Sato M, Shakutsui S, Watanabe M, Fufita T: Effects of dopamine on immunoreactive growth hormone-releasing factor and somatostatin secretion from rat hypothalamic slices perfused in vitro. Endocrinology 1989;124:69–76.
4 Massara F, Camanni F: Effect of various adrenergic receptor stimulating and blocking agents on human growth hormone secretion. J Endocrinol 1972;54:195–206.
5 Masala A, Delitala G, Alagna S, Devilla L: Effect of pimozide on levodopa-induced growth hormone release in man. Clin Endocrinol (Oxf) 1977;7:253–256.
6 Cammani F, Massara F: Phentolamine inhibition of human growth hormone secretion induced by L-DOPA. Horm Metab Res 1972;4:128–
7 Bluet-Pajot MT, Mounier F, Durand D, Kordon C: Involvement of dopamine D1 receptors in the control of growth hormone secretion in the rat. J Endocrinol 1990;127:191–196.
8 Ferone D, de Herder WW, Pivonello R, Kros JM, van Koetsveld PM, de Jong T, Minuto F, Colao A, Lamberts SW, Hofland LJ: Correlation of in vitro and in vivo somatotropic adenoma responsiveness to somatostatin analogs and dopamine agonists with immunohistochemical evaluation of somatostatin and dopamine receptors and electron microscopy. J Clin Endocrinol Metab 2008;93:1412–1417.
9 Saveanu A, Jaquet P, Brue T, Barlier A: Relevance of coexpression of somatostatin and dopamine D2 receptors in pituitary adenomas. Mol Cell Endocrinol 2008;286:206–213.
10 Saveanu A, Lavaque E, Gunz G, Barlier A, Kim S, Taylor JE, Culler MD, Enjalbert A, Jaquet P: Demonstration of enhanced potency of a chimeric somatostatin-dopamine molecule, BIM-23A387, in suppressing growth hormone and prolactin secretion from human pituitary somatotroph adenoma cells. J Clin Endocrinol Metab 2002;87:5545–5552.
11 Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC: Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 2000;288:154–157.

