ENDOCRINE REVIEWS

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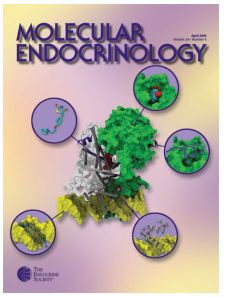

Genetic Regulation of Pituitary Gland Development in Human and Mouse

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Normal hypothalamopituitary development is closely related to that of the forebrain and is dependent upon a complex genetic cascade of transcription factors and signaling molecules that may be either intrinsic or extrinsic to the developing Rathke's pouch. These factors dictate organ commitment, cell differentiation, and cell proliferation within the anterior pituitary. Abnormalities in these processes are associated with congenital hypopituitarism, a spectrum of disorders that includes syndromic disorders such as septo-optic dysplasia, combined pituitary hormone deficiencies, and isolated hormone deficiencies, of which the commonest is GH deficiency. The highly variable clinical phenotypes can now in part be explained due to research performed over the last 20 yr, based mainly on naturally occurring and transgenic animal models. Mutations in genes encoding both signaling molecules and transcription factors have been implicated in the etiology of hypopituitarism, with or without other syndromic features, in mice and humans. To date, mutations in known genes account for a small proportion of cases of hypopituitarism in humans. However, these mutations have led to a greater understanding of the genetic interactions that lead to normal pituitary development. This review attempts to describe the complexity of pituitary development in the rodent, with particular emphasis on those factors that, when mutated, are associated with hypopituitarism in humans. **(*Endocrine Reviews* 30: 790–829, 2009)**

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Abbreviations: αGSU, α-Glycoprotein subunit; AFB, anterior forebrain; BMP, bone morphogenetic protein; CH, congenital hypopituitarism; CNS, central nervous system; CPHD, combined pituitary hormone deficiency; dpc, days post coitum; EGR1, Early growth response 1; FGF, fibroblast growth factor; GHD, GH deficiency; IGHD, isolated GH deficiency; *Isl1*, Islet-1; MRI, magnetic resonance imaging; NR5A1, nuclear receptor 5A1; POMC, proopiomelacortin; PRL, prolactin; PROP1, Prophet of Pit1; SF1, steroidogenic factor 1; SHH, Sonic Hedgehog; SOD, septo-optic dysplasia; WNT, Wingless-type MMTV integration site family.

I. Introduction

Congenital hypopituitarism (CH) is a syndrome with a wide variation in severity and with many underlying causes. It may present early in the neonatal period or later in childhood. It can be associated with single or multiple pituitary hormone deficiencies, and the endocrinopathy can evolve to include other hormonal deficits. These conditions are often associated with significant morbidity and occasional mortality, and despite many advances and increased understanding of some of the genetic mechanisms involved, their etiology remains unknown in the majority of cases. The frequent association of CH with other abnormalities, notably of the eye and forebrain, suggests that many cases are the result of disordered embryogenesis because these are all structures that depend on normal development of the anterior midline. The genetic cascade of signaling molecules and transcription factors thought to orchestrate development of the pituitary, but also of the surrounding regions, is gradually being pieced together. However, the definitive identity of mutant genes and/or their relationship with phenotypes remain to be determined in the majority of cases.

Nevertheless, as we describe in this review, mutations in a number of developmental genes have been linked with, and probably account for, several combined pituitary hormone deficiency (CPHD) syndromes, often in association with a number of extrapituitary defects such as optic nerve hypoplasia, anophthalmia/microphthalmia, and forebrain defects such as agenesis of the corpus callosum and absence of the septum pellucidum. Additionally, mutations in a number of genes that are associated with cellular differentiation, proliferation, or hormone production have been discovered in association with isolated hormone deficiencies. This review will describe the current state of knowledge in our understanding of the etiology of congenital CPHD in both mouse and human. In doing so, we cover a wide range of factors implicated in pituitary development and attempt to convey the rapid progress in understanding made particularly over the last few years, while also highlighting how much more remains to be learned.

II. Embryonic Development of the Mouse Pituitary Gland

A. Gross structure and function of the mature pituitary gland

The pituitary gland is a central regulator of growth, reproduction, and endocrine physiology and functions to relay signals from the hypothalamus to various target organs. The hypothalamus is the principal neural structure regulating homeostasis in vertebrates and coordinates complex signals from other regions of the brain and the periphery to monitor and maintain the body's internal balance. Its primary neuroendocrine output is via neural terminal arborizations in the median eminence, releasing factors that control the release of hormones from pituitary endocrine cells. It is also likely to provide an equally important trophic stimulus to the maintenance and plasticity of the gland, although these factors are much less understood. The pituitary, in turn, signals to peripheral organs to regulate vital processes such as growth, puberty, metabolism, stress responses, reproduction, and lactation. The gland is situated within the sella turcica, a recess in the sphenoid bone, at the base of the brain. The mature pituitary gland is comprised of the adenohypophysis (consisting of the anterior and intermediate lobes) and the neurohypophysis (posterior lobe), which are functionally and morphologically distinct structures whose close apposition nevertheless raises intriguing developmental and functional possibilities that are yet to be seriously explored.

The anterior lobe of the pituitary secretes hormones from five different specialized hormone-producing major cell types. In humans, in contrast to the mouse, the intermediate lobe largely disappears during embryogenesis with essentially no intermediate lobe in the human pituitary, although the mechanism underlying this species difference is unclear. This simplified description conceals rather more complex cellular relationships because there are subpopulations within the same hormone cell types [*e.g.*, lactotropes (1)] and subsets of cells that coexpress more than one hormone (*e.g.*, somatomammotropes). The proportion of these coexpressing cells can alter under different physiological (2) or pathological conditions (3). Their adult function can be selectively regulated (1), and it is probable that they are also specified by genetic cascades that extend from fetal to adult life; factors such as Ikaros, that might be involved in such coregulation, have been identified (4). The processes determining cell fate, migration patterns, and final location of the different hormone-producing cells during development of the embryonic Rathke's pouch (RP) into the adult anterior lobe requires a correct genetic program, and individual gene deletions can result in misspecification and/or mislocation of cells, although these sometimes still achieve a terminally differentiated hormone phenotype (5–7).

The posterior lobe contains the terminal axonal projections of magnocellular neurons from the paraventricular and supraoptic nuclei of the hypothalamus and specialized supportive astroglia known as pituicytes, which surround the projections. The magnocellular neurons produce oxytocin, required during parturition and lactation, and arginine vasopressin, which is involved in the regulation of osmotic bal-

ance. These peptide hormones are transported to the axon terminals within the posterior pituitary where they are released as required under hypothalamic control.

Hypothalamic factors are rapidly transported via the hypophyseal portal blood system to the adenohypophysis where they regulate endocrine cell proliferation, hormone synthesis, and hormone release from all the pituitary cell types. Classically, these factors are thought of as specific secretagogues, defined by the specificity of receptors on their target cells. However, this is an oversimplification because there are a much larger number of peptides and other factors known to be coexpressed in subsets of these hypophysiotropic neurons (8). These are also transported and released into portal blood, probably in much smaller quantities than the hypophysiotropic peptides, and may modulate the secretion of anterior pituitary hormones and possibly regulate anterior pituitary plasticity.

The neural and vascular connections are carried by the pituitary stalk, which is responsible for conveying all the information from the hypothalamus to the pituitary gland. This is a vulnerable essential bridge between brain and pituitary, and any damage to the pituitary stalk, *e.g.*, through childbirth or other physical head trauma throughout life, results in both anterior and posterior pituitary dysfunction, although to a surprisingly variable extent. The stalk can be visualized noninvasively using magnetic resonance imaging (MRI) in both rodents and humans, and flow can be imaged directly using fluorescence in rodents (I. C. A. F. Robinson, unpublished data). The presence or absence of an intact stalk or ectopic localization of the posterior lobe on imaging is an important part of the description of the phenotype in human pituitary dysfunction syndromes.

B. Morphogenesis of the pituitary gland

The three lobes of the mature pituitary gland have a dual embryonic origin; the anterior and intermediate lobes are derived from oral ectoderm, whereas the posterior pituitary originates from the infundibulum, a specific region of the developing central nervous system (CNS) that forms in the midline of the ventral diencephalon. This review focuses on the mouse as a model organism for pituitary development in mammals, given the increasing number of mouse mutants that have been analyzed in which morphogenesis of the pituitary has been affected. However, fate map studies have shown that these processes are similar in all vertebrate species studied, including zebrafish, amphibians, chick, and rodents (9–13).

In the mouse, the first sign of pituitary development occurs at 7.5 days post coitum (dpc) with the development of the hypophyseal placode, a thickening of the ectoderm in the midline of the anterior neural ridge. It is already associated at this stage with the presumptive hypothalamic territories, posteriorly adjacent, as described in chick (14, 15). During the next 24 h, as the anterior neural tube bends and rapidly expands, the hypophyseal placode is displaced ventrally, within the ectoderm at the roof of the future oral cavity. At approximately 9 dpc, the placode forms a rudimentary Rathke's pouch, the primordium of the anterior and intermediate lobe. By 10.5 dpc, a restricted region of the ventral diencephalon above the pouch gives rise to the infundibulum from which the posterior pituitary and pituitary stalk will derive. The juxtaposition of Rathke's pouch and the diencephalon is maintained throughout the early stages of pituitary organogenesis. This close relationship is required for tissue interactions between neural and oral ectoderm, which are critical for the initial stages of pituitary specification. By 10.5 dpc, the pouch is fully developed, and at 12.5 dpc it is completely separated from the underlying oral ectoderm (Fig. 1). The lumen of the pouch persists as the pituitary cleft, separating the anterior and intermediate lobes in the mature gland. The iterative nature of the inductive interactions required for pituitary morphogenesis makes it very

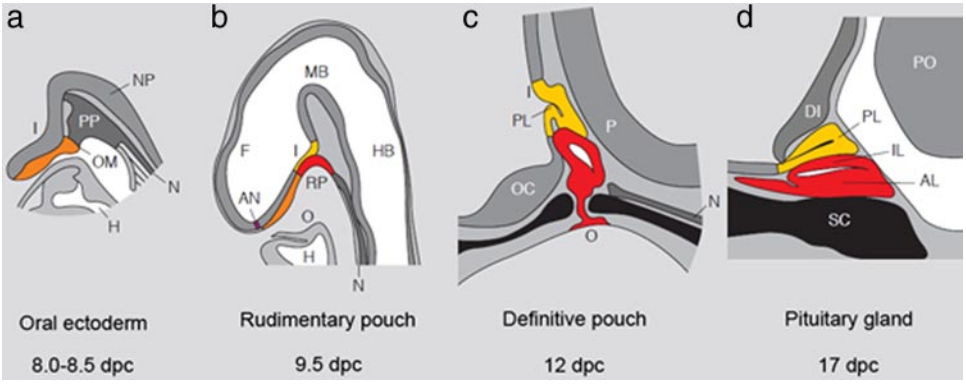

FIG 1. Mouse pituitary development in sagittal section. Stages of development are indicated in dpc. AL, Anterior lobe; AN, anterior neural pore; DI, diencephalon; F, forebrain; H, heart; HB, hindbrain; I, infundibulum; IL, intermediate lobe; MB, midbrain; N, notochord; NP, neural plate; O, oral cavity; OC, optic chiasma; OM, oral membrane; P, pontine flexure; PL, posterior lobe; PO, pons; PP, prechordal plate; RP, Rathke's pouch; SC, sphenoid cartilage. [Adapted from H. Z. Sheng and H. Westphal: *Trends Genet* 15:236–240, 1999 (317), with permission from Elsevier.]

sensitive to both loss- and gain-of-function mutations. In turn, such mutations can be informative as to the underlying mechanisms driving invagination and shaping of the pouch (16), cell migration, tissue patterning, and the specification of individual cell fates. Although much remains to be learned, we discuss the current state of knowledge below.

C. Endocrine cellular differentiation in the developing pituitary gland

The definitive Rathke's pouch comprises proliferative progenitors that will gradually relocate ventrally, away from the lumen as they differentiate. A proliferative zone containing progenitors is maintained in the embryo in a perilumenal area and was recently found to persist in the adult (17, 18). Ventrally, relocalization is associated with cell cycle exit (19), and it is not known whether this is an active migration event or a passive process of cells driven away by new cells proliferating in the perilumenal area.

The earliest phenotypic marker of differentiation within the anterior pituitary is the expression of *Cga*, the gene encoding α-glycoprotein subunit (αGSU), which appears at 11.5 dpc in a restricted patch of cells in the ventral region of Rathke's pouch. These αGSU-positive cells also express the transcription factor Islet-1 (*Isl1*) and correspond to prospective thyrotropes, which differentiate after initiating expression of *Tshb* at 12.5 dpc. However, this early population of thyrotropes is short-lived and disappears at birth (20–22). In the mature adult pituitary, *Cga* expression is only detected in the thyrotrope and gonadotrope lineages (20).

At 12.5 dpc, corticotrope cells start to differentiate in a domain just dorsal to thyrotropes, producing proopiomelanocortin (POMC) (22, 23). In the intermediate lobe, as development proceeds, *Pomc*-expressing cells begin to be detected at 14.5 dpc with gradually increasing expression in melanotropes (21). Definitive thyrotropes are observed at 14.5 dpc, characterized by the expression of *Tshb* in a restricted number of cells within the anterior lobe. The expression of *Gh* and *Prl* marks the differentiation of somatotrope and lactotrope lineages, respectively, beginning at 15.5 dpc with the number of somatotropes increasing dramatically and extending throughout the central and lateral areas of the anterior lobe. Lactotropes remain localized to a more restricted medial zone adjacent to the ventral surface of the intermediate lobe. The gonadotropes are the last cell type to emerge, beginning at 16.5 dpc with the onset of *Lhb* expression followed by *Fshb* 1 d later.

Inevitably, much of this description relies on the use of hallmark differentiation markers to identify cell types. However, recent unpublished birthdating studies (S. Camper and S. Davis, Department of Human Genetics, University of Michigan, personal communication) imply that the first waves of many endocrine cell types may be specified earlier and then migrate some distance before these markers are expressed. This raises the question of whether there are sorting cues for migrating cells leaving progenitor pools, and how they end up in the three-dimensional homotypic networks, shown recently in the adult mouse pituitary gland (24). Many developmental studies of the pituitary concentrate their analysis by careful registration of sections in the midline to permit comparisons of anatomical sections over time. However, these are likely to have given a very incomplete description of cellular distribution in three dimensions, especially because the majority of endocrine cells end up in the lateral rather than medial portions of the gland. Advanced imaging and reconstruction techniques, combined with fluorescent tags for different endocrine cell populations, will likely change our views on the way the gland assembles its networks of cells and maintains these through cell turnover and replacement (P. Mollard, Institute of Functional Genomics, Montpelier, France, personal communication).

D. Mouse genetic models unravel aspects of pituitary development

The existence of spontaneous mutations and the ease of generating engineered mutant strains of mice that exhibit disrupted pituitary development place the mouse as the major model for studying pituitary development. We will consider several of the genes and their protein products that are present from early stages of pituitary development and which are required endogenously within Rathke's pouch progenitor cells for their maintenance and proliferation. Next, we will discuss those that are active within cells of the ventral diencephalon, but which are required for the induction, regionalization, and maintenance of Rathke's pouch. Finally, we will review some of the molecules involved in endocrine cell differentiation from 12.5 dpc, *i.e.*, after Rathke's pouch is fully developed and has separated from the oral ectoderm. Naturally occurring mutations and targeted ablation of several hypothalamic hormones including GnRH, GHRH, and TRH has shown that mice lacking these peptide hormones generally have normal pituitary development. Hormone deficiencies and the associated effects on downstream targets for these respective factors develop after birth and are beyond the scope of this review (25–28). Similarly, the description of isolated hormonal deficiencies will not be reviewed because most of the effects of these hormonal deficiencies are only observed postnatally.

III. Factors and Signaling Pathways Present in the Pituitary Primordium and Initially Involved in Its Formation and Maintenance

A. SIX homeodomain proteins

These proteins contain a DNA binding homeodomain homologous to that of *Drosophila sine oculus* protein.

They form a family of six members in mammals that can either activate or repress transcription. These factors work in a complex network with other proteins, including EYA, and DACH (encoded by the mammalian homologs of the Eyes absent and dachshund genes in *Drosophila*). EYA is a phosphatase, whereas DACH is able to recruit corepressors, therefore, depending on the activity of the former, transcription is either induced or repressed. This transcription factor network is iteratively used during organogenesis; retinal cell determination is a well-characterized example (29–31). The SIX3/6 proteins can also interact with the Groucho family of transcriptional repressors (32, 33), which implies yet further links with, *e.g.*, Wingless-type MMTV integration site family (WNT) signaling pathways.

