Published in final edited form as:
Mol Cell Endocrinol. 2010 July 8; 323(1): 4–19. doi:10.1016/j.mce.2009.12.012.

Molecular mechanisms of pituitary organogenesis: in search of novel regulatory genes

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Abstract

Defects in pituitary gland organogenesis are sometimes associated with congenital anomalies that affect head development. Lesions in transcription factors and signaling pathways explain some of these developmental syndromes. Basic research studies, including the characterization of genetically engineered mice, provide a mechanistic framework for understanding how mutations create the clinical characteristics observed in patients. Defects in BMP, WNT, Notch, and FGF signaling pathways affect induction and growth of the pituitary primordium and other organ systems partly by altering the balance between signaling pathways. The PITX and LHX transcription factor families influence pituitary and head development and are clinically relevant. A few later-acting transcription factors have pituitary-specific effects, including PROP1, POU1F1 (PIT1), and TPIT (TBX19), while others, such as NeuroD1 and NR5A1 (SF1), are syndromic, influencing development of other endocrine organs. We conducted a survey of genes transcribed in developing mouse pituitary to find candidates for cases of pituitary hormone deficiency of unknown etiology. We identified numerous transcription factors that are members of gene families

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Note added in proof: Recent analysis of Lhx2 by in situ hybridization shows a broader range of expression in the ventral diencephalon than we report by immunohistochemistry. This report also reveals a direct role for LHX2 in the development of the posterior lobe of the pituitary gland. It is required in the infundibulum to restrict growth factor expression and suppress proliferation, and it is necessary for vasopressin expression in the posterior lobe. LHX2 deficiency causes dysmorphology of the intermediate and anterior lobes indirectly because no expression is detected there. LHX2 is not required for specification of the hormone producing cells of the anterior lobe. (Zhao Y, Mailloux CM, Hermesz E, Palkóvits M, Westphal H. A role of the LIM-homeobox gene Lhx2 in the regulation of pituitary development. Dev Biol. 2009 Nov 6. [Epub ahead of print]

with roles in syndromic or nonsyndromic pituitary hormone deficiency. This collection is a rich source for future basic and clinical studies.

Keywords

bHLH; beta helix-loop-helix; HMG; high mobility group; T-box; forkhead; hypopituitarism

Introduction

Human growth insufficiency

Height of 2 or more standard deviations (SD) below the mean for age and sex is defined as short stature. Metabolic or endocrine disorders usually cause proportionate short stature, while skeletal defects often cause disproportionate short stature [1-4]. The sitting height to standing height ratio can be used to distinguish proportionate and disproportionate short stature in cases where the distinction is not immediately obvious. Skeletal and hypothalamic-pituitary axis-based growth insufficiencies occur with similar frequencies. Genetic causes of growth hormone deficiency (GHD) are thought to occur in approximately 1/4,000 to 1/10,000 births [5,6]. These can be syndromic, including pituitary and head defects as well as defects in the development of other organs, or non-syndromic, with pituitary gland-specific effects (Table 1). Because the pituitary gland is critical for the development and function of many other organs, all defects in pituitary organogenesis cause secondary effects on target organs. Syndromic pituitary deficiencies include effects on non-pituitary tissues that are not within the expectations for secondary effects on target organs. Genetic defects in the GH gene itself and mutations in the growth hormone releasing hormone receptor (GHRHR) cause isolated GHD (IGHD), but most cases of IGHD are idiopathic (reviewed in: [7]). Patients with mutations in the growth hormone receptor gene (Laron dwarfism, a growth hormone insensitivity syndrome) have a clinical presentation similar to patients with IGHD, but they have elevated levels of GH and are treated with insulin like growth factor because they are unable to respond to GH therapy (Reviewed in: [8]).

The availability of recombinant growth hormone has generally led to effective correction of growth insufficiency in children with multiple or IGHD due to pituitary developmental defects, but it is not always efficacious for some idiopathic short stature patients or for other problems associated with syndromic pituitary hormone deficiency (i.e. septo-optic dysplasia and other severe craniofacial abnormalities) [9-13]. Thus, treatment of children with pituitary hormone deficiency can be challenging, as well as expensive.