12 Ferone D, Pivonello R, Lastoria S, Faggiano A, Del Basso de Caro ML, Cappabianca P, Lombardi G, Colao A: In vivo and in vitro effects of octreotide, quinagolide and cabergoline in four hyperprolactinaemic acromegalics: correlation with somatostatin and dopamine D2 receptor scintigraphy. Clin Endocrinol (Oxf) 2001;54:469–477.
13 Huseman CA, Hassing JM: Evidence for dopaminergic stimulation of growth velocity in some hypopituitary children. J Clin Endocrinol Metab 1984;58: 419–425.
14 Miyake H, Nagashima K, Onigata K, Nagashima T, Takano Y, Morikawa A: Allelic variations of the D2 dopamine receptor gene in children with idiopathic short stature. J Hum Genet 1999:44:26–29.
15 Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Grandy DK, Low MJ: Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron 1997;19:103–113.
16 Cristina C, García-Tornadú I, Diaz-Torga G, Rubinstein M, Low MJ, Becu-Villalobos D: The dopaminergic D2 receptor knockout mouse: an animal model of prolactinoma. Front Horm Res 2006; 35:50–63.
17 Asa SL, Kelly MA, Grandy DK, Low MJ: Pituitary lactotroph adenomas develop after prolonged lactotroph hyperplasia in dopamine D2 receptor-deficient mice. Endocrinology 1999;140:5348–5355.
18 Diaz-Torga G, Feierstein C, Libertun C, Gelman D, Kelly MA, Low MJ, Rubinstein M, Becu-Villalobos D: Disruption of the D2 dopamine receptor alters GH and IGF-I secretion and causes dwarfism in male mice. Endocrinology 2002;143:1270–1279.
19 Simonian SX, Murray HE, Gillies GE, Herbison AE: Estrogen-dependent ontogeny of sex differences in somatostatin neurons of the hypothalamic periventricular nucleus. Endocrinology 1998;139:1420–1428.
20 Cuttler L, Welsh JB, Szabo M: The effect of age on somatostatin suppression of basal, growth hormone (GH)-releasing factor-stimulated and dibutyril adenosine 3′-5′-monophosphate-stimulated GH release from rat pituitary cells in monolayer culture. Endocrinology 1986;119:152–158.
21 Garcia-Tornadu I, Rubinstein M, Gaylinn BD, Hill D, Arany E, Low MJ, Diaz-Torga G, Becu-Villalobos D: GH in the dwarf dopaminergic D2 receptor knockout mouse: somatotrope population, GH release, and responsiveness to GH-releasing factors and somatostatin. J Endocrinol 2006;190:611–619.
22 Waxman DJ, O'Connor C: Growth hormone regulation of sex-dependent liver gene expression. Mol Endocrinol 2006;20:2613–2629.
23 Norstedt G, Palmiter R: Secretory rhythm of growth hormone regulates sexual differentiation of mouse liver. Cell 1984;36:805–812.
24 Logan DW, Marton TF, Stowers L: Species specificity in major urinary proteins by parallel evolution. PLoS ONE 2008;3:e3280–
25 al Shawi R, Wallace H, Harrison S, Jones C, Johnson D, Bishop JO: Sexual dimorphism and growth hormone regulation of a hybrid gene in transgenic mice. Mol Endocrinol 1992;6:181–190.
26 McGuinness L, Magoulas C, Sesay AK, Mathers K, Carmignac D, Manneville JB, Christian H, Phillips JA III, Robinson IC: Autosomal dominant growth hormone deficiency disrupts secretory vesicles in vitro and in vivo in transgenic mice. Endocrinology 2003;144:720–731.
27 Lin SC, Lin CR, Gukovsky I, Lusis AJ, Sawchenko PE, Rosenfeld MG: Molecular basis of the little mouse phenotype and implications for cell type-specific growth. Nature 1993;364:208–213.
28 Cella SG, Locatelli V, Broccia ML, Menegola E, Giavini E, De Gennaro Colonna V, Torsello A, Wehrenberg WB, Muller EE: Long-term changes of somatotrophic function induced by deprivation of growth hormone-releasing hormone during the fetal life of the rat. J Endocrinol 1994;140:111–117.
29 Robinson GM, Spencer GS, Berry CJ, Dobbie PM, Hodgkinson SC, Bass JJ: Evidence of a role for growth hormone, but not for insulin-like growth factors I and II in the growth of the neonatal rat. Biol Neonate 1993;64:158–165.
30 Maheshwari HG, Silverman BL, Dupuis J, Baumann G: Phenotype and genetic analysis of a syndrome caused by an inactivating mutation in the growth hormone-releasing hormone receptor: Dwarfism of Sindh. J Clin Endocrinol Metab 1998;83:4065–4074.
31 Wehrenberg WB, Brazeau P, Luben R, Ling N, Guillemin R: A noninvasive functional lesion of the hypothalamo-pituitary axis for the study of growth hormone-releasing factor. Neuroendocrinology 1983;36:489–491.
32 Bloch B, Ling N, Benoit R, Wehrenberg WB, Guillemin R: Specific depletion of immunoreactive growth hormone-releasing factor by monosodium glutamate in rat median eminence. Nature 1984; 307:272–273.
33 Flavell DM, Wells T, Wells SE, Carmignac DF, Thomas GB, Robinson IC: Dominant dwarfism in transgenic rats by targeting human growth hormone (GH) expression to hypothalamic GH-releasing factor neurons. EMBO J 1996;15:3871–3879.
34 Alba M, Salvatori R: A mouse with targeted ablation of the growth hormone-releasing hormone gene: a new model of isolated growth hormone deficiency. Endocrinology 2004;145:4134–4143.

35 Le Tissier PR, Carmignac DF, Lilley S, Sesay AK, Phelps CJ, Houston P, Mathers K, Magoulas C, Ogden D, Robinson IC: Hypothalamic growth hormone-releasing hormone (GHRH) deficiency: targeted ablation of GHRH neurons in mice using a viral ion channel transgene. Mol Endocrinol 2005; 19:1251–1262.
36 Mathews LS, Hammer RE, Brinster RL, Palmiter RD: Expression of insulin-like growth factor I in transgenic mice with elevated levels of growth hormone is correlated with growth. Endocrinology 1988;123:433–437.
37 Reid JD: Congenital absence of the pituitary gland. J Pediatr 1960:56:658–664.
38 Fodor M, Kordon C, Epelbaum J: Anatomy of the hypophysiotropic somatostatinergic and growth hormone-releasing hormone system minireview. Neurochem Res 2006;31:137–143.
39 Bouyer K, Loudes C, Robinson IC, Epelbaum J; Faivre-Bauman A: Sexually dimorphic distribution of sst2A somatostatin receptors on growth hormone-releasing hormone neurons in mice. Endocrinology 2006;147:2670–2674.
40 Slama A, Bluet-Pajot MT, Mounier F, Videau C, Kordon C, Epelbaum J: Effects of neonatal administration of octreotide, a long-lasting somatostatin analogue, on growth hormone regulation in the adult rat. Neuroendocrinology 1996;63:173–180.
41 Ezzat S, Walpola IA, Ramyar L, Smyth HS, Asa SL: Membrane-anchored expression of transforming growth factor-α in human pituitary adenoma cells. J Clin Endocrinol Metab 1995;80:534–539.
42 Mann K, Rossbach W, Muller MJ, Muller-Siecheneder F, Pott T, Linde I, Dittmann RW, Hiemke C: Nocturnal hormone profiles in patients with schizophrenia treated with olanzapine. Psychoneuroendocrinology 2006;31:256–264.

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