Four members of the SIX family are expressed during pituitary development; but the exact role of these proteins is difficult to determine due to likely functional redundancy between them (*Six1* and *4*) and the severity of the phenotype affecting anterior forebrain structures in mouse mutants (*Six3*). The first demonstration of a role for these proteins in pituitary development emerged from studies on *Six6*. This is first expressed in the invaginating pouch, but it soon becomes restricted dorsally in the perilumenal region, where cell proliferation continues, distant from regions involved in differentiation. In the adult pituitary, the protein is still strongly expressed (K. Rizzoti, unpublished observations), implying a potential continuing role in pituitary plasticity. *Six6* is also expressed in the developing eye and hypothalamus (31, 34). Liveborn $Six6^{-/-}$ mice possess hypoplastic pituitaries, likely due to an early impairment of progenitor proliferation (12). Arguing by analogy with the retinal phenotype seen in the same animals, the reduced pituitary proliferation is strongly suggestive of a lack of repression of cell cycle kinase inhibitors such as *p27kip1* whose promoter is directly bound *in vivo* by the SIX6/DACH1 complex (31). In contrast, SIX3 heterozygous deletion (in $Six3^{+/-}$; $Hesx1^{+/-}$ embryos) has recently been linked to increased cell proliferation (see *Section III.B* and Ref. 35).

To date, disease-causing mutations in *SIX6* have not been identified in human patients with either eye defects or pituitary hormone deficiencies (M. T. Dattani, unpublished observations; and Ref. 36).

B. Paired-like homeodomain proteins

1. Hesx1

The transcription factor HESX1 is a member of the paired-like class of homeodomain proteins (37), and it functions as a transcriptional repressor (38, 39). TLE1, the mammalian ortholog of the *Drosophila* protein Groucho, and the nuclear corepressor N-COR both bind to HESX1 to exert repression (38). DNA methyltransferase 1 is also capable of partnering with HESX1 to repress transcription, possibly through CpG methylation of HESX1 target genes (40). *Hesx1* is one of the earliest markers of Rathke's pouch, initially present in the anterior neural ridge midline, where the hypophyseal placode develops, and in the adjacent rostral neural plate, a region fated to form the forebrain and ventral diencephalon. It becomes restricted to Rathke's pouch by 9 to 9.5 dpc, where expression is maintained until 13.5 dpc (41, 42). Its down-regulation is absolutely required for cell determination to occur, in particular for the related paired-like homeodomain activator Prophet of Pit1 (PROP1) to promote these events (see below). Activation of the *5′Hesx1* promoter by the LIM homeodomain proteins *Lhx1* and *Lhx3* (see below) is required for its early expression, whereas its 3′ downstream region contains elements both necessary and sufficient for later expression in the developing Rathke's pouch (43).

Hesx1 is essential for normal forebrain development, and mice with a homozygous targeted disruption of *Hesx1* display a range of forebrain defects (44, 45). The most severely affected *Hesx1* null embryos exhibited a significant reduction in anterior forebrain (AFB) structures, especially of midline telencephalic commissural tracts such as the corpus callosum and anterior commissure and of olfactory bulbs, fully penetrant eye defects ranging from microphthalmia to anophthalmia, and absence of the infundibulum, in addition to dysmorphology of Rathke's pouch (45, 46). Andoniadou *et al.* (45) have shown that the reduction of AFB tissue in the null mutants is caused by the posteriorization of AFB precursors at early somite stages. This in turn is related to the ectopic activation of WNT/β-catenin signaling in the prospective AFB. Although the pituitary gland is severely dysplastic and cellular proliferation is enhanced, terminal differentiation of the hormone-producing cell types is not affected at later stages of development (38). A small percentage (5%) of the most severely affected neonates are reported to lack an anterior pituitary gland (38). However, recent studies have shown that, in mice bearing a homozygous null mutation in *Hesx1* ($Hesx1^{R160/R160C}$) that were severely affected with defects of the telencephalon, eyes, and craniofacial structures, the anterior pituitary was ectopically located in the roof of the nasopharyngeal cavity, as was also observed in $Hesx1^{-/-}$ embryos (47). In contrast, the more mildly affected homozygous mutants show the presence of a Rathke's pouch that is correctly located, albeit one that is morphologically abnormal with additional bifurcations and cell overproliferation. This results in the apparent formation of multiple pituitary glands (38). However, postnatally the overgrown gland becomes hypoplastic (38), perhaps due to defects in the hypothala-

mus, because this controls the growth of the gland postnatally and it is also affected by *Hesx1* deletion (35).

Recently, embryos heterozygous for both *Hesx1* and *Six3* null mutations were shown to display a phenotype reminiscent of that of mildly affected $Hesx1^{-/-}$ embryos. These show increased cell proliferation in the pouch and a hyperplastic, dysmorphic gland, and it has been suggested that derepression of the WNT pathway could be involved (35). Thus both HESX1 and SIX3 seem to control progenitor proliferation in the same or parallel pathways, and the later switch of expression between the repressor HESX1 and the activator PROP1 is an important step toward proper cell determination (see *Section V*).

The forebrain, eye, and pituitary deficits identified in the *Hesx1* null mutants subsequently led to the identification of mutations in the human homolog *HESX1* in patients with various forms of hypopituitarism including septo-optic dysplasia (SOD), CPHD, and isolated GH deficiency (IGHD) (46, 48, 49).

2. Otx2

OTX2 is a homolog of the *Drosophila* orthodenticle protein. In mammals there are two related proteins, OTX1 and OTX2, and their requirement for the formation of anterior structures has been conserved throughout evolution from flies to mice (for review, see Ref. 50).

In the mouse, *Otx2* is expressed early in development, and in its absence gastrulation is impaired. As a consequence, mutant embryos lack anterior structures corresponding to the future head (51). Later in development, it is still required for maintenance of the forebrain (52, 53). Although its role during pituitary development has not been precisely investigated, its requirement in anterior structures implies that it is also necessary for Rathke's pouch development. It is in particular necessary for correct *Hesx1* expression in the forebrain (54).

Recently, mutations in *OTX2* have been identified in patients with eye disorders such as anophthalmia or microphthalmia, with or without variable hypopituitarism (see below).

3. Pitx1

PITX1 contains a DNA binding homeodomain related to that of *Drosophila* bicoid and orthodenticle (55, 56). There are three Pitx proteins in mammals, and they play important roles in different tissues during embryogenesis (57). Interaction between PITX1 and the pituitary transcription factor POU1F1 (previously termed PIT1; see below) results in synergistic activation of the promoter of the *Prl* gene and also, to a lesser extent, the *Gh* promoter (58, 59). PITX1 can also synergize with the transcription factors NEUROD1 and TBX19 (previously called TPIT) to activate *Pomc* expression, whereas in gonadotrope cells it activates *Lhb* expression in conjunction with the transcription factors nuclear receptor 5A1 [NR5A1; also termed steroidogenic factor 1 (SF1)] and Early growth response 1 (EGR1). PITX1 also appears to be essential for maintaining the expression of αGsu and the GnRH receptor (23, 59–62), and it is an essential upstream regulator of *Lhx3* (59, 63, 64).

Expression of *Pitx1* is first detected in the anterior ectoderm at 8.0 dpc. By 9.5 dpc, *Pitx1* is expressed throughout the oral ectoderm and in Rathke's pouch. Expression is maintained throughout anterior pituitary development in all hormone-producing cell types (58, 65). In the adult pituitary, *Pitx1* expression is highest in αGSU-expressing thyrotropes and gonadotropes and some POMC-producing cells, with lower levels in other hormone-producing cell types (66). Mice with a homozygous disruption of *Pitx1* die before or shortly after birth, reflecting the pleiotropic functions of the protein during development, with a small subset exhibiting embryonic lethality at 11.5 dpc. Morphogenesis of the pituitary in *Pitx1* null embryos appears normal. However, at birth an increase in the levels of *Acth* transcripts and protein was noted in corticotropes, whereas the number of gonadotropes and thyrotropes, as well as levels of LHβ and TSHβ, were greatly diminished (67). The presence of the closely related transcription factor PITX2 in Rathke's pouch could explain the absence of early defects (see below).

4. Pitx2

PITX2 exhibits a large degree of sequence identity with PITX1 (68, 69) and is similarly capable of activating the promoters of most of the pituitary hormone genes (59, 70, 71). Expression of *Pitx2* is initiated in the oral ectoderm at 8.5 dpc and is maintained throughout development of Rathke's pouch and in the surrounding mesenchyme (68, 72). Its expression is maintained in the adult gland mainly in thyrotropes and gonadotropes (64). It is expressed in numerous endocrine cell lines *in vitro*, as is PITX1 (64, 68).

Homozygous loss of *Pitx2* results in early embryonic lethality with a severe phenotype, consistent with the widespread expression of this gene (73–76). In contrast to *Pitx1*, pituitary development is severely affected in *Pitx2* null embryos. Rathke's pouch undergoes initial specification, but *Hesx1* expression is not maintained and development of the pouch is arrested by 12.5 dpc (76). Studies have shown that activation of the WNT signaling pathway or constitutive activation of β-catenin can induce *Pitx2* expression. In turn, PITX2 controls genes such as *cyclin D1* and *Cyclin D2* that regulate the cell cycle (77, 78). It has therefore been proposed that pituitary hypoplasia in *Pitx2* null mice may result from decreased cell proliferation (77). Another study shows however that lack of *Pitx2* results in excessive cell death during

early pituitary development, suggesting that it is required for survival rather than proliferation (64). Indeed, it could be involved in both survival and proliferation.

The expression patterns of *Pitx1* and *Pitx2* overlap during early pituitary development, suggesting a possible redundancy as intimated above; indeed animals mutant in both genes show a more severe phenotype. Expression of *Lhx3* is undetectable in these, whereas single mutants do show *Lhx3* expression. This supports the notion of some functional redundancy between PITX1 and PITX2; however, the single mutant phenotypes show that early development is more dependent on PITX2 than on PITX1 (64).

By creating a hypomorphic allele of *Pitx2*, Gage *et al.* (76) were able to assess its role in later stages of pituitary differentiation. Generation of an allelic series containing different combinations of *Pitx2* wild-type, hypomorphic, and null alleles showed that reduced dosage of *Pitx2* is proportional to the extent of pituitary hypoplasia and cellular differentiation (79). Examination of hypomorphic *Pitx2* homozygous embryos, which survive postnatally, revealed that the gonadotrope lineage was most profoundly affected. Numbers of differentiated somatotropes and thyrotropes were also reduced in mice homozygous for the hypomorphic allele; however, the corticotrope population appeared unaffected, as judged by the normal expression of *Pomc* (79). Finally, overexpression of *Pitx2* in the gonadotrope and thyrotrope lineages under the control of αGSU regulatory sequences results in exclusive expansion of the gonadotrope population (64). This specificity may rely on the requirement of PITX2 for the expression of the genes encoding gonadotrope-specific transcription factors GATA2, EGR1, and NR5A1 (SF1) (79). In conclusion both factors are involved in progenitor maintenance, or expansion, with a prominent role for PITX2. Later on they are individually necessary within specific endocrine cell populations.

The third member of this family, PITX3, which has a major role in left-right asymmetry, has been implicated in pituitary development in lower vertebrates, where it defines an equivalence domain for the lens and anterior pituitary placode (80). Mutations affecting *PITX3* have been reported in human patients with anterior segment dysgenesis and cataract, and loss of expression in the lens is associated with aphakia in mice (81, 82); however, because there is currently little information pertaining to its role in pituitary development in mammals, we will not discuss it further.

C. LIM homeodomain transcription factors

1. Isl1

ISL1 is a member of the LIM homeodomain family of transcription factors, characterized by two tandemly repeated cysteine/histidine-rich LIM domains, involved in protein-protein interactions between the N-terminal end of the protein and the DNA-binding homeodomain. Combinatorial expression of LIM transcription factors has been shown to be important in different cell specification events (83), and it is becoming clear that related proteins and cofactors for LIM proteins interact to regulate target genes in pituitary cells (84, 85).

ISL1 is the first LIM protein to be expressed during pituitary development; initially detectable at 8.5 dpc throughout the oral ectoderm, it becomes restricted to the pouch at 9.5 dpc. Between 10.5 and 11.5 dpc, its expression is gradually restricted to the ventral portion of the pouch, which will begin to express *Cga* and subsequently *Tshb* because the cells become rostral tip thyrotropes after 12.5 dpc (22). This dynamic expression pattern appears to be dictated by interactions with the surrounding tissues (see below).

Homozygous loss of *Isl1* results in developmental arrest by 10 dpc, at which time the oral ectoderm of mutant mice has invaginated, but pouch formation is blocked at an early stage. This suggests that the gene is necessary for pituitary progenitor cell proliferation and/or maintenance (86). Its function at later stages has not been described, and to date no human mutations in *ISL1* have been identified.

2. Lhx3

Lhx3 is expressed early during anterior pituitary development, initially with strong uniform expression within Rathke's pouch at 9.5 dpc (87). Subsequently, although its expression is maintained throughout the pouch, from 12.5 dpc it forms a gradient of expression with higher protein levels dorsally (88–90). By 16.5 dpc, *Lhx3* is present throughout the pituitary, and expression persists into adulthood (89). Mice with a targeted homozygous disruption of *Lhx3* die shortly after birth and exhibit pituitary aplasia, whereas heterozygous mice are normal. Although Rathke's pouch develops, its expansion is arrested in null embryos by 12.5 dpc (87). Moreover, whereas *Hesx1* and *Isl1* are expressed normally at 9.5 dpc in these mutants, their expression fails to be maintained from 12.5 dpc (87, 90). Defects in cell proliferation have been reported (87); however, increased apoptosis appears to be the major contributor to the pituitary hypoplasia (63, 90). Later during pituitary development, the phenotype of $Lhx3^{-/-}$ pituitaries involves defects in the differentiation of all endocrine cell types. Dorsoventral cell specification appears impaired, and this may, in part, be due to disrupted Notch signaling because *Notch2* fails to be expressed in the mutants.

Failure to activate *Pou1f1* (*Pit1*) results in a predictable loss of lactotropes, somatotropes, and thyrotropes (87, 90) (see *Section V*). Gonadotropes also fail to fully differentiate, probably due to the down-regulation of *Foxl2* and αGSU. A similar loss of both TBX19 (TPIT) and NEUROD1

results in a dramatic reduction of corticotropes (90). In the intermediate lobe, down-regulation of *Tbx19* expression is consistent with the observed absence of melanotropes. Therefore, LHX3 is required for early progenitor survival as well as late endocrine cell differentiation events, reflecting its wide expression pattern both spatially and temporally. Its pleiotropic functions are reflected by its different target genes: *Fshb*, *Cga*, *Prl*, *Tshb*, *Gnrhr*, and *Pou1f1* (91–94) and partners including Pou1f1 and Isl1 (91, 95).

Mutations in *LHX3* have now been identified in a number of human pedigrees characterized by CPHD, a short stiff neck, and variable sensorineural hearing loss (see *Section VI*).

3. Lhx4

Lhx4 is closely related to *Lhx3* and is also expressed throughout the invaginating Rathke's pouch at 9.5 dpc. Subsequently, however, the expression patterns of the two genes differ (89, 96, 97). Although *Lhx3* is widely expressed and maintained throughout the developing pituitary, *Lhx4* expression is transient, becoming restricted to the future anterior lobe by 12.5 dpc and eventually down-regulated at 15.5 dpc (89).

Homozygous $Lhx4^{-/-}$ mice die shortly after birth, whereas heterozygous animals appear normal (89). As is the case with *Lhx3* null mice, loss of *Lhx4* does not prevent definitive pouch formation but results in the formation of a hypoplastic pituitary. However, in contrast with $Lhx3^{-/-}$ mice, the anterior lobe of $Lhx4^{-/-}$ mice contains all five of the differentiated cell types (89). Although there is a slight reduction in cell proliferation, the small size of the anterior pituitary is clearly due to a wave of apoptosis completed by 14.5 dpc (88). LHX4 is also required for the proper expression of *Lhx3*, specifically during early pituitary development. This may also involve another pituitary-specific transcription factor, PROP1, because embryos lacking both LHX4 and PROP1 fail to express *Lhx3* at early stages of pituitary morphogenesis (88).