Are mouse studies informative for clinical endocrinologists?

Studies in genetically engineered and mutant mice have advanced the understanding of the mechanisms underlying pituitary organogenesis defects that lead to short stature [14,15]. In many cases, genes discovered in the mouse led to the discovery of lesions in human patients and have revealed the mechanism of action and genetic hierarchy of control of pituitary cell specification and growth [16]. For example, the discovery of the etiology of the Snell and Ames dwarf mutations (*Pou1f1* and *Prop1*, respectively), and characterization of the phenotypes of genetically engineered mice with mutations in *Tpit* (officially *Tbx19*), *Hesx1*, *Lhx3* and *Lhx4*, paved the way for identification of the mutated human genes. Some genes necessary for normal growth in mice, i.e. *Aes*, have not yet been reported to have lesions in human patients, but there can be a considerable lag between discovery in mice and identification of rare human patients [17,18].

The ability of mouse mutants to predict the correct human patient characteristics for screening is remarkable, as evidenced by *Pou1f1, Prop1, Tpit, Hesx1, Lhx3* and *Lhx4*, although the correspondence is imperfect. For example, *LHX4* mutations cause similar hormone deficiencies in humans and mice, and while the mouse mutations are recessive and cause perinatal lethality, the human mutations are haploinsufficient and viable [19-23]. PROP1 mutations are another example of an imperfect correspondence between the mouse and human features. In mice, lesions in *Prop1* cause pituitary hypoplasia and congenital pituitary hormone deficiency, including GH, TSH, PRL, and gonadotropin deficiencies [24,25]. Both male and female mutant mice go through puberty and become fertile with GH, thyroid hormone and PRL supplements, suggesting that the gonadotropin deficiency is secondary to the lack of POU1F1 [26,27]. In contrast, humans have variable pituitary size and progressive hormone deficiency, usually with failure to undergo puberty and the additional involvement of evolving ACTH deficiency, which can be fatal if untreated [28-31]. The missense mutation (S83P) in the spontaneous Ames dwarf mutant, *Prop1*$^{df/df}$, minimally transactivates an artificial paired homeodomain binding site in cell transfection assays, while the most common human PROP1 mutation creates a frame shift and likely complete loss of function [32,33]. This does not account for the differences in pituitary dysfunction between the species, as mice homozygous for genetically engineered *Prop1* loss of function alleles have features similar to the missense mutation, and the S83P mutant appears to have no activity in culture on the *Pou1f1* early enhancer, which is considered a bona fide target [32,34,35].

Dissimilarities in the features that characterize mouse mutants and human patients may be attributable to differences in the effect of the mutations (i.e. partial vs. complete loss of function), species differences in temporal or spatial expression, overlapping gene function amongst gene family members, and/or “genome variation.” Genome variation means different phenotypic manifestations of the same genetic defect due to the influence of other genes in the genome that have functional variant alleles segregating in the population. Analysis of mutant mice on different strain backgrounds can be exploited to uncover the influence of these modifier genes that magnify or minimize the manifestations of reduced function in other genes [36]. For example, genetic background has a profound effect on the survival of *Prop1* mutant mice, ranging from neonatal lethal, juvenile lethal to completely viable [34]. The utility of genetically engineered mice and well defined inbred strains may make it possible to tease out the genetic risk factors that could cause some human mutations to have mild effects in some individuals and more severe ones in other patients with the same mutation [36,37].

Cell-cell signaling plays a critical role in pituitary organogenesis

Classic embryology experiments involving tissue transplantation and recombination reveal that diffusible molecules produced by the neural tissue located dorsal to Rathke’s pouch, the primordia for the intermediate and anterior lobes of the pituitary gland, are essential for pouch induction and growth [38-43]. Subsequently, members of the WNT, BMP, FGF, Notch, and hedgehog pathways were discovered to have profound effects on pituitary development [35,41,43-56]. Some essential signaling molecules are expressed in the infundibulum, but there are some in the mesenchyme surrounding the pituitary, i.e. *Tgfbi*, and some in the pouch itself [57]. This suggests that the regulation of pituitary development by signaling molecules is complex.