Generation of mice with various combinations of *Lhx3* and *Lhx4* gene dosage using single and double knockouts has revealed that a single wild-type allele of either *Lhx3* or *Lhx4* is sufficient for the formation of a definitive pouch structure, and that homozygous loss of both genes does not prevent formation of a rudimentary pouch at 9.5 dpc, but there is no progression beyond this stage. This strongly suggests early functional redundancy when expression of the two proteins overlaps (98). Later, however, normal specification and terminal differentiation of the pituitary cell types is entirely dependent on the presence of at least one copy of *Lhx3*, not *Lhx4*, showing that functional redundancy is temporally limited (98).

Heterozygous mutations within *LHX4* have now been described in a number of human patients with CPHD (see *Section VI*).

D. SOX transcription factors

Sox2 together with *Sox1* and *Sox3* are all grouped into the B1 subfamily of *Sox* genes (of which there are 20 in mice and humans) based on their extensive homology. The SOX proteins bind and bend DNA with their HMG domain; they can modulate gene activity as classical transcription factors but, due to the nature of the acute bend they induce in the DNA, they are also involved in the assembly of transcriptional complexes (99–101). During pituitary development, SOX2 is expressed in the early ectoderm and maintained throughout the pouch. Its expression is down-regulated as endocrine cell differentiation proceeds. Expression is maintained in the prospective progenitor proliferative zone, around the Rathke's pouch lumen, during embryogenesis but also in the mature gland (17). It is also expressed in the ventral diencephalon (see below).

$Sox2^{-/-}$ null embryos die shortly after implantation and therefore provide no information about its role in the pituitary (102). However, about one third of *Sox2* heterozygous mice show perinatal lethality, and we have recently shown that the remaining animals are affected by a mild hypopituitarism (103). We also detected in a proportion of heterozygous mutants a mild hypoplasia of the anterior pituitary gland from Rathke's pouch stages until adulthood (K. Rizzoti, unpublished observations), which could underlie the hypopituitarism. SOX2 could therefore be required for the maintenance or proliferation of pituitary progenitor cells in the embryo. It may also be involved in the regulation of expression of *Hesx1* (103, 104). Along with the size reduction, the embryonic pituitaries of *Sox2* mutants displayed abnormal bifurcations resulting in the presence of extra clefts in the adult gland (103). These may be caused indirectly by the reduction of SOX2 in the overlying neuroepithelium (see below).

Sox2 expression is also seen in specific subpopulations of hormone-negative cells in the postnatal and adult pituitary (17). Some of these line the cleft and are probably direct descendents of the luminal cells lining Rathke's pouch; others are scattered in adjacent tissue of the anterior pituitary. It has recently been shown that SOX2-positive cells, dissociated from adult pituitaries, are able to form pituispheres in culture (17). These are free-floating balls of cells derived clonally by proliferation of a single cell. The majority of cells within the spheres retain *Sox2* expression when grown in growth factors [epidermal growth factor (EGF) and fibroblast growth factor (FGF)], but they can be induced to differentiate by removing growth factors and allowing the spheres to attach to a substrate. They quite rapidly lose SOX2 and give rise to endocrine-producing cells. All the endocrine cell types found in the anterior lobe can be found (essentially at

random) in the attached clumps of cells. If the pituispheres are kept in growth medium and dissociated into single cells, these can give rise to secondary pituispheres, which have similar properties to the primary spheres. Together these data provide *in vitro* evidence for the presence of self-renewing progenitor or stem cells, marked by *Sox2* expression. It is therefore possible that at least some of the SOX2-positive cells in the adult pituitary also represent multipotent pituitary progenitor/stem cells. These could play a role in pituitary plasticity when changing physiological situations, such as lactation or pregnancy, lead to changes in numbers and types of endocrine cells (17).

Lineage tracing studies will be necessary to show the contribution of such adult stem progenitors to normal endocrine cell turnover. A subsequent report attempted to do so, although this did not concern *Sox2* expression (18). Although the conclusions were in broad agreement with the notion that there are stem/progenitor cells located in the region of the cleft, additional methods are required to prove this. Moreover, further work is required to show whether SOX2 is important for the identity and properties of these cells as stem/progenitor cells as it is for other stem cells types, such as embryonic stem cells and neural stem cells (102, 105).

De novo SOX2 mutations have now been identified in a number of patients with eye defects such as anophthalmia or microphthalmia in association with hypogonadotropic hypogonadism and variable GH deficiency (GHD), as well as a number of other features such as agenesis of the corpus callosum, hypothalamic hamartomata, esophageal atresia, and sensorineural hearing loss (see *Section VI*). Unlike the murine phenotype, where haploinsufficiency is associated with a generalized reduction in pituitary cell numbers, in humans the phenotype consistently appears to include hypogonadotropic hypogonadism, whereas GHD is rarer and other cell types do not seem to be affected.

E. WNT/β-catenin and Notch signaling pathways

1. WNT/β-catenin signaling

WNT signaling pathways are repeatedly involved during embryogenesis and are implicated in cell proliferation, determination, and differentiation events and also in determining cell polarity. WNT ligands activate their signaling cascade by binding to a complex comprising Frizzled receptors and LRP proteins (low density lipoprotein-related receptor). At least three alternative pathways can then be activated: 1) a β-catenin-dependent pathway that affects transcriptional activity, usually through binding TCF/LEF proteins, which can then function as transcriptional activators; 2) the planar cell polarity pathway; and 3) a Ca^{2+}-mediated pathway related to cell adhesion (for review, see Ref. 106 and references therein).

A pathway involving signaling via WNT/Disheveled/β-catenin activates the expression of *Pitx2*, promoting the proliferation of pituitary precursors (77). The identity of the WNT ligand inducing this pathway is not known, but the expression of several candidate ligands has been detected within the developing gland (107–109). In particular, *Wnt4* is expressed in the pouch from 9.5 dpc, and its deletion results in pituitary hypoplasia (108, 110). However, the origin of this defect is not clear. It was originally shown that αGSU expression, in particular, was reduced in $Wnt4^{-/-}$ embryonic pituitaries (110), whereas a more recent report finds reduced POU1F1 (PIT1), but normal levels of αGSU (108). TCF4, a downstream effector of WNT/β-catenin signaling, is present in Rathke's pouch and also in the ventral diencephalon. Its deletion induces the presence of abnormal bifurcations of the pouch, although this may be due to its deletion in the overlying CNS (see below). However, *Tcf4* mutants also show pituitary hyperplasia (111, 112), which could be explained by the expansion of *Six6* expression in the pouch at 11.5 dpc (113).

The phenotypes of WNT4 and TCF4 appear contradictory, but in the absence of WNT signaling, TCFs actively repress transcription, so the two loss of function mutations are not comparable. Nevertheless, these data suggest that in the absence of WNT ligand, TCF4 restricts SIX6 expression to control progenitor cell proliferation; but in the presence of WNT, proliferation is encouraged. This may also reflect a more complex crosstalk of pathways controlled by different ligands secreted both by the ventral diencephalon and within the pouch (107, 108). Furthermore WNTs and β-catenin can cooperate with other signaling pathways, in particular the Notch signaling pathway (106), and it may therefore not be surprising to obtain different phenotypes when manipulating the ligand *vs.* either intermediate central effectors like β-catenin or specific downstream effectors.

2. Notch signaling

Notch proteins are transmembrane receptors displaying an extracellular domain made of epidermal growth factor-like repeats and an intracellular domain comprising Ankyrin-like repeats. Four Notch receptors are present in mammals. Upon activation by ligands (in mammals, Delta1, -2, -3, and -4; Jagged1 and -2), the intracellular domain of Notch is released and translocates to the nucleus where it activates transcription via its main effector, the protein RBP-J/CSL. Among its transcriptional target genes are members of the Hairy enhancer of Split (Hes) and bHLH transcription factors (for review, see Ref. 106). This signaling pathway mediates the process of lateral inhibition where cells within groups are singled out to adopt a particular fate. It is used iteratively during development,

and from flies to mammals, to direct or influence cell fate decisions.

During pituitary development, *Notch2*, *Notch3*, *Jagged1*, and *Hes1* are expressed at 9.5 dpc in the invaginating pouch. They are then quickly down-regulated from the differentiating zone ventrally but maintained dorsally around the lumen of Rathke's pouch (114, 115). Notch signaling pathway components are expressed by cells in the adult gland in the same locations that are proposed to contain progenitor/stem cells as those mentioned above (see *Section III.D*) (116).

To gain insight into Notch signaling function, Zhu *et al.* (115) deleted *Rbp-J* exclusively in Rathke's pouch, resulting in the premature differentiation of corticotropes. Such a phenotype is also observed when a downstream effector of Notch, *Hes1*, is deleted (117). This latter phenotype was correlated with decreased proliferation (115, 117); however one study also reported increased cell death (7). In *Rbp-J*$^{-/-}$ embryos, *Prop1*, a direct target of RBP-J (115), fails to be up-regulated at 12.5 dpc. As a consequence, *Pou1f1* (*Pit1*) fails to be expressed (see below). Therefore Notch signaling could be required to prevent early (corticotrope) differentiation and maintain undifferentiated progenitors fated for a later (POU1F1-dependent) fate. In this way, Notch signaling would allow the generation of different endocrine cell types by controlling the time and therefore the context in which they differentiate.

IV. Regulation of Rathke's Pouch Development by the Ventral Diencephalon

Experimental manipulation of embryos from several species, as well as Rathke's pouch explant experiments in rodents, have shown that signals from the diencephalon are essential not only for the induction and maintenance of Rathke's pouch, but also for the regionalization within the pouch that allows the emergence of the different endocrine cell types (22, 110, 118–123). Recently, however, careful examination of the expression patterns of signaling molecules within the ventral diencephalon has revealed their presence for longer than previously thought, raising questions about a prolonged influence of the developing infundibulum on the pituitary (124).

Genetic evidence that signals from the neural ectoderm are crucial for pituitary morphogenesis initially came from the targeted disruption of the transcription factor *Nkx2.1* (also known as *T/ebp*, *Ttf1*, *Titf1*). This results in the loss of the ventral forebrain and the complete absence of Rathke's pouch, although *Nkx2.1* is not expressed in the latter. This confirmed a role for the ventral diencephalon in the maintenance and survival of the pouch (125). It does not provide the only influence, however, because the surrounding mesenchyme is also involved (126) (see also *Sections IV.A and IV.B*).

A. Bone morphogenetic proteins and fibroblast growth factors: synergy and antagonism

1. Bmp4

Bone morphogenetic proteins (BMPs) belong to a family of 20 secreted molecules, which bind to serine-threonine receptor kinases that in turn transduce an intracellular activation cascade. Some members of the BMP family are involved in multiple events during embryogenesis. BMP4 is the earliest signaling molecule known to be expressed in the prospective infundibulum, arising at 8.5 dpc, as Rathke's pouch is first visible; it is maintained there until 14.5 dpc (22, 124).

Complete deletion of *Bmp4* usually results in early embryonic lethality (127); however, histological analysis of a few mice that survived to 10 dpc failed to show any sign of pouch formation or even a thickened ectodermal placode (86). In contrast, in *Nkx2.1* null mutants where the *Bmp4* expression domain is initially present, a rudimentary pouch forms but fails to enlarge by cell proliferation (86). Moreover, ectopic expression of the BMP2/BMP4 antagonist *noggin* within the oral ectoderm and Rathke's pouch results in early arrest of pouch development at 10 dpc (110). Also deletion of the gene encoding for the BMP receptor, *Bmpr1a*, in Rathke's pouch at 9.5 dpc resulted in an underdeveloped structure at 10.5 dpc (early lethality prevented investigations at later stages) (124). In contrast, *noggin* null embryos showed expanded domains of *Bmp4* activity within the ventral diencephalon resulting in a range of Rathke's pouch phenotypes from an enlarged, rostrally displaced pouch to induction of a second pouch resulting in pituitary duplication (124). All these data implicate BMP4 as the earliest secreted molecule required in the ventral diencephalon for induction and maintenance of Rathke's pouch.

Several lines of evidence show that expression of *Isl1* in the developing anterior pituitary is regulated by BMP4 signaling from the ventral diencephalon. First, the timing of expression of *Isl1* throughout Rathke's pouch coincides with the maximum expression of *Bmp4* at 9.5 dpc, which declines by 11.5 dpc as *Isl1* becomes down-regulated. Additionally, ectopic expression of *Bmp4* within the pouch expands the *Isl1* expression domain within the anterior lobe (110), whereas noggin null mice show ectopic expression of *Isl1* in the mesenchyme adjacent to Rathke's pouch. Furthermore, loss of BMPR1A within Rathke's pouch prevents expression of *Isl1* (124). Therefore regulation of *Isl1* expression could, at least partially, be responsible for the maintenance and survival effects of BMP4 on Rathke's pouch progenitor population.

2. FGFs

Members of the FGF family activate receptor tyrosine kinases, and their extracellular association with heparan-sulfate proteoglycans is crucial for their activity in multiple processes during embryogenesis.

Three of the 23 members of this family (*Fgf8*, *Fgf10*, and *Fgf18*) begin to be expressed in the infundibulum by 9.5 dpc, 24 h later than the onset of *Bmp4* (22, 110, 123) Transcripts from *Fgfr2*, encoding an FGF receptor, have been detected in Rathke's pouch adjacent to the domain of *Fgf8* expression (86). This timing of expression corresponds to the ability of the infundibulum to inhibit differentiation dorsally, by down-regulating the expression of *Isl1* and thereby restricting it to ventral prospective thyrotropes, and also to maintain proliferative progenitors by inducing the expression of *Lhx3* and *Lhx4* in the pouch. Indeed, treatment of pouch explants by *Fgf8* induced *Lhx3* expression, whereas *Isl1* expression was restricted away from the source of the factor (22). At later stages (16.5 dpc), *Lhx3* expression appears to be independent of FGFs (124). In contrast, treatment with a specific FGF receptor antagonist, mimicking the loss of FGF signaling, induced down-regulation of *Lhx3* and ectopic differentiation of prospective thyrotropes ($Isl1^+$;αGsu^+) and corticotropes ($Acth^+$) in the dorsal region along with significantly reduced proliferation (128). Later, at around 11.5 dpc, FGF8 can restrict corticotrope differentiation to an intermediate zone of the pouch in between ventral thyrotropes and dorsal proliferative progenitors in contact with the factor source (22). Moreover, early ectopic expression of *Fgf8* under the control of the *Cga* (αGSU) promoter within Rathke's pouch results in severe dysmorphogenesis and enlargement of the pituitary, with an expansion of *Pomc*-expressing cells (corticotropes and melanotropes) and loss of the other cell lineages (110, 123). Finally, terminal differentiation requires exit from the cell cycle and FGF8 signaling down-regulation (22). Although homozygous disruption of *Fgf8* results in early embryonic lethality before gastrulation, precluding any study of its role in Rathke's pouch development (129), deletions of *Fgf10* or the gene encoding its receptor, *Fgfr2IIIb*, confirmed the proposed proliferative effect of FGFs on the pouch. They both result in a poorly formed Rathke's pouch with widespread apoptosis resulting in absence of the pituitary (130, 131). All these data show a proliferative role for FGFs from the ventral diencephalon onto Rathke's pouch after an initial induction by BMP4; however, an additional signaling source is also required for correct patterning of the pouch. Intriguingly, in humans, mutations of *FGF8* and the receptor *FGFR1* appear to be associated with Kallmann syndrome, resulting in isolated hypogonadotrophic hypogonadism (132–134).

3. Bmp2

In contrast with *Bmp4*, *Bmp2* is first expressed in the ventral mesenchyme adjacent to Rathke's pouch at 10.5 dpc along with *Bmp7*, but then gradually it is also found throughout the pouch itself, first ventrally then expanding dorsally, from 10.5 to 12.5 dpc (22, 124). *In vitro*, BMP2, -4, or -7 is able to mimic the influence of ventral mesenchyme on pouch explants by inducing *Isl1* expression and therefore favoring a ventral thyrotrope cell lineage (22). Therefore, because BMP4 regulates early expression of *Isl1* throughout the pouch, BMP2 may subsequently be responsible for its ventral maintenance. Maintenance of *Bmp2* expression under control of the *Cga* promoter resulted in a hyperplastic gland with strong expansion of the expression domain of the transcription factor *Gata2*, which is present in gonadotropes and thyrotropes but is also involved in determination of POU1F1 (PIT1) lineages (see below) (110). However, as observed with FGF8, down-regulation of BMP signaling is necessary for terminal differentiation (110). In agreement with these data, expression of a dominant-negative BMPR1A receptor (as deletion of the gene is lethal early in development) induced the formation of a hypoplastic gland where POU1F1 (PIT1) cell lineages are essentially absent and the gonadotrope population is reduced, whereas corticotropes and intermediate lobe melanotropes are present (110).

In vitro, BMP signaling inhibits *Pomc* transcription (135). Based on these results, it has been classically proposed that BMP2 (and BMP7 in ventral mesenchyme) may induce the proliferation and determination of ventral cell types at least partially through regulation of *Isl1* and *Gata2*, whereas at the same stages FGFs induce dorsal cell proliferation and restrict, along with BMPs, corticotrope determination to an intermediate domain (summarized in Fig. 2).

The field has therefore been stimulated by the notion, derived from a reasonable and consistent interpretation of data obtained a while ago, that organizing gradients across the developing pouch are crucial for spatial differentiation signals. However, the presence of a dorsal FGF source antagonizing a ventral BMP signal has not been formally demonstrated *in situ*, and whereas the expression pattern of BMP2 is indeed restricted ventrally at 10.5 dpc, it is present throughout the pouch at 12.5 dpc, which is not consistent with a gradient model (124). Therefore, the presence of several BMP inhibitors, including *chordin*, *noggin*, *NblI*, and *FstlI*, within the infundibulum, the pituitary, and surrounding mesenchyme, as well as receptor tyrosine kinase inhibitors of the Sprouty family in the pouch, may all shape and modulate the range of activities of BMP and FGF signaling in a rather more complex local fashion. Moreover, they may interact with migrating

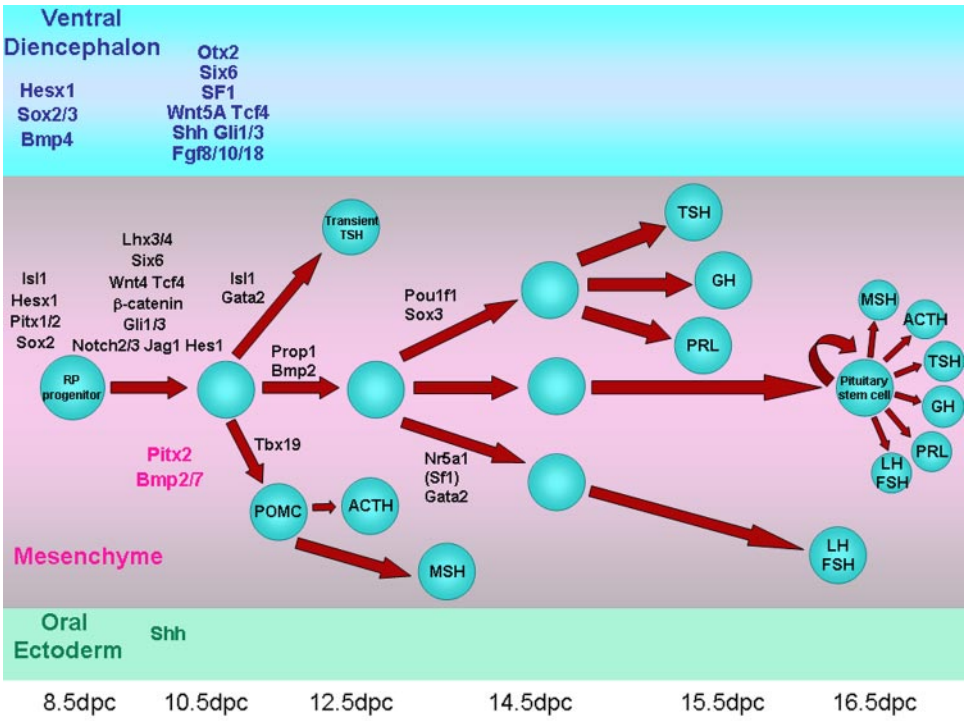

FIG 2. Schematic representation of the developmental cascade of genes implicated in human pituitary development with particular reference to pituitary cell differentiation.

streams of cells specified much earlier than previously thought, where the simple gradient model may not apply.

This suggests that simple gradients are not sufficient and that proper morphogenesis of the pouch depends on more interactions, including the program of cellular responsiveness interacting with concentration, and time of exposure of individual opposing extracellular factors. Ultimately, however, it is the downstream signal transduction pathways that integrate and converge these signals to a set of transcription factors that are critical for cell-fate specification, and the challenge will be to identify what these are, how they are controlled in temporal sequence (both on and off) and how they function to activate specific differentiation pathways (110, 124, 136).

B. Sonic Hedgehog signaling

There are three mammalian hedgehog proteins—Sonic Hedgehog (SHH), Indian Hedgehog (IHH), and Desert Hedgehog (DHH)—that can act as morphogens during development to elicit different cellular responses and induce different cell fates. Again, these are thought to act according to the distance from the source of hedgehog (affecting the "quantity" of signal), duration of this signal, and competence of the receiving cells. These pathways are also involved for different purposes in adults. Hedgehog ligands bind to and activate the transmembrane receptor Patched, whereupon the Smoothened coreceptor is released, which activates transcription factors of the GLI family that can either activate or repress transcription of target genes.

Sonic Hedgehog (*Shh*) is expressed in the ventral diencephalon, as well as throughout the oral ectoderm, from which expression becomes excluded specifically within Rathke's pouch as soon as it appears (110, 123). *Shh* expression is subsequently lost within the oral epithelium at 12 dpc and within the ventral diencephalon by 14 dpc. In contrast, the *patched* receptor is highly expressed in Rathke's pouch (123), and three members of the *Gli* gene family of transcription factors (*Gli1*, *Gli2*, and *Gli3*) are also expressed in the ventral diencephalon and within Rathke's pouch (137); therefore the developing gland is competent to receive and respond to SHH signaling.

The effect of complete loss of SHH on pituitary development in mice cannot be directly assessed because *Shh* null mice exhibit cyclopia and a generalized loss of midline structures of the brain, including the regions expressing *Nkx2.1* (138). By expressing an antagonist of hedgehog signaling, *Hip*, ectopically within the oral ectoderm and Rathke's pouch, Treier *et al.* (123) showed that pouch development was arrested in transgenic embryos. A rudimentary pouch was formed because BMP4 and FGF signaling from the ventral diencephalon were not disrupted; however, the pouch was severely hypoplastic. *Lhx3* expression was gradually lost, except from the cells in contact with the infundibulum, whereas *Bmp2*, *Gata2*, then subsequently *Pomc* and *Tsh*, were not expressed.

In a complementary experiment, overexpression of *Shh* under the control of *Cga* regulatory sequences led to upregulation and maintenance of *Bmp2* expression within the developing pituitary at 17.5 dpc, resulting in overexpansion of the thyrotrope and gonadotrope cell types (123). In contrast to a previous study where levels of induced BMP2 were much higher (110), terminal differentiation in the presence of BMP2 was observed (123). Additionally, *Lhx3* expression was slightly increased, and pituitary volume was dramatically enhanced. Explant experiments further showed that SHH along with FGF8 is involved in *Lhx3* induction and therefore progenitor proliferation (123). These observations suggest that *Shh* signaling in both the ventral diencephalon and the oral ectoderm is important for normal pituitary development, regulating progenitor proliferation probably in part through regulation of *Bmp2* and *Lhx3* expression. However, the spatial and temporal specificities of its action are unclear: how could SHH, which is present both dorsally and ventrally, favor a "ventral" fate in the pouch or set up a simple dorsoventral gradient signaling model? Also, how does it interact with other signaling pathways both in time and space?

Clearly, while some actors, described here, are now identified, their temporospatial roles and interactions remain to be determined.

C. WNT/β-catenin signaling

During pituitary development, WNT signaling pathways are required both within the pouch (see above) and in the ventral diencephalon. *Wnt5a* is the only member of this extensive gene family known to be expressed in the ventral diencephalon (110), and deletion of the gene induces in particular a misshaping of the pouch with the presence of extra bifurcations (139), similar to that observed in *Sox2* or *Sox3* mutant embryos (see below). This phenotype may be the consequence of a disruption of the sharp ventral boundary of FGFs and BMP4 expression domains in the infundibulum at 10.5 dpc, resulting in the recruitment of a wider region of the oral ectoderm contributing to Rathke's pouch with consequent multiple bifurcations within it (112).

A similar phenotype as seen in $Wnt5a^{-/-}$ mutants is also observed in *Tcf4* null mice (111, 112), and the abnormal bifurcations observed are similarly mirrored by an expansion of FGF and BMP expression patterns in the ventral diencephalon (112). However, TCF4 is present not only in the ventral diencephalon but also in Rathke's pouch itself, where it may control progenitor cell proliferation. Therefore, in the ventral diencephalon, the function of WNT signaling might be more to control the infundibular expression of FGFs and BMP4 to ensure correct proper shaping and/or extent of the pouch. It should also be borne in mind that the WNT signaling pathway can interact with that of Notch, so phenotypes may not solely be due to the effects of β-catenin on LEF/TCF-mediated transcription (106).

D. *Sox3* and *Sox2*

Sox3, along with *Sox2*, is expressed throughout the CNS with particularly high levels of expression noted in the ventral diencephalon, including the infundibulum and presumptive hypothalamus (140, 141). Deletion of *Sox3*, which is situated on the X chromosome, induces a variable phenotype mainly characterized by hypopituitarism and craniofacial defects (141–143). Rathke's pouch in $Sox3^{y/-}$ hemizygous embryos presents abnormal bifurcations that persist in the adult gland as extra clefts. The protein is not normally present in the pouch, but it is strongly expressed in the ventral diencephalon, suggesting that impaired signaling from the overlying mutant CNS could induce the formation of additional bifurcations. Expansion of the expression domains of BMP4 and FGF8 observed at 10.5 dpc could explain these. This expansion is probably due to an abnormal morphology of the infundibulum, which is less evaginated toward the pouch and therefore artificially expanded ventrally. Additionally, a reduction in cell proliferation was observed within this region (141).

In humans, both duplications and loss of function mutations in the gene are associated with variable hypopituitarism and variable learning deficits, often associated with abnormal midline structures such as hypoplasia/agenesis of the corpus callosum (see *Section VIA*).

SOX2 is also expressed in the ventral diencephalon, and additional bifurcations have also been observed in the pouches of $Sox2^{+/-}$ embryos, although at a lower frequency (103). Although we cannot rule out a direct effect of a reduction of SOX2 in the pouch (see above) to explain the additional bifurcations, it is possible that SOX2 is also involved in development of the ventral diencephalon. In agreement with this, deletion of one copy of *Sox2* on the $Sox3^{y/-}$ background results in a much more severe ventral forebrain phenotype (K. Rizzoti, unpublished data).

As observed in *Sox2* heterozygotes, the anterior lobe is hypoplastic in $Sox3^{y/-}$ animals, but the origin of this reduction is not known. By 15.5 dpc, expression of *Sox3* is initiated in the developing pituitary, in a small subset of *Pou1f1* (*Pit1*)-positive cells, and later in some lactotropes. This expression is maintained in the adult (K. Rizzoti and C. Galichet, unpublished data). However, the hypopituitarism phenotype in the $Sox3^{y/-}$ animals is unlikely to be a consequence of the deletion of the gene in the pituitary itself because the phenotype appears only after birth, as the hypothalamus starts to control pituitary secretion. This suggests that deletion of *Sox3* in the CNS may have been the cause of the hypopituitarism in the global mutants.

The similarity in the morphological abnormalities of Rathke's pouch between *Sox3* mutants and mutants involving the WNT pathway suggests their interplay during pituitary organogenesis. Studies in *Xenopus* have demonstrated that XSOX3, as well as XSOX17α and XSOX17β, are capable of interacting with β-catenin and repressing the activity of TCF-mediated signaling (144). Other members of the SOXB1 family have also been reported to suppress β-catenin-mediated TCF signaling (145). SOX2 has also been reported to inhibit WNT/β-catenin signaling in murine osteoblasts, where it represses the activity of a TCF responsive reporter, via an association of β-catenin with the C-terminal domain of SOX2 (146). These experimental models suggest that interactions between the SOX proteins and β-catenin and the consequent effects on β-catenin target genes may be important for pituitary development.

V. Factors Regulating Cellular Differentiation

The definitive Rathke's pouch, formed by 11.5 dpc, initially contains undifferentiated proliferative progenitors. Gradually, these will differentiate and give rise to the five endocrine cell types present in the anterior pituitary. Naturally occurring mutations, but also studies on the role of specific genes during development, have led to the characterization of several transcription factors involved during endocrine differentiation. Regulated expression of these, both in time and space, is required to obtain the full set of endocrine cells at birth. We will now discuss the best studied/most representative among these factors.

A. *Prop1*

PROP1 is, like HESX1, a member of the paired-like family of homeodomain transcription factors. It is expressed exclusively during pituitary development where, in contrast to HESX1, data suggest it is capable of functioning both as a transcriptional activator and repressor in a context-dependent manner (38, 147). *Prop1* expression is first apparent at 10 dpc within Rathke's pouch, at a stage when *Hesx1* is still present. Expression of *Prop1* peaks at 12 dpc throughout the pouch and is then markedly decreased, being maintained until 15.5 dpc only in the perilumenal area where progenitors are located (39, 147).

The Ames dwarf mouse (*df*) was found to harbor a naturally occurring mutation in the homeodomain of PROP1, lowering its DNA binding activity (39). In fact, mice homozygous for a targeted deletion of *Prop1* show a nearly identical phenotype to the Ames mutation, so it is more or less equivalent to a null allele (148). *df/df* mice are deficient in GH, prolactin (PRL), TSH, and gonadotropins and exhibit severe proportional dwarfism, hypothyroidism, and infertility (39, 149, 150). Moreover, no intermediate lobe differentiation marker is expressed, and vascularization of the developing gland is abnormal (151). Morphologically, the gland is clearly hypoplastic in adults, whereas at 14.5 dpc, it appears enlarged and dysmorphic. In fact, this is due to a failure of dorsal progenitors in the perilumenal area to relocate ventrally and differentiate (152). Normally, these progenitors express *Notch2* from 12.5 dpc (116), and ventral relocalization is accompanied by down-regulation of *CyclinD2* (cell cycle exit). However, in *Prop1* mutants, dorsal cells fail to express *Notch2*, and *CyclinD2* expression is down-regulated while cells are still in the perilumenal area (116, 149, 152). Ward *et al.* (152) suggest that down-regulation of NOTCH2 may be coupled with premature cell cycle exit and defects in localization, in parallel with the role of Notch in CNS precursors; however, another study failed to show this down-regulation and suggested instead that Notch signaling is essential for PROP1 maintenance (see above) (117). Finally, a late wave of apoptosis and a reduction in proliferation in *df/df* mice postnatally probably explain the subsequent pituitary hypoplasia (114, 151, 152).

The switch of expression from the repressor HESX1 to the activator PROP1 is an important step during development of the gland because it is required for emergence of both the POU1F1 (PIT1; GH, PRL, and TSH) and gonadotrope lineages (38). It has been known for some time that PROP1 is directly involved in the activation of *Pou1f1* (*Pit1*) expression (149, 153), but more recently, Olson *et al.* (107) showed that *in vitro* PROP1 and β-catenin form a complex, along with other cofactors. Genetic approaches combined with chromatin immunoprecipitation then suggest that these complexes directly repress *Hesx1* while activating *Pou1f1* (*Pit1*) expression (107). This represents a significant progression during pituitary development from progenitor proliferation and maintenance (as a result of the repressive actions of HESX1) to cell determination [activation of *Pou1f1 (Pit1)*] (107). Finally, overexpression of *Prop1* under *Cga* promoter control results in a delay in *Fsh*β expression leading to delayed puberty and also an increased risk of pituitary adenomas. This highlights the importance of correct temporal regulation of *Prop1* expression (154, 155).

The gonadotropin deficiency observed in the Ames dwarf (df) remains unexplained. In elegant studies that unraveled the phenotype of the Ames dwarf, Bartke (156) showed that male mice produced spermatozoa that were motile, histologically normal, and capable of fertilizing ova. However, most remained sterile although a few untreated males were fertile and sired litters. Administration of T_4 or GH singly or in combination resulted in fertility

of most males (157). Similar treatment regimens resulted in sexual maturation in females, although fertility did not ensue, possibly due to the absence of LH. Hence, the impaired fertility in Ames mice could be explained/exacerbated by concomitant untreated GHD and hypothyroidism, although these interactions are complex because thyroid hormone deficiency worsens GH deficiency, and replacement of both is necessary to restore growth and metabolism and for achieving functional sexual maturation. Interestingly, the administration of T_4 to $\alpha GSU^{-/-}$ mice led to gonadotrope hypertrophy, which had previously been lacking in these hypogonadal mice. Appropriate thyroid function and timing may be required to establish the sensitivity of gonadotropes to feedback regulation by gonadal steroids (158).