Expression of noggin, an antagonist of BMP signaling, TCF7L2, an effector of canonical WNT signaling, and WNT5A, typically acting in the non-canonical pathway, are critical for maintaining the balance of signaling pathways necessary for normal pituitary growth and morphology [17,44-47]. For example, excessive BMP signaling in noggin mutant mice is associated with reduced FGF10 expression, alteration in the SHH signaling domain, and

multiple invaginations of Rathke's pouch [46]. TCF7L2 deficient mice exhibit expansion of the FGF10 and BMP signaling domains and an abnormally large Rathke's pouch and subsequently oversized anterior lobe [47]. Finally, WNT5A deficient mice also have expanded FGF and BMP signaling domains, and the pouch is dysmorphic but not markedly oversized [44]. In each of these cases, disruption of one signaling pathway has pleiotropic effects on other signaling pathways. This paradigm is emerging as a common theme for signaling pathway function in pituitary development.

Popular models suggest that signaling molecules influence the spatial patterns of pituitary transcription factor expression, leading to the emergence of specialized cell types that produce pituitary hormones, yet there is also compelling evidence that alterations in signaling pathways affect the morphology and size of the organ more than cell specification [17,41,44-47,56]. The noggin, WNT5A, and TCF7L2 mutants are each able to generate the 5 major hormone-producing cells of the anterior lobe despite variations in size and shape of the organ. Rizzoti et al. recently reviewed the effects of various genetic lesions on pituitary growth and shape [58].

The developing pituitary transcriptome contains many members of the BMP, FGF, WNT, Notch and SHH signaling pathways [57]. Using Genomatix software we identified 61 additional genes in these pathways that are expressed at a time when they could influence pituitary development. 17 genes may be involved in cross talk between the pathways. Gene ontology terms revealed an additional 72 genes that could contribute to cell signaling in the developing pituitary gland. RT-PCR surveys of WNT genes expressed in and around the developing organ have identified many different candidates for regulation of β-catenin activity, but little is known about the functional significance of many of these genes [35,44]. Several pituitary transcription factors are regulated by β-catenin, including the EGR1, NR5A1 complex, PITX2, and the HESX1, PROP1 complex [35,59-62]. Because β-catenin is regulated by G-protein coupled receptors, some of the pituitary transcription factors that respond to β-catenin could be independent of WNT molecules themselves, which is an area for future study [63]. Many of the signaling pathways involved in pituitary development play important roles in ontogeny of other organs, leading to lethality in mice homozygous for complete loss of function alleles. Thus, it seems unlikely, but not impossible, that genes in these pathways will be responsible for hypopituitarism in humans.

Transcription factor regulation of pituitary development

Many transcription factors play important roles in pituitary development and hormone production (Table 1). The early-acting genes are not pituitary specific, and lesions in these genes cause defects in development of craniofacial or other structures. Some of these are homeobox genes with overlapping functions and multiple roles during ontogeny, i.e. *Pitx1* and *Pitx2, Lhx3* and *Lhx4* [19,64-69]. Defects in some of these genes cause apoptosis, reduced cell proliferation, or both, which ultimately results in pituitary hypoplasia. The functions of genes like *Nr5a1, Pitx2*, and *Gata2*, a downstream target of *Pitx2*, with broad expression patterns and roles in the pituitary as well as other critical organs, can be dissected by tissue specific disruption in mice [70,71]. Such studies reveal roles for *Gata2* in thyrotropin and gonadotropin production, and implicate *Gata3* as a gene with potential for compensatory activity [72,73].

Prop1 and *Pou1f1* are examples of homeodomain transcription factors critical for pituitary development, specifically. Mutations in the human ortholog of *Prop1* are the most common known cause of multiple pituitary hormone deficiency in humans [16,33,74,75]. There are dramatic differences in the effects of *Prop1* and *Pou1f1* mutations on fetal and neonatal pituitary development in mice. *Prop1* mutants have poor pituitary vascularization and dysmorphology that appears to result, in part, from the failure of progenitors to migrate

away from the proliferative zone and undergo differentiation [76,77]. The defect may result from failure to undergo epithelial to mesenchymal transition, as *Prop1* is required for normal N-cadherin expression, and changes in cadherin gene expression are typically associated with epithelial to mesenchymal transition [78-80]. In contrast, there are no obvious effects on pituitary vascularization or morphology in *Pou1f1* mouse mutants.