In humans, *PROP1* mutations are the most common cause of CPHD, including GH, TSH, gonadotropin, and evolving ACTH deficiencies. These are variable in onset and phenotype and may be associated with a pituitary mass that spontaneously involutes to result in a hypoplastic anterior pituitary (see *Section VI.B*).

B. *Pou1f1*

POU1F1 (previously termed PIT1) is a member of the POU homeodomain family of transcription factors characterized by two highly conserved protein domains, a POU-specific domain and a POU-DNA binding homeodomain. *Pou1f1* (*Pit1*) is expressed relatively late during pituitary development, becoming detectable from 13.5 dpc in prospective somatotrope, lactotrope, and thyrotrope cells (159). It reaches maximum expression in differentiating GH, PRL, and TSH cells by 16 dpc, persisting in these into adulthood (20, 159). It is required for the production of GH, PRL, and TSHβ, respectively, as well as for the expression of *Ghrhr* (160, 161). Recently, analysis of *Pouf1* (*Pit1*) regulatory regions has shown that its early activation involves different enhancers, one of them bound by the giant zinc finger protein ATBF1 (162).

Two naturally occurring recessive mouse mutants initiated the dissection of POU1F1 (PIT1) function. First, the Snell dwarf (*dw*) mouse harbors a point mutation within the POU homeodomain (p.W261C) affecting DNA binding. Second, the Jackson dwarf (*dwJ*) mouse fails to express *Pou1f1 (Pit1)* as a consequence of a chromosomal rearrangement (163). Both strains exhibit an identical phenotype comprising postnatal, but not embryonic, anterior pituitary hypoplasia and GH, TSH, and PRL deficiencies (163). In *dw/dw* mice, expression of *Pou1f1* is normal until 18.5 dpc, at which time it is significantly reduced, eventually becoming extinguished postnatally. The presence of POU1F1 binding sites within its own regulatory sequences suggests that *Pou1f1* (*Pit1*) can regulate itself (164, 165). The presence of normal GH, TSH, and PRL populations in the embryo shows that POU1F1 (PIT1) is dispensable for their initial emergence but required for their postnatal expansion; indeed, in $Pou1f1^{-/-}$ ($Pit1^{-/-}$) pups, it is a reduction in proliferation along with some apoptosis that leads to hypoplasia (151). In postnatal endocrine cells, expansion is known to be under hypothalamic control, but there are also other signals involved locally in the gland. However, the basis of the $Pou1f1^{-/-}$ ($Pit1^{-/-}$) postnatal phenotype is still unknown.

It has been shown that POU1F1 (PIT1) is able to inhibit the transcription factor GATA2 (see below) independently of its DNA binding properties to prevent gonadotrope fate, whereas in thyrotropes, the two act in synergy to promote the thyrotrope fate (166). This may explain how overexpression of *Prop1*, which activates POU1F1 (PIT1), delays gonadotrope differentiation (see above). In somatotropes, the POU1F1 (PIT1) target gene *Math3*, encoding a bHLH transcription factor, allows terminal differentiation and is repressed by Notch signaling, probably preventing premature differentiation (115). POU1F1 (PIT1) function is therefore more than "promoting its own lineage"; it impacts on the regulation of other cell types ensuring that the proper balance of endocrine cell types is achieved.

POU1F1 (PIT1) is reported to regulate its target genes by binding to response elements of their promoter regions and recruiting coactivator proteins, such as the cAMP response element-binding protein-binding protein (167) and other transcription factors like LHX3 to the transcriptional complex (168).

In humans, mutations in *POU1F1* are associated with GH, PRL, and variable TSH deficiency, with a small anterior pituitary gland identified in the majority of cases (see *Section VI.B*).

C. *Gata2*

GATA2 belongs to a family of six transcription factors characterized by the presence of at least one N-terminal transactivation domain and a zinc finger DNA binding domain. It has dual functions as a stem cell maintenance factor in some tissues (169), but also promotes differentiation in others (for review, see Ref. 170). *Gata2* starts to be expressed at 10.5 dpc in the ventral Rathke's pouch, where it is induced by BMP2 (166) along with α-GSU, the common subunit of LH, FSH, and TSH. It therefore marks prospective, then definitive, gonadotropes and thyrotropes; its expression is maintained in the adult (166). GATA2 has been shown *in vitro* to activate the *Cga* promoter (171) and also to induce *Tsh*β expression in synergy with POU1F1 (PIT1) (166, 172).

Deletion of *Gata2* results in embryonic lethality at 10.5 dpc because of yolk sac hematopoiesis defects (173).

Therefore, to study its function, a dominant-negative form of the molecule was expressed under control of *Cga* regulatory elements. As a consequence, gonadotropes were lost and thyrotropes reduced, whereas the *Pou1f1* (*Pit1*) expression domain was expanded (166). In contrast, ectopic expression of *Gata2* under control of the *Pou1f1* (*Pit1*) promoter resulted in dorsal expansion of the gonadotrope population to the detriment of *Pou1f1* (*Pit1*)-dependent lineages (166). Therefore, GATA2 may promote and be required for specification of the gonadotrope and thyrotrope lineages, respectively, in opposition and in synergy with POU1F1 (PIT1) (166).

Further evidence of the function of GATA2 in promoting gonadotrope and thyrotrope fate has recently been provided by a specific inactivation of the protein in αGSU-positive cells. In these mutants, where more than 90% of the αGSU-positive cells inactivate GATA2, the TSH and LH population is reduced in neonates, but only transiently. However, in adult animals, levels of circulating TSH and FSH stay low, and the function of gonadotropes and thyrotropes is abnormal. The transient nature of the reduction in endocrine populations could be due to the observed up-regulation of the closely related GATA3 (174). The differences in phenotypes observed between expression of a dominant negative and the deletion of the gene may be explained by the wider range of action of the dominant negative, which may also inhibit other members of the family such as GATA3, and possible residual GATA2 function in the conditionally ablated mice (175). However, both studies clearly highlight the requirement for GATA2 in the differentiation of gonadotropes and thyrotropes and also for the function of these cells in the postnatal gland.

D. *Nr5a1*

Nuclear receptor 5a1, encoded by the gene *Nr5a1* (also named steroidogenic factor-1, SF1) is an orphan nuclear receptor involved as a transactivating factor in steroid hormone biosynthesis. Phospholipid binding has been shown to increase its transactivation activity, although it is uncertain whether these should be considered as conventional ligands (for review, see Ref. 176). NR5A1 (SF1) is expressed throughout the adrenal and reproductive axes during development and postnatal life, regulating several genes involved in sex determination, reproduction, and steroidogenesis, including in the hypothalamus and pituitary those encoding the GnRH receptor, LH, FSH, and αGSU (61, 177–179). In the developing pituitary, GATA2 is capable of inducing *Nr5a1* (*Sf1*) expression in the developing gonadotropes, with initial onset of expression at 13.5 dpc; therefore, after the initiation of αGSU expression but before that of LHβ and FSHβ (166, 180). It has been shown *in vitro* that interaction of NR5A1 (SF1) with the transcription factors EGR1 and PITX1 (61), and also more recently with β-catenin (181), is involved in activation of *Lhβ*.

Nr5a1 (*Sf1*) null mutant mice exhibit adrenal and gonadal agenesis, male-to-female sex reversal, ablation of the ventromedial hypothalamic nucleus, and selective loss of gonadotropin, αGSU, and *Gnrhr* expression (180, 182–185). However, exogenous GnRH treatment at very high doses in *Nr5a1* (*Sf1*) null mice is capable of inducing gonadotropin expression, demonstrating that gonadotropes are present and can respond to stimulation (185). Mice with a conditional deletion of *Nr5a1* (*Sf1*) specifically within the pituitary (as a result of conditional ablation in αGSU expressing cells) also have gonadal hypoplasia with a dramatic decrease in pituitary gonadotropin expression, and fail to develop normal secondary sexual characteristics, whereas the adrenal glands and hypothalamus are unaffected. Also in these mice, supraphysiological doses of GnRH can result in LHβ expression, suggesting that in the absence of NR5A1 a cofactor, possibly EGR1, can alone activate *Lhβ* (186). These data show that pituitary expression of *Nr5a1* (*Sf1*) is necessary for gonadotrope maturation, representing one of the functions of this factor in reproductive axis development.

Mutations of *NR5A1* in humans are associated with 46XY sex reversal with adrenal failure, 46XY gonadal dysgenesis, and 46XX ovarian insufficiency and premature ovarian failure (187, 188). The detailed description of the role of this gene in human disease is beyond the scope of this review.

E. *Tbx19*

TBX19 (previously referred to as TPIT) is a member of the T-Box family of transcription factors comprising 17 members in mouse. The T-box is the DNA binding domain of these factors, and some of them also have a transactivation domain. In the case of TBX19 (TPIT), transcriptional activation requires association with the coactivators sarcoma virus/p160, but it can also interact with other partners (for review, see Ref. 189). *Tbx19* (*Tpit*) is exclusively expressed in the developing pituitary, first at 12.5 dpc in POMC-positive cells, then in corticotropes and melanotropes, where it is maintained in the adult gland (23). It is capable of directly activating *Pomc* expression in association with PITX1 (23).

Tbx19 (*Tpit*) deletion in mice induces severe ACTH and glucocorticoid deficiencies, in addition to adrenal hypoplasia and pigmentation defects. In the pituitary of these mice there is very little POMC expression, and the intermediate lobe is hypoplastic. In the embryo, the transient expression of *NeuroD1* in precorticotropes is not affected, and premelanotropes are also present. Therefore both cell lineages are initially specified in normal numbers. This

suggests that TBX19 (TPIT) is required for the maturation and maintenance of both populations (5). Interestingly, the hypoplastic intermediate lobe of *Tbx19*$^{-/-}$ (*Tpit*$^{-/-}$) embryos contains both gonadotropes and POU1F1-independent thyrotropes (therefore similar to those arising from the early transient populations), suggesting that TBX19 (TPIT) may normally inhibit acquisition of these fates. Indeed, when it is overexpressed, the number of αGSU-positive cells in mice is reduced. *Tshβ* expression is normal, but the population of gonadotropin-expressing cells is greatly reduced. Different functions of TBX19 (TPIT) may underline its ability to repress gonadotrope fate. First, it is able to directly repress *Cga* (αGSU) expression *in vitro*; and second, there is a mutual antagonism between TBX19 (TPIT) and NR5A1 (SF1), independent of DNA binding activity (5). Therefore, TBX19 (TPIT) promotes and is required for corticotrope and melanotrope fate while actively repressing gonadotrope identity.

Mutations in *TBX19* (*TPIT*) are the commonest cause of isolated ACTH deficiency presenting in the neonatal period in humans (see *Section VI.C*).

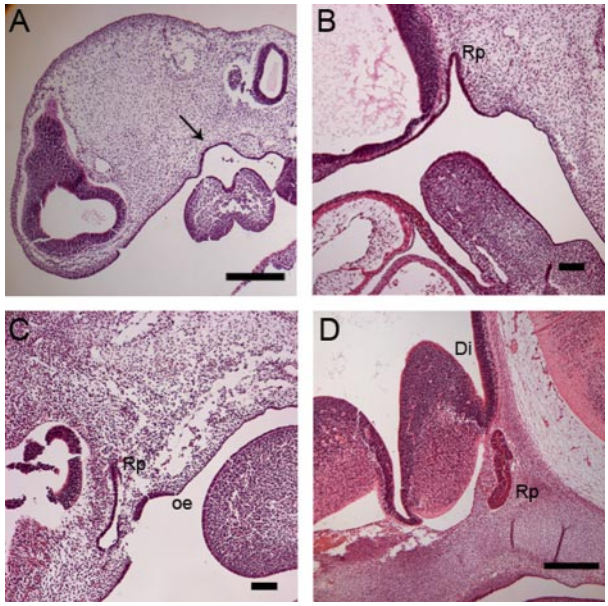

FIG 3. Midline sagittal hematoxylin and eosin-stained sections showing pituitary organogenesis during human embryonic development. A, Midline sagittal section of a Carnegie stage (CS) 13 embryo (approximately 5 wk of development) showing the invagination of the oral ectoderm to form Rathke's pouch (*arrow*). B, Sagittal section of CS14 embryo showing the developing Rathke's pouch (Rp) coming into contact with the overlying neuroectoderm. C, Sagittal section of CS15 embryo showing the definitive Rathke's pouch becoming separated from the oral ectoderm (oe). D, Definitive Rathke's pouch (Rp) shown in sagittal section fully separated from the oral ectoderm maintaining contact with the neural ectoderm of the diencephalon (Di) at CS17. Scale bars: A and D, 300 μm; B and C, 100 μm. [Images were kindly provided courtesy of D. Gerrelli, Medical Research Council-Wellcome Trust Developmental Biology Resource, University College London Institute of Child Health, London, United Kingdom.]

VI. Disorders of Pituitary Development in Humans

In this section, we discuss pituitary development in humans. It is clear that useful knowledge has been gained from comparing genotype/phenotype analyses in mice and humans. In general, there are great similarities (Fig. 3 and Table 6), although there are notable exceptions, so one should remain cautious in extrapolating detailed mouse phenotypes to humans. In contrast to the mouse, there are obvious limitations in what can be directly investigated in human pituitary development. Human studies are also prone to ascertainment or referral bias, and often cases are too rare to generalize with confidence about phenotypic prevalence. What is less often appreciated is that many mouse studies are light on phenotypic detail, particularly postnatally, which hampers comparison with most human data, and that strain background effects in mice can also have major effects on phenotypic penetrance.

Congenital hypopituitarism encompasses a group of different etiological disorders. It may manifest as isolated deficiency of a single pituitary hormone; for example IGHD, ACTH deficiency, gonadotropin deficiency (hypogonadotropic hypogonadism), TSH deficiency, or central diabetes insipidus. Alternatively, several pituitary hormone axes may be defective, resulting in a CPHD that may also be associated with extrapituitary defects such as optic nerve hypoplasia or midline forebrain abnormalities. Clinical features of hypopituitarism are often variable and may occur early in the neonatal period or later with growth failure or abnormal pubertal development. IGHD is by far the most common endocrinopathy and may present with growth failure with an incidence ranging from 1 in 3,500 to 1 in 10,000 births (190–194). At the more severe end of the spectrum, SOD is a rare congenital anomaly with a prevalence of approximately 1 in 10,000 (195). It is a genetically and phenotypically heterogeneous disorder characterized by a clinical triad of midline forebrain abnormalities, optic nerve hypoplasia, and hypopituitarism. Each of these components can occur in isolation or in combination. The majority of cases of hypopituitarism are idiopathic in origin; however, familial inheritance, which may be either dominant or recessive, accounts for between 5 and 30% of all cases (196). The etiology of many cases still remains unknown and is likely to involve a combination of both genetic and environmental factors. Both genetic and environmental factors have been implicated in the etiology of SOD, for example (195, 197). Environmental agents such as viral infections, vascular or degenerative changes, and exposure to alcohol or drugs have been implicated in the

etiology of SOD. The condition presents more commonly in children born to younger mothers, and it clusters in geographical areas with a high frequency of teenage pregnancies (195, 198, 199).

Several genes causing abnormal pituitary function when mutated in the mouse have also been implicated in human pituitary development by the identification of mutations in their human orthologs in patients with various hypopituitary phenotypes. These can be broadly categorized into three groups: 1) mutations in genes involved in early development and patterning of the forebrain and pituitary; these tend to result in syndromic forms of hypopituitarism in association with extrapituitary defects affecting other tissues where they are expressed, most often the eyes, optic nerves, or midline forebrain structures; 2) genes that are involved at initial stages of pituitary cell differentiation often resulting in CPHD; and 3) mutations in genes encoding specific hormone subunits or required for specification of particular cell types giving rise to isolated pituitary hormone deficiencies. This review will focus on the early developmental genes as well as those involved in cellular differentiation.

A. Syndromic hypopituitarism: early developmental genes

1. HESX1

The variable phenotype of midline forebrain defects, ocular abnormalities, and pituitary dysplasia observed in *Hesx1* null mice is highly similar to that observed in human SOD. Consistent with this observation, five homozygous and eight heterozygous mutations have been identified in *HESX1* (OMIM 601802) in a small proportion of hypopituitary patients, although these have highly variable phenotypes and no obvious phenotype-genotype correlation. Dattani *et al.* (46) reported a homozygous mutation at a highly conserved arginine residue of the homeodomain (p.R160C) resulting in loss of DNA binding of the mutant protein, which was identified in two siblings (born to consanguineous parents) who manifested a severe SOD phenotype with panhypopituitarism. MRI revealed anterior pituitary hypoplasia, an ectopic or undescended posterior lobe, agenesis of the corpus callosum with an absent septum pellucidum, and optic nerve hypoplasia with a small optic chiasm (Fig. 4B) (46, 197, 200). A second homozygous mutation was identified in a girl presenting with GH and gonadotropin deficiency, subsequently evolving to deficiencies of ACTH and TSH. She had hypoplasia of the anterior pituitary and an undescended posterior pituitary, but with normal optic nerves and no midline forebrain defects. This mutation, a threonine/isoleucine substitution at residue 26 (p.I26T), lies in a highly conserved engrailed homology domain in the amino-terminal part of the protein that is crucial for transcriptional repression. The mutation was shown to result in partial loss of repressor function *in vitro*, in part due to impaired interaction with the TLE1 corepressor (48). The milder phenotype associated with the latter mutation was supported by recent data in the mouse (47).

Two siblings from a third consanguineous family were found to be recessive for an Alu-element insertion in exon 3 of *HESX1*, which contains the homeobox (201). Affected individuals homozygous for the mutation had aplasia of the anterior pituitary and undetectable anterior pituitary hormone levels, although the posterior pituitary and infundibulum were normal. One sibling had unilateral blindness as a result of coloboma of the right eye, whereas the other had no ophthalmic abnormalities but displayed a left-sided diaphragmatic hernia and aortic coarctation and died shortly after birth. Two additional patients with recessive *HESX1* mutations and anterior pituitary aplasia in the absence of a posterior pituitary or optic nerve malformation have also recently been reported. Sequencing *HESX1* in these unrelated individuals revealed that one was homozygous for a 2-bp deletion (c.449_450delAC) resulting in a frameshift, whereas the other was homozygous for a mutation in the splice donor site in intron 2 (c.357 + 2T>C). Both mutations would be predicted to disrupt the homeodomain and constitute null alleles (202).

To gain further insights into the molecular basis of impaired HESX1 function by the p.R160C and p.I26T mutations, Sajedi *et al.* (47) recently generated mouse mutants carrying these alleles. Mice homozygous for the p.R160C mutation displayed pituitary and forebrain defects identical to those observed in *Hesx1* null embryos, whereas those homozygous for the p.I26T allele showed pituitary defects and ocular abnormalities comparable to those of *Hesx1* null mice, but no defects in the telencephalon, suggesting that the p.I26T mutation yields a hypomorphic allele, whereas p.R160C effectively produces a null allele; this could explain its more severe phenotype in both mice and humans. The fact that the p.I26T mutant protein retains DNA binding but has impaired transcriptional repressor function due to its inability to interact with TLE1 suggests that this particular protein-protein interaction is absolutely required for normal pituitary and eye development. The p.R160C substitution yields a protein with no DNA binding properties but is able to repress transcription, indicating the necessity of HESX1:DNA interactions for normal HESX1 function during development. The expression pattern of *HESX1* in human embryos parallels that of other vertebrates, suggesting a conserved evolutionary role in forebrain, eye, and pituitary development (47). Additionally, these data as well as

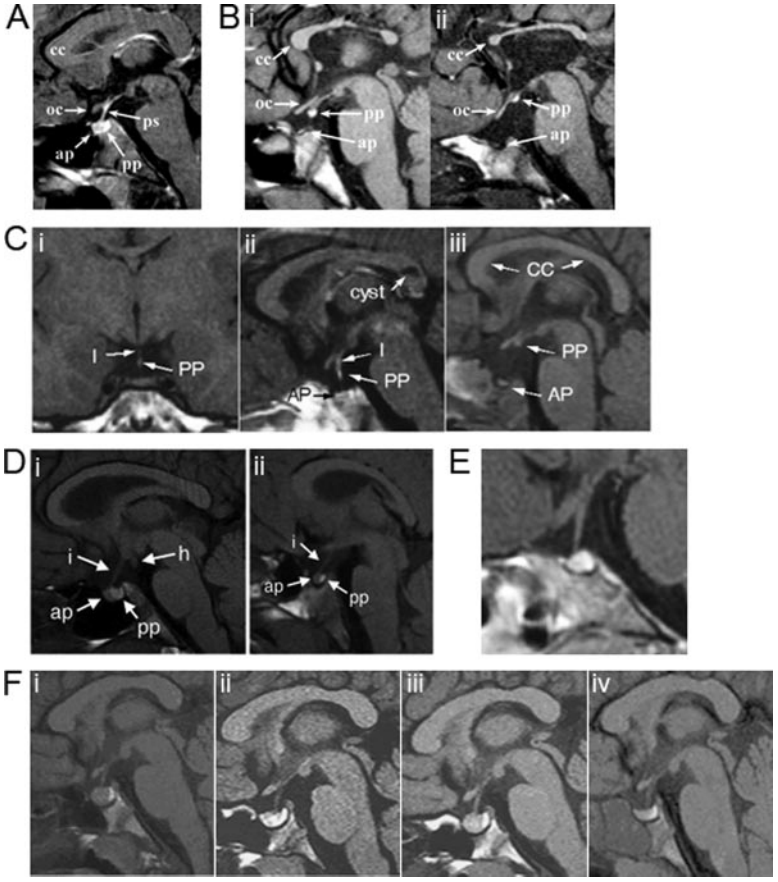

FIG 4. A, Midsagittal MRI scan of the head of a normal child. Note the well-formed corpus callosum (CC), the optic chiasm (OC), and the posterior pituitary (PP), which appears as a bright spot within the sella turcica. B, Sagittal MRI scan of two siblings with a homozygous p.R160C mutation in *HESX1*. In the first sibling (i) the splenium of the corpus callosum is more hypoplastic than the rest of the structure and the posterior pituitary is partially descended as compared with the other sibling (ii) who has a severely hypoplastic corpus callosum, ectopic posterior pituitary, and lack of visible pituitary stalk (PS). C, Coronal and sagittal MRI scans from one patient [panels (i) and (ii)] and sagittal scan from a second patient (iii) with *SOX3* duplication showing anterior pituitary (AP) hypoplasia, partial hypoplasia of the infundibulum (I) in the first patient, which is completely absent in the second, and an ectopic posterior pituitary which is more severe in patient 2. D, MRI scan from patients with *SOX2* mutations. Sagittal section from patient with c60insG mutation showing anterior pituitary (ap) hypoplasia with normal posterior pituitary (pp) and infundibulum (i) and a hypothalamic hamartoma (h). E, Sagittal MRI scan in patient with compound heterozygosity for p.E230K and p.R172Q mutations in *POU1F1*, showing hypoplasia of the anterior pituitary gland with a normal posterior pituitary and infundibulum. F, Sequential MRI scanning of a patient with a 13-bp deletion (c.112_124del13) in *PROP1* reveals waxing and waning of a pituitary mass (*arrow*); (i) on initial presentation, (ii) after 4 months, (iii) after 12 months, and (iv) 21 months after initial MRI. [Panels A and B were derived from Brickman *et al.* (200); panel C was derived from Woods *et al.* (220) and reproduced with permission from Elsevier (University of Chicago Press); panel D was derived from Kelberman, *et al.* (103) and reproduced with permission from the American Society for Clinical Investigation. Panels E (copyright 2005, The Endocrine Society) and F, derived from Turton *et al.* (Refs. 274 and 250, respectively), were reproduced with permission.]

those of Andoniadou *et al.* (45) support the hypothesis that the pituitary gland is most sensitive to reductions in HESX1 dosage, followed by the eyes and then the forebrain. This would appear to be the case in both humans and mice (45, 47). Eight additional heterozygous mutations of *HESX1* have been identified in individuals with various degrees of hypopituitarism and SOD (Table 1). In general, these heterozygous *HESX1* mutations are associated with milder phenotypes, and haplotype analysis in familial cases has revealed that such mutations are associated with incomplete penetrance, with some affected individuals inheriting the mutation from an apparently unaffected parent. This raises the possibility that such heterozygous mutations are not acting independently to cause disease and that affected individuals may harbor additional mutations in *HESX1* (*e.g.*, promoter/enhancer regions) or in other gene products interacting with HESX1.

We have screened approximately 850 patients for mutations in *HESX1* including more than 300 with SOD; 410 with isolated pituitary dysfunction, optic nerve hypoplasia, or midline neurological abnormalities; and 126 patients with familial inheritance of the condition. The overall incidence of coding region mutations in *HESX1* within this cohort is approximately 1%, showing that mutations in *HESX1* are a rare cause of hypopituitarism and SOD (199). It is possible that homozygous mutations in *HESX1* result in early embryonic lethality and that the few patients identified with *HESX1* mutations represent the minority of surviving individuals. If so, it follows that there is a greater likelihood of identifying mutations in genes that are not associated with other major embryonic defects in surviving patients with CPHD.

2. PITX2

Mutations in *PITX2* in humans are associated with Axenfeld Rieger syndrome (OMIM 601542), a genetically and phenotypically heterogeneous disorder characterized by malformation of the anterior segment of the eye, dental hypoplasia, a protuberant umbilicus, and brain abnormalities (72). Reduced GH concentrations and a small sella turcica, probably reflecting pituitary hypoplasia, have been noted in some patients (203), suggesting a role for PITX2 in pituitary development and hypopituitarism in humans, but its importance and prevalence remains unclear.

3. SOX2

Heterozygous mutations within *SOX2* in humans have been associated with bilateral anophthalmia or severe microphthalmia. Additionally, the phenotype of hypopitu-

TABLE 1. Mutations identified in the *HESX1* gene in patients with SOD and hypopituitarism

Mutation	Inheritance	Endocrine phenotype	Neuroradiological findings	Ref.
p.Q6H (2 reports)	Dominant	GH, TSH, LH, FSH deficiency; GH deficiency, evolving TSH, ACTH deficiency	AP hypoplasia, ectopic PP	49, 295
p.I26T	Recessive	GH, LH, FSH deficiency; evolving ACTH, TSH deficiency	AP hypoplasia, ectopic PP, normal ON	48
c.306_307insAG	Dominant	GH, LH, FSH deficiency; hypothyroidism	AP hypoplasia, ON hypoplasia	296
p.Q117P	Dominant	GH, TSH, ACTH, LH, FSH deficiency	AP hypoplasia, ectopic PP	297
c.357 + 2T>C	Recessive	GH, TSH, ACTH, PRL deficiency	AP aplasia, normal PP, normal ON	202
Alu insertion (exon 3)	Recessive	Panhypopituitarism	AP aplasia, hypoplastic sella, normal PP and infundibulum	201
p.E149K	Dominant	GH deficiency	AP hypoplasia, ectopic PP, infundibular hypoplasia	199
c.449_450delCA	Recessive	GH, TSH, ACTH deficiency	AP aplasia, normal PP, normal ON, thin CC, hydrocephalus	202
p.R160C	Recessive	GH, TSH, ACTH, LH, FSH deficiency	AP hypoplasia, ectopic PP, ON hypoplasia, ACC	46
p.S170L	Dominant	GH deficiency	Normal AP, ON hypoplasia, ectopic PP, partial ACC	49
p.K176T	Dominant	GH deficiency, evolving ACTH, TSH deficiency	Ectopic PP	297
g.1684delG	Dominant	GH deficiency	AP hypoplasia, ON hypoplasia, ACC, absent PP bright spot	298
p.T181A	Dominant	GH deficiency	AP hypoplasia, normal ON, absent PP bright spot	49

AP, Anterior pituitary; PP, posterior pituitary; ON, optic nerve; (A)CC, (agenesis of the) corpus callosum.

itarism characterized by anterior pituitary hypoplasia and gonadotropin deficiency (hypogonadotropic hypogonadism) with genital abnormalities in males is present in all cases where neuroimaging and endocrine investigations have been performed to date (103, 104, 204, 205). Forebrain defects reported in some patients include hypoplasia of the corpus callosum, hypothalamic hamartoma, and hippocampal malformation (Fig. 4D) (103, 206), associated with additional abnormalities including esophageal atresia, sensorineural hearing loss, and learning difficulties. To date, 22 *de novo* intragenic heterozygous mutations have been identified in 27 patients, including eight nonsense, 10 frameshift, and three missense mutations (103, 204, 205, 207–212). Additionally, seven *de novo* heterozygous deletions of the entire gene and one case of a partial 3′ deletion have been reported (204, 205, 212, 213), as well as three heterozygous nonsynonymous changes identified in individuals who inherited the variant from a clinically unaffected parent (103, 214). *SOX2* genetic variation is catalogued online (see http://lsdb.hgu.mrc.ac.uk/home.php?select_db=SOX2). GHD has been described in some patients (103), and many patients with *SOX2* mutations manifest hypogonadotropic hypogonadism (103, 104, 210), which has obvious implications for their clinical management. Continued endocrine follow-up of individuals with *SOX2* mutations and timely diagnosis and treatment of GH and sex steroid deficiencies would help prevent their associated long-term morbidities.

It is of interest to note that *SOX2* expression is not uniform in the developing hypothalamus in humans (104), suggesting that haploinsufficiency for *SOX2* may affect only certain populations of glia, neuroendocrine neurons, their progenitors, or their afferent inputs. The report of a patient with isolated hypogonadotropic hypogonadism without pituitary hypoplasia on imaging (210) suggests that *SOX2* might be involved independently at multiple levels during the development of the hypothalamo-pituitary-gonadal axis, including the neural processes necessary for establishing or maintaining the population of GnRH neurons in the hypothalamus, their migration, and/or formation of an appropriate terminal field in the median eminence. These could be additional to the more direct involvement of SOX2 in Rathke's pouch morphogenesis. Because mice with haploinsufficiency of *Sox2* show more generalized pituitary deficits, the apparent selectivity for *SOX2* mutations impairing primarily the gonadotrope axis in human subjects is intriguing and unexplained, and it is a reminder that phenotypic details differ significantly between human and mouse.

SOX2 shows overlapping domains of expression with *LHX3* and *HESX1* within Rathke's pouch and the developing anterior lobe during human embryonic development (47, 104, 215, 216). Moreover, SOX2 can bind to sequences within the proximal promoters of both genes and activate transcription *in vitro*, suggesting that it might be a regulator of *LHX3* and/or *HESX1* during pituitary development. SOX2 is also capable of repressing β-catenin mediated transcriptional activation *in vitro*. Mutations that result in a truncated protein lose this inhibitory activity, which is mediated by the carboxyl-terminal do-

main and is independent of either the HMG domain or the ability to bind DNA (104, 146). Disruption of interactions with β-catenin and the consequent effects on regulation of β-catenin target genes may also be one mechanism by which loss-of-function mutations in *SOX2* can result in abnormal pituitary morphogenesis.

4. SOX3

A number of pedigrees have been described with X-linked hypopituitarism and mental retardation, mapping involving duplications of Xq26–27 encompassing *SOX3* (OMIM 313430) (217–219), with the smallest described duplication to date being approximately 690 Kb (220). Of the three annotated genes in this interval, only *Sox3* was expressed in the murine infundibulum. The phenotypes of affected males with X-linked hypopituitarism involving duplications within this region are variable. All affected males manifest GH deficiency associated with anterior pituitary and infundibular hypoplasia with an undescended posterior pituitary and abnormalities of the corpus callosum (218). Some individuals are also deficient in ACTH, TSH, or gonadotropins, and panhypopituitarism has been documented (219), with varying degrees of developmental delay or mental retardation.

Further implication of *SOX3* in X-linked hypopituitarism came from the identification of patients harboring an expansion of one of the polyalanine tracts within the gene (220, 221). Laumonnier *et al.* (221) identified an in-frame duplication of 33 bp occurring between nucleotides 711 to 743 and cosegregating in affected males in a large family with X-linked mental retardation and GH deficiency. This duplication encodes an additional 11 alanine residues, predicts an expansion of this polyalanine tract from 15 to 26 residues, and was associated with a phenotype of short stature, IGHD, and mental retardation, with facial anomalies in some, but not all, patients. A second expansion of seven alanine residues within the same tract has been identified in three siblings of a consanguineous pedigree presenting with profound and complete panhypopituitarism in association with anterior pituitary hypoplasia, an absent or hypoplastic infundibulum, and an ectopic/undescended posterior pituitary (Fig. 4C) (220). Interestingly, there was no evidence of mental retardation or craniofacial dysmorphism in these individuals. A deletion resulting in contraction of the same polyalanine repeat by nine residues has also been reported in two brothers with mental retardation; however, the significance of this finding remains unknown because functional studies have not been performed and the deletion was also present in the maternal grandfather of the patients who was clinically unaffected (221).