Pou1f1 is generally accepted as a direct downstream target of *Prop1*. This is based on the ability of PROP1 to transactivate a DNA fragment of *Pou1f1* that contains the early enhancer in cell culture and the occupancy of PROP1 at that site by chromatin immunoprecipitation in extracts of microdissected embryonic pituitary glands at e12.5 and e13.5 [32,35]. Careful review of the evidence suggests that the story may be more complicated. First, there is a profound temporal delay (approximately 4 days) between activation of *Prop1* and *Pou1f1* expression in mice, which is unusual for a direct downstream target [32]. Second, human newborns with loss of function alleles in PROP1 have low but biologically significant levels of TSH, GH and PRL initially, suggesting that *PROP1* is not required for initial expression of *POU1F1* in humans [30]. Similarly, mice with *Prop1* mutations express limited amounts of *Pou1f1* and its targets *Tshb, Gh*, and *Prl* [81,82]. More work needs to be done to reconcile these apparently conflicting observations and clarify the role of PROP1 in humans and mice.

We hypothesize that the role of PROP1 is to generate precursor cells that are capable of becoming hormone-producing cells of the anterior lobe and promote the transition from proliferation to differentiation. It may also play a role in regulating the accessibility of the POU1F1 regulatory elements. POU1F1 is activated in some of the precursor cells to promote differentiation into somatotrophs, thyrotrophs and lactotrophs and to expand the proliferation of that lineage after birth. If this hypothesis is true, the progressive hormone deficiency in humans with *PROP1* mutations could arise by depletion of the progenitor pool, and the more severe, congenital hormone deficiency in *Prop1* mutant mice could be due to a stronger and/or earlier requirement of *Prop1* for establishing the precursor pool in mice than humans. Investigation of genes expressed in the developing pituitary gland between peak *Prop1* and *Pou1f1* expression may uncover direct targets of *Prop1* that are intermediates between *Prop1* and *Pou1f1*. *Neurod4* (also known as *Math3*) is a candidate for an intermediate, as it is activated at e13.5 before *Pou1f1* is generally detected, although maintenance of *Neurod4* expression requires *Pou1f1* [53]. Novel genes expressed at these early developmental times will be candidates to explain pituitary deficiency diseases of unknown etiology.

Several of the known pituitary transcription factors were discovered using the approach of defining the key cis-acting sequences in hormone genes and the trans-acting factors that bind to them [83-88]. Additional advances could be made by pursuing this strategy more extensively and/or by identifying the regulatory sequences for some of the early-acting pituitary specific transcription factors and their binding factors, as well as the downstream targets of key transcription factors. For example, we used comparative genomics and bioinformatics to identify regulatory sequences in *Prop1*, and confirmed their relevance in cell culture and transgenic mice [89]. A highly conserved intragenic enhancer that affects spatial expression of a *Prop1* transgene is a target of Notch signaling [53,89]. This suggests that screening for *PROP1* mutations in human patients should include a scan of the intronic enhancer that controls spatial expression of the gene in mice [90].

Another approach is to identify gene expression differences in the pituitary glands of normal and mutant mice to identify potential downstream targets of *Prop1* and *Pou1f1* [57,91-94]. This gene discovery approach has revealed new members of transcription factor families that are exciting candidates for regulating pituitary development and the basis of human

hormone deficiency disease. Here we report the discovery of transcription factors expressed in the developing mouse pituitary gland that are members of several important gene families including basic helix-loop-helix, high mobility group, and T-box. These genes are intriguing candidates for future functional studies and evaluation in human patients. In addition, we present a summary of the clinical features associated with hypopituitarism caused by known transcription factors, with the purpose of streamlining molecular diagnostic studies.