Consistent with the presence of the *SOX3* locus on the X chromosome, all patients described to date are male, with female carriers appearing clinically unaffected. However, no mutations involving *SOX3* have been found in patients with sex reversal, gonadal dysgenesis, or infertility (222, 223). It is striking that patients presenting with duplications involving *SOX3*, or loss-of-function polyalanine tract expansion mutations, show essentially similar phenotypes, comprised of infundibular hypoplasia and variable hypopituitarism. This suggests that the dosage of *SOX3* is quite critical for normal hypothalamopituitary development. Given the observation that *Sox3* is not normally expressed in Rathke's pouch in the mouse, our current view is that morphological defects of the anterior pituitary might be secondary to disruption of infundibular development and signals thereof.

5. LHX3

Eight homozygous mutations in *LHX3* (OMIM 600577) have been identified in 13 patients from eight unrelated consanguineous families, in addition to a single patient who was found to be compound heterozygous for two missense mutations within the gene (Table 2). These patients usually present with a multiple anterior pituitary hormone deficit, with sparing of ACTH in the majority of cases, although patients with ACTH deficiency have also recently been described (216). Pituitary morphology is variable between patients with *LHX3* mutations: most patients have a hypoplastic anterior pituitary with a normal posterior pituitary and midline structures (216, 224, 225); conversely, an enlarged anterior pituitary has also been reported in a patient that was not evident in a previous MRI scan performed 10 yr earlier (224). Additionally, a patient with a hypointense lesion in the anterior pituitary consistent with a microadenoma has also been described (226).

The majority of patients with *LHX3* mutations reported to date have also exhibited a short rigid cervical spine with limited neck rotation and trunk movement. Again, the skeletal phenotypes can vary, and a single patient with normal neck rotation and no other syndromic features has been reported (225). Analysis of *LHX3* expression during human development shows a pattern of expression in the developing embryonic pituitary highly similar to that observed in the mouse. Expression is detected within Rathke's pouch at 5 wk of development and later in the anterior and intermediate region of the pituitary, but not in the posterior lobe. Expression of *LHX3* is also observed in specific regions of the spinal cord corresponding to interneuron and motor neuron populations (215). The underlying mechanism of the vertebral and skeletal defects in patients with *LHX3* mutations is unclear because expression is not detected in the sclerotome or myotome, the tissues giving rise to the vertebrae and skeletal muscle (Ref. 215, and our unpublished observa-

TABLE 2. Mutations identified in *LHX3* in patients with CPHD and associated extrapituitary phenotypes

Mutation	Endocrine phenotype	Associated abnormalities	Deafness phenotype	Ref.
p.Y116C (3 patients)	GH, TSH, PRL, LH, FSH deficiency. Severe anterior pituitary hypoplasia. Severe growth retardation.	Elevated and anteverted shoulders, restriction of cervical spine rotation. No vertebral malformation.	Mild to moderate bilateral sensorineural hearing loss on reinvestigation.	216, 224
23-bp deletion	GH, TSH, PRL, LH, FSH deficiency. Enlargement of anterior pituitary. Growth retardation.	Abnormal steepness of cervical spine, no vertebral malformation.	Profound sensorineural deafness on reinvestigation.	216, 224
g.159delT	GH, TSH, PRL, LH, FSH deficiency. Hypointense pituitary lesion. Growth retardation.	Normal alignment and configuration of cervical spine. Limited neck rotation. Large anterior and posterior fontanelle, hypertelorism and jaundice.	Not described.	226
p.A210V (2 patients)	GH, TSH, PRL, LH, FSH deficiency. Enlarged anterior pituitary. Growth retardation.	Short neck, elevated shoulders, limited neck rotation, loss of lordosis of cervical spine. Hypoglycemia, prolonged jaundice, facial dysmorphism.	Not described.	225
p.E173X	GH, TSH, PRL, LH, FSH deficiency. Anterior pituitary hypoplasia.	Short neck with limited rotation, short arms, hypoglycemia, hyponatremia, dry skin, depressed nasal bridge.	Not described.	225
p.W224X	GH, TSH, PRL, LH, FSH deficiency. Growth retardation.	No syndromic features, normal neck rotation.	Not described.	225
LHX3 gene deletion	GH, TSH, PRL, LH, FSH deficiency. Anterior pituitary hypoplasia.	Short neck with limited rotation, loss of cervical lordosis. Hypoglycemia, prolonged jaundice, retarded psychomotor development.	Not described.	225
c.80–32_775 + 454 del3,088 (3 patients)	GH, TSH, PRL, LH, FSH, ACTH deficiency. Anterior pituitary hypoplasia. Severe growth retardation.	Short neck with limited rotation, vertebral abnormalities. Spinal stenosis, hyperextensible joints, hyperextensible skin.	Mild to moderate bilateral sensorineural hearing loss.	216
p.K50X	GH, TSH, PRL, LH, FSH, ACTH deficiency. Anterior pituitary hypoplasia.	Short stiff neck, skeletal dysplasia. Prominent cranial frontal bones.	Profound bilateral sensorineural deafness.	216
Splice acceptor site mutation in intron 3 (c.455–2A>G) (6 patients)	GH, TSH, PRL, LH, FSH deficiency. Aplastic/hypoplastic/cystic anterior pituitary.	Short neck with restricted rotation. Hypoglycemia, neonatal jaundice, cervical lordosis and thoracolumbar scoliosis. Facial dysmorphism.	Moderate to severe sensorineural deafness.	229

tions). More recently, we have reported an additional phenotype of sensorineural deafness in association with homozygous loss of *LHX3*. The severity of hearing loss is also highly variable and can range from profound to very mild and may be missed in some cases (216). A direct role may be implicated here because *LHX3* is expressed in specific regions of the inner ear in a pattern highly conserved between humans and mice, and it is likely to have a role in cochlea hair cell development (216, 227–229).

Most missense mutations identified in patients have diminished capacity to activate transcription of the promoters of several potential *LHX3* target genes including *CGA*, *PRL*, *FSHB*, *TSHB*, and *POU1F1* (230, 231). Frameshift mutations or deletions of several exons or the entire gene have also been reported and are likely to represent null alleles as a result of premature protein truncation or nonsense-mediated degradation. The results suggest that whereas complete lack of *LHX3* results in pathogenesis, a single functional copy may be sufficient for normal pituitary development.

6. LHX4

To date, four separate reports have described six heterozygous mutations within *LHX4* (OMIM 602146; Table 3), with all patients exhibiting GH deficiency and associated short stature on presentation, again with variable additional endocrine deficits and extrapituitary abnormalities. A heterozygous intronic mutation that abolishes normal splicing of *LHX4* was initially reported by Machinis *et al.* (232) in a three-generation family segregating in a dominant and fully penetrant manner. The probands presented with short stature and were found to be GH, TSH, and ACTH deficient, with anterior pituitary hypoplasia, an undescended posterior pituitary, and ab-

TABLE 3. Reported mutations in the *LHX4* gene

Mutation	Associated endocrine phenotype	Neuroradiological findings	Ref.
c.607–1G>C	GH deficiency, variable TSH, ACTH, gonadotropin deficiency	AP hypoplasia, normal PP or undescended PP with absent pituitary stalk, poorly formed sella turcica, pointed cerebellar tonsils	232
p.P366T	Panhypopituitarism	AP hypoplasia, undescended PP, poorly developed sella turcica, Chiari malformation	234
p.R84C	GH, TSH, evolving gonadotropin deficiency	AP hypoplasia, ectopic PP	235
p.L190R	GH, TSH, ACTH deficiency	AP hypoplasia, undescended PP	235
p.A210P	GH deficiency, variable TSH, ACTH, gonadotropin deficiency	Hypoplastic AP, normal PP	235
c.293_294insC	GH deficiency, variable TSH, gonadotropin deficiency	AP hypoplasia, variable undescended PP	233

AP, Anterior pituitary; PP, posterior pituitary.

sent pituitary stalk on MRI (232). Other affected family members presented with short stature associated with IGHD and a normal posterior pituitary. Additional manifestations included a poorly formed sella turcica and pointed cerebellar tonsils. Subsequent follow-up revealed that the male patient developed gonadotropin deficiency at the age of 18, whereas his younger sister began spontaneous puberty (233). A second, *de novo* mutation, producing a p.P366T substitution, was associated with a more severe panhypopituitary phenotype. MRI demonstrated a hypoplastic anterior pituitary, an undescended posterior lobe, a poorly developed sella turcica, Chiari malformation, and respiratory distress syndrome. Functional studies confirming the pathogenic nature of this mutation on the resultant protein were not performed (234); however, the mutation was absent in both unaffected parents, and the similarity in phenotype is consistent with other patients harboring mutations demonstrated to disrupt LHX4 function *in vitro*.

In a screen of 253 patients, Pfaeffle *et al.* (235) identified an additional three heterozygous missense mutations—one occurring between the two LIM domains of the protein (p.R84C) and two within the homeodomain (p.L190R, p.A210P). The p.A210P mutation was identified in two female siblings presenting with short stature and GH deficiency; MRI showed that both had a hypoplastic anterior lobe with cystic lesions but a eutopic posterior pituitary. One sister had a more severe hypopituitary phenotype with additional TSH, ACTH, and gonadotropin deficiencies, whereas the other had only partial TSH deficiency. The mutation was inherited from their father who had short stature and low GH, but no evidence of other hormone deficiencies (235). The p.R84C mutation was identified in a single male patient presenting with short stature and was found to be GH and TSH deficient, later developing gonadotropin deficiency with pubertal failure. The p.L190R mutation was associated with GH, TSH, and ACTH deficiency. Patients with both of the latter mutations had a small anterior pituitary and an undescended posterior pituitary on imaging, with no abnormalities in other regions of the brain. More recently, two brothers have been described with a single base insertion in exon 3 (c.293_294insC) resulting in a frameshift after codon 99. Both siblings presented with GH and TSH deficiencies with pituitary hypoplasia and a poorly developed sella turcica. The youngest brother also had a hypoplastic corpus callosum and an undescended posterior pituitary. Their father, who also harbored the mutation, was GH deficient and had experienced delayed puberty (233). The same genetic screen also identified two other nonsynonymous variants (p.T90M and p.G370S); however, these changes did not alter LHX4 function *in vitro* and may represent rare neutral variation.

Functional *in vitro* analyses of mutant *LHX4* proteins showed that they either fail to bind DNA or show reduced transactivation properties at the promoters of potential LHX4 target genes including *POU1F1*, *CGA*, and *TSHB* (233, 235, 236). This could account for *LHX4* haploinsufficiency leading to GH, TSH, and gonadotropin deficiencies. The additional brain malformations suggest that LHX4 may be involved in the coordination of brain development and skull shaping. It is interesting to note that $Lhx4^{+/-}$ heterozygous mice display no observable phenotype, suggesting a difference in the requirement of *LHX4* dosage between the two species.

7. OTX2

Mutations in OTX2 (OMIM 600037) have been implicated in the etiology of 2–3% of anophthalmia/microphthalmia syndromes in humans (237–239). To date, two complete deletions and 12 heterozygous intragenic mutations (of which six have been shown to be associated with functional compromise) have been associated with severe ocular and CNS phenotypes, including developmental delay and seizures. The association of deletions of 14q22–23 (which also includes the candidate genes *BMP4*, *RTN1*, *SIX6*, *SIX1*, and *SIX4*) with anophthalmia, hypopituitarism, and ear abnormalities (240) led to the investigation of the role of OTX2 in hypothalamopituitary development. A heterozygous *de novo* 2-bp in-

sertion (c.576_577insCT) in exon 3 of *OTX2* was identified in a Japanese patient with bilateral anophthalmia and panhypopituitarism associated with a small anterior pituitary gland, an ectopic/undescended posterior pituitary, and an absent infundibulum with a Chiari malformation (241). The mutation generates a protein lacking the C-terminal transactivation domain, which failed to activate the promoters of *HESX1* and *POU1F1* as compared with the wild-type protein. No dominant-negative activity was observed.

A *de novo* heterozygous frameshift mutation (c.402insC) was identified in a Japanese female with bilateral anophthalmia in association with short stature and partial GHD with a normal anterior pituitary gland on MRI (242). The mutation resulted in a truncated protein that was associated with reduced transactivation at the *IRBP*, *POU1F1*, and *HESX1* promoters. A further heterozygous point mutation (p.N233S) in the C-terminal transactivation domain was identified in two unrelated children with CPHD who presented with neonatal hypoglycemia (243). The first patient had a small anterior pituitary associated with an undescended/ectopic posterior pituitary and an absent or severely hypoplastic infundibulum, whereas the second patient had a hypoplastic anterior pituitary gland. The mutant p.N233S protein (243) was able to bind to a consensus OTX2 binding sequence but showed reduced transcriptional activation properties compared with wild-type OTX2. Furthermore, the mutant protein acts as a dominant-negative inhibitor of the *HESX1* promoter *in vitro*, suggesting that the hypopituitary phenotypes of these patients may be due to disrupted expression of *HESX1* (243). The *OTX2* gene consists of three exons that encode a 297-aa protein that contains a homeodomain and a proline, serine, threonine-rich C-terminal region that encompasses a highly conserved SIWSPA peptide sequence and a tandemly repeated OTX tail, and is required for anterior neural plate induction. Because it appears to regulate the expression of *HESX1*, it is possible that mutations in the protein compromise the development of the forebrain and ventral diencephalon, in addition to any direct effect within Rathke's pouch.

8. GLI2

Recently, the Sonic Hedgehog (*SHH*) signaling pathway has been implicated in more complex disorders of pituitary development. Mutations within *SHH* (OMIM 600725) are associated with holoprosencephaly (244). Three members of the Gli gene family of transcription factors have been implicated in the mediation of SHH signals, and heterozygous loss of function mutations within the *GLI2* gene (OMIM 165230) have been identified in patients with holoprosencephaly (245). Phenotypic penetrance was variable, with the disorder transmitted through a parent carrying the mutation but showing no obvious phenotype in one family. In all affected individuals with *GLI2* mutations, pituitary gland function was abnormal, accompanied by variable craniofacial abnormalities. Other features included postaxial polydactyly, single nares, single central incisor, and partial agenesis of the corpus callosum.

B. Combined pituitary hormone deficiency: genes involved in pituitary cell differentiation

1. PROP1

As a result of the identification of *Prop1* as the gene underlying the Ames dwarf phenotype, the first mutations in *PROP1* (OMIM 601538) were identified in human patients with hypopituitarism characterized by GH, TSH, and PRL deficiencies in addition to reduced gonadotropins and failure to enter spontaneous puberty (246). Subsequently, 26 distinct mutations have been identified in more than 180 patients from over 21 different countries, implicating *PROP1* mutations as the most common genetic cause of CPHD accounting for approximately 50% of familial cases (247–249), although the incidence in sporadic cases is much lower (247, 250). All affected individuals exhibit recessive inheritance, and the majority of mutations identified involve the DNA binding homeodomain, which is highly conserved between human and mouse, showing 91% identity at the nucleotide level (246, 251). The mutations in *PROP1* identified to date include nonsense, missense, frameshift, intronic, and deletion mutations (Table 4). The majority of the mutations are predicted to result in complete loss of function by ablating DNA binding and transcriptional activation, although some missense mutations retain partial activity (246, 252, 253). By far the most common mutation, accounting for 50–72% of all familial *PROP1* mutations in multiple unrelated families (247, 248, 254), is a 2-bp deletion within exon 2 resulting in a frameshift at codon 101 and the introduction of a termination codon at position 109 (often referred to as p.S109X). The deletion occurs within three tandem GA repeats, so the 2 bp deleted cannot be defined; consequently this mutation has been referred to as c.296delGA and c.301_302delAG in different reports. This mutation is likely to represent a mutational hot spot within the gene, rather than a common founder mutation (247), and combined with the incidence of the c.150delA mutation, accounts for approximately 97% of all known *PROP1* mutations.

Homozygosity for mutations in *PROP1* is typically associated with a deficit of GH, TSH, PRL, and gonadotropins, although the time of onset and severity of hormone deficiency varies. Most patients present with early-onset GH deficiency and growth retardation; however, normal growth in early childhood has been reported in a patient who attained normal final height without GH replacement therapy (255, 256). In this case, the patient presented with

TABLE 4. Reported mutations in the *PROP1* gene

Nucleotide change	Location	Type of mutation	Effect on protein	Ref.
c.2T>C	Exon 1	Missense (initiation codon)	No translation	299
c.109 + 1G>T	Intron 1	Splice site	Aberrant splicing	265
c.112_124del13	Exon 2	Frameshift	Premature truncation	264
c.149_150delAG	Exon 2	Frameshift	Premature truncation	300
c.150delA	Exon 2	Frameshift	Premature truncation	263
c.157delA	Exon 2	Frameshift	Premature truncation	301
c.211C>T	Exon2 (HD)	Missense	p.R71C	302
c.212G>A	Exon2 (HD)	Missense	p.R71H	302
c.217C>T	Exon 2 (HD)	Missense	p.R73C	248, 251
c.218G>A	Exon 2 (HD)	Missense	p.R7^{3}H	259
c.247C>T	Exon 2 (HD)	Nonsense	p.Q83X	303
c.263T>C	Exon 2 (HD)	Missense	p.F88S	252
c.295C>T	Exon 2 (HD)	Nonsense	p.R99X	259
c.296G>A	Exon 2 (HD)	Missense	p.R99Q	304
c.296_297delGA c.301_302delAG	Exon 2 (HD)	Frameshift	Premature truncation	246
c.310delC	Exon 2	Frameshift	Premature truncation	253
c.343–11C>G	Intron 2	Splice site	Aberrant splicing (loss of exon 3)	253
c.343–2A>T	Intron 2	Splice site	Aberrant splicing	251
c.349T>A	Exon 3 (HD)	Missense	p.F117I	246
c.358C>T	Exon 3 (HD)	Missense	p.R120C	246, 257
c.373C>T	Exon 3	Missense	p.R125W	253
c.467insT	Exon 3	Frameshift	Premature truncation	305
c.582G>A	Exon 3	Nonsense	p.W194X	255
c.629delC	Exon 3	Frameshift	Altered transactivation domain at codon 210	306

HD, Homeodomain.

gonadotropin deficiency with the evolution of other hormone deficiencies later in life. TSH deficiency is also highly variable and has been reported as the initial presenting symptom in some cases (248, 257, 258), whereas other patients show delayed onset (257, 259). Onset of ACTH deficiency is significantly correlated with increasing age, and most patients exhibit normal ACTH and cortisol levels in early life (260–263); however, patients as young as 6 yr have been described with cortisol deficiency emphasizing the necessity for complete and continued clinical assessment of patients with *PROP1* mutations (264, 265).

Although PROP1 has been shown to play a critical role in mouse gonadotrope differentiation, the spectrum of human gonadotropin deficiency is extremely variable in patients with *PROP1* mutations, ranging from early hypogonadism with a micropenis and undescended testes and complete lack of pubertal development to spontaneous, albeit often delayed, onset of puberty with subsequent deficiency of gonadotropins, requiring hormone replacement therapy (248, 257, 259, 262). Variation in the timing and severity of gonadotropin deficiency could mean that hypogonadism in patients with *PROP1* mutations is acquired and late evolving rather than early congenital, consistent with a role for PROP1 in maintenance or terminal differentiation of gonadotropes rather than initial specification (266). However, a number of individuals with mutations may have congenital gonadotropin deficiency, given the presence of a micropenis and bilaterally undescended testes at birth (250).

The pituitary morphology in patients with *PROP1* mutations is unpredictable; most cases show a hypoplastic or normal-sized anterior pituitary gland on imaging, with a normal pituitary stalk and posterior lobe, although some reports have documented an enlarged anterior gland (246, 259, 260, 267). Longitudinal analyses of anterior pituitary size over time have revealed that several patients with an enlarged anterior gland at initial scanning in childhood show spontaneous regression and involution, so that MRI in older patients often demonstrates anterior pituitary hypoplasia, although the size of the pituitary can wax and wane during this time (Fig. 4F) (250, 258, 263). The pituitary enlargement consists of a mass lesion interposed between the anterior and posterior lobes, possibly originating from the intermediate lobe (258) or Rathke's pouch remnant in the cleft, although the underlying mechanism for the mass remains unknown. Evidence from the mouse (see above) suggests that PROP1 regulates the migration of progenitor cells from Rathke's pouch into the developing anterior pituitary, and in the absence of functional PROP1, undifferentiated cells are trapped in the perilumenal area resulting in enlargement of the anterior pituitary followed by apoptosis (152). Such a mechanism would be an attractive explanation for the human imaging findings, but of course would be difficult to establish and cannot account for the waxing and waning of the mass. Earlier biopsies of such a mass failed to reveal any definitive histopathology, with no cell types identified (266), and such material is now likely to prove elusive because the

masses no longer require surgical removal in patients with identified *PROP1* mutations.

The evolving nature of hormone insufficiencies in patients with *PROP1* mutations suggests a progressive decline in the anterior pituitary axis, so such patients require regular monitoring for the development of hormone deficits that may not be apparent on initial presentation. The highly variable nature of the phenotype associated with *PROP1* mutations, even between siblings within the same family carrying identical mutations (253, 257), together with the observation of phenotypic differences in *Prop1* mutant mice on different genetic backgrounds, again implicate unidentified genetic modifiers playing a role in the severity and onset of disease pathogenesis.

2. POU1F1 (PIT1)

Mutations within *POU1F1* (*PIT1*; OMIM 173110) were first reported in 1992 by four independent groups (268–271) and are generally associated with GH, PRL, and TSH deficiencies with variable anterior pituitary hypoplasia, consistent with the phenotype of Snell and Jackson dwarf mice. Deficiencies of GH and PRL are generally complete and present early in life, whereas TSH deficiency can be highly variable. The majority of cases present with early TSH deficiency; however, in some cases hypothyroidism occurs later in childhood (269, 272, 273). We have recently described a 21 yr old with GH and PRL deficiency who has normal thyroid function to date (274) with a *POU1F1* mutation identical to that found in an unrelated patient who developed central hypothyroidism in the second year of life. MRI of patients with *POU1F1* mutations demonstrates a small or normal-sized anterior pituitary gland with a normal posterior pituitary and infundibulum, but with no extrapituitary abnormalities (Fig. 4E). A total of 28 different mutations in *POU1F1* have been described to date; 23 of these show recessive inheritance, including a complete gene deletion and a recent report of a splice site mutation (275), whereas five are dominant mutations, found in over 60 patients from several different countries (Tables 5 and 6). Of these, the amino acid substitution p.R271W is the most frequent having been identified in several unrelated patients from a variety of different ethnic backgrounds (270, 271, 276–282).

The p.R271W mutant protein is capable of binding to DNA and appears to act as a dominant-negative inhibitor of transcription by wild-type POU1F1 (PIT1) protein (270, 283), although this has been disputed (284). The only other mutations reported in more than one pedigree are the recessively inherited mutations p.R172X (reported in three pedigrees) (268, 273, 285), p.A158P (two pedigrees) (269), p.P239S (three pedigrees) (286), and p.E230K identified in five pedigrees from three different countries (274, 287). Studies of *POU1F1* in CPHD patient cohorts suggest that the incidence of mutations in cases of sporadic CPHD is quite low (approximately 3–6%), whereas the incidence among familial patients with hypopituitarism is much greater (25%) (274, 288).

TABLE 5. Mutations identified in the *POU1F1* gene in patients with CPHD

Mutation	Type of mutation	Location	Inheritance	Ref.
p.Q4X	Nonsense	Exon 1	Recessive	307
p.P14L	Missense	Exon 1	Dominant	308
p.P24L	Missense	Exon 1	Dominant	271
p.F135C	Missense	Exon 3	Recessive	272
p.R143Q	Missense	Exon 3	Recessive	271
p.R143L	Missense	Exon 3	Recessive	288
p.K145X	Nonsense	Exon 3	Recessive	309
p.A158P	Missense	Exon 4 (POU)	Recessive	310
p.Q167K	Missense	Exon 4 (POU)	Dominant	311
p.R172Q	Missense	Exon 4 (POU)	Recessive	274
p.R172X	Nonsense	Exon 4 (POU)	Recessive	268
p.E174G	Missense	Exon 4 (POU)	Recessive	285
p.W193R	Missense	Exon 4 (POU)	Recessive	312
p.W193X	Nonsense	Exon 4 (POU)	Recessive	287
p.L194Q	Missense	Exon 4 (POU)	Recessive	288
c.682 + 1G>A	Splice site	Intron 4	recessive	275
p.K216E	Missense	Exon 5	Dominant	290
p.E230K	Missense	Exon 6 (HD)	Recessive	287
p.F233L	Missense	Exon 6 (HD)	Recessive	313
p.P239S	Missense	Exon 6 (HD)	Recessive	286
c.725_726delAA (reported as p.Q242R)	Frameshift	Exon 6 (HD)	Recessive	314
c.747delA	Frameshift	Exon 6 (HD)	Recessive	312
p.E250X	Nonsense	Exon 6 (HD)	Recessive	315
c.778insA	Frameshift	Exon 6 (HD)	Recessive	274
p.F262L	Missense	Exon 6 (HD)	Recessive	314
p.R271W	Missense	Exon 6 (HD)	Dominant	270
p.V272X	Nonsense	Exon 6 (HD)	Recessive	317

HD, Homeodomain; POU, POU specific.

Functional analysis has been performed for a relatively small number of mutations and has revealed effects on DNA binding (274), protein-protein interactions [p.S179R mutation (289)], and retinoic acid induction of the *Pou1f1* (*Pit1*) gene distal enhancer either alone or in combination with wild-type POU1F1 (PIT1) [dominant-negative p.K216E mutation (290)]. Further analysis is required to understand the molecular mechanisms underlying mutations such as the dominant-negative p.P14L and p.P24L mutations.

C. *TBX19* and ACTH deficiency

Several recessive mutations have been identified in the *TBX19* (*TPIT*) gene located at 1q23–24, encoding the transcription factor TBX19 (TPIT), resulting in severe ACTH deficiency, profound hypoglycemia associated with seizures in some cases, and prolonged cholestatic jaundice in the neonatal period. Mutations within *TBX19* (*TPIT*) were initially identified in two patients who pre-

TABLE 6. Comparison of murine and human genes, proteins, and phenotypes

Gene	Protein	Murine loss of function phenotype	Human phenotype	Inheritance murine/ human
HESX1	HESX1	Anophthalmia or microphthalmia, agenesis of corpus callosum, absence of septum pellucidum, pituitary dysgenesis or aplasia	Variable: SOD, CPHD, IGHD with EPP. Anterior pituitary hypoplastic or absent. Posterior pituitary ectopic or eutopic Frequency of mutations: <1% (199)	Dominant or recessive in humans, recessive in mouse
OTX2	OTX2	Lack of forebrain and midbrain, olfactory placode, optic placodes	Anophthalmia, APH, ectopic posterior pituitary, absent infundibulum. Frequency of mutations: 2–3% of anophthalmia/microphthalmia cases (239)	Heterozygous: haploinsufficiency/ dominant negative
SOX2	SOX2	Homozygous null mutants: embryonic lethal	Hypogonadotropic hypogonadism; APH, abnormal hippocampi, bilateral anophthalmia/ microphthalmia, abnormal corpus callosum, learning difficulties, esophageal atresia, sensorineural hearing loss, hypothalamic hamartoma	*De novo* haploinsufficiency in humans, heterozygous mutation associated with haploinsufficiency in mouse
		Heterozygous mice and further dose reduction: poor growth, reduced fertility, CNS abnormalities, anophthalmia; pituitary hypoplasia with reduction in all cell types	Frequency of mutations: 8/235 (103); 10% (212)	
SOX3	SOX3	Poor growth, weakness, craniofacial abnormalities, ACC, hypothalamic and infundibular abnormalities	IGHD and mental retardation, hypopituitarism; APH, infundibular hypoplasia, EPP, midline abnormalities Frequency of mutations 6% (duplications), 1.5% (mutations) (220)	X-linked recessive in both mice and humans
GLI2	GLI2	N/A	Holoprosencephaly, hypopituitarism, craniofacial abnormalities, polydactyly, single nares, single central incisor, partial ACC Frequency of mutations: 1.5% (245)	Haploinsufficiency In humans
LHX3	LHX3	Hypoplasia of Rathke's pouch	GH, TSH, gonadotropin deficiency with pituitary hypoplasia. ACTH insufficiency variable. Short, rigid cervical spine. Variable sensorineural hearing loss Frequency of mutations: 1.3% (225)	Recessive in both
LHX4	LHX4	Mild hypoplasia of anterior pituitary	GH, TSH, cortisol deficiency, persistent craniopharyngeal canal and abnormal cerebellar tonsils; APH, ectopic/eutopic posterior pituitary, absent infundibulum Frequency of mutations: 1.2% (235)	Recessive in mouse, dominant in humans
PROP1	PROP1	Hypoplasia of anterior pituitary with reduced somatotropes, lactotropes, thyrotropes, corticotropes and gonadotropes	GH, TSH, PRL, and gonadotropin deficiency. Evolving ACTH deficiency. Enlarged pituitary with later involution Frequency of mutations: 1.1% sporadic cases, 29.5% familial cases (250)	Recessive in both
POU1F1	POU1F1 (PIT1)	Anterior pituitary hypoplasia with reduced somatotropes, lactotropes and thyrotropes	Variable anterior pituitary hypoplasia with GH, TSH, and PRL deficiencies Frequency of mutations: 3.8% sporadic cases, 18% familial cases (274)	Recessive in mouse, dominant/recessive in humans

ACC, Agenesis of the corpus callosum; APH, anterior pituitary hypoplasia; EPP, ectopic posterior pituitary; N/A, not available.

sented with very similar symptoms of isolated ACTH deficiency (23), with very low basal plasma cortisol concentrations and no ACTH response to CRH, but a cortisol response to corticotropin administration. Consistent with its pituitary expression restricted to POMC cells, mutations in *TBX19* (*TPIT*) are frequently associated with neonatal isolated ACTH deficiency, but never with cases of juvenile onset deficiency. Twelve independent mutations have been identified, including nonsense, missense, frameshift, and splicing mutations in addition to a 5.2-Kb genomic deletion encompassing exons 2 and 3 (23, 291–294). All of these mutations have been shown or are predicted to result in loss of TBX19 (TPIT) function (291, 292), and all patients appear to be homozygous or compound heterozygous for *TBX19* (*TPIT*) mutations, with unaffected heterozygous parents, implying a recessive mode of inheritance.

Vallette-Kasic *et al.* (292) have reported the largest series to date and demonstrated *TBX19* (*TPIT*) mutations in 17 of 27 patients from 21 unrelated families, suggesting that mutations in this gene are the principal molecular cause of congenital neonatal isolated ACTH deficiency. Three patients carry only one mutant *TBX19* (*TPIT*) allele, although their family history implied recessive inheritance, so additional, as yet unidentified, mutations may be present in regulatory regions of the gene in some cases (292). In contrast with most other developmental gene mutations, *TBX19* (*TPIT*) mutations present a relatively selective endocrine phenotype with normal function of all other pituitary axes. Severe hypoglycemia, associated with seizures and failure to thrive in some cases, and prolonged cholestatic jaundice are classically associated with ACTH deficiency presenting in the neonatal period (23, 292). Furthermore, Vallette-Kasic *et al.* (292) noted that in their series about 25% of families with segregating *TBX19* (*TPIT*) mutations (five of 21) suffered a neonatal death, suggesting that isolated ACTH deficiency may be an underestimated cause of this.

VII. Conclusion

Over the past decade, there has been an explosion in the knowledge of the genetic cascade that orchestrates hypothalamo-pituitary development. Several transcription factors and signaling molecules are critical for organ commitment and cell differentiation and proliferation at a very early stage of gestation. Knowledge of the expression pattern of these genes has helped explain the wider phenotype in patients with hypopituitarism, for example those with SOD who have associated forebrain and eye defects. The mouse has served as an excellent model for understanding the genetic basis of congenital hypopituitarism in humans, although the correlation between mouse and human disease phenotypes is variable (Table 6). This candidate gene approach, based on mouse studies, has led to the identification of several human mutations that disrupt hypothalamopituitary development resulting in specific patterns of hormone dysfunction. However, there are many genes that remain to be identified and in future will enable more extensive screening of the large number of patients who lack a definitive etiology. Our understanding of the etiology and molecular mechanisms of these disorders will undoubtedly lead to improvements in the management of these patients.

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