Results and Discussion

Prioritizing genes for molecular studies in human patients

There are approximately a dozen different transcription factor genes that are mutated in cases of short stature and/or pituitary gland dysfunction (Table 1). These are classified based on the type of pituitary defect that they produce as well as any other clinical features. Several of these genes are expressed in the developing hypothalamus and are likely to affect anterior pituitary gland development by disrupting the normal balance of signaling pathways and inductive factors produced by the hypothalamus. For example, GLI2, SOX2, SOX3, and TCF7L2 are primarily expressed in the neural ectoderm [17,47,95-100]. Some of the pituitary transcription factor genes are large and can pose difficulties for DNA sequence analysis because of high GC content. Thus, it is useful to predict which of the many genes are most likely to be mutated given a set of clinical characteristics.

The primary sources of diagnostic information for a clinical endocrinologist are the family history, evidence of growth retardation based on the longitudinal height and weight curve of population matched controls, basal and stimulated levels of circulating hormones, and imaging to analyze the shape of the sella turcica, the size and position of the anterior pituitary, the pituitary stalk and the posterior pituitary. Non-pituitary related syndromic features such as Chiari malformation, craniofacial abnormalities, limited neck rotation, eye abnormalities, and hearing deficits can suggest a causative gene that could account for hypopituitarism and the syndromic features (Fig. 1). Congenital hypopituitarism associated to dysmorphic features are generally linked to early-expressed genes during pituitary development as *GLI2*, *SOX2*, *SOX3*, *HESX1*, *LHX3*, *LHX4*, whereas non-syndromic hypopituitarism is generally due to defects in later-expressed, pituitary-specific genes such as *PROP1* and *POU1F1* (Fig. 1). There can be a large spectrum of clinical features associated with any one gene. For example, *HESX1* mutations can cause multiple pituitary hormone deficiencies or occasionally, isolated GH deficiency [101,102]. Obviously, not all patients with mutations in the same gene have identical clinical findings, but there are trends that suggest prioritization of the molecular diagnostic tests. Distinguishing between hypothalamic and pituitary origins of hormone deficiency is useful in planning the strategy for genetic analysis, although this is not without controversy [7,103]. There is a lack of data in the literature correlating TRH stimulation pattern in patients with known genetic defects associated to hypothalamic or pituitary cells differentiation. In a study of 43 patients with MPHD, 26% were compatible with pituitary hypopituitarism, and 70% had hormonal responses suggestive of hypothalamic hypopituitarism [104]. Ectopic posterior lobe was a characteristic of putative hypothalamic hypopituitarism.

Mutations in *HESX1*, *LHX3*, *LHX4*, *GLI2*, *SOX2*, and *SOX3* are rare causes of congenital hypopituitarism, while *PROP1* defects are a common cause of familial, congenital hypopituitarism [15,75,105,106]. Although *PROP1* mutations have a variable effect the size of the gland, all cases described to date have an intact stalk, eutopic (normally placed) posterior pituitary, and hormone deficiencies that tend to worsen progressively [28-30,33]. Other genes that can be mutated in patients with MPHD, eutopic posterior lobe and normal pituitary stalk are *POU1F1* and *LHX3*, although *LHX3* mutations can present with cervical stiffness (Fig. 1). Despite the common occurrence of *PROP1* mutations in multiple ethnic

groups, and the rare mutations reported in other transcription factor genes, most patients with hypopituitarism have no identifiable genetic lesions [104]. Ectopic posterior pituitary and stalk disruptions are common features of patients with unknown etiology [101].

There have been impressive advances in identifying the molecular basis for pituitary hormone deficiency over the past 20 years. It is also appreciated that non-genetic traumatic events can cause hypopituitarism [104]. Yet birth trauma does not appear to be an obvious contributor to the majority of cases of hypopituitarism without a molecular diagnosis. Thus, additional genes are likely to be involved, and alternative approaches must be taken to identify these genes.

Gene discovery to identify new candidate genes

In an effort to identify novel candidate genes for hypopituitarism, we undertook a project to discover genes expressed during a critical period of mouse pituitary gland development [57,93]. A brief description of library preparation and analysis follows. We dissected pituitary glands from normal mice at e12.5 (CD1 strain) and from DF/B-*Prop1*$^{df/df}$ mutants and their wild type littermates at e14.5 and genotyped individual fetuses using previously described protocols [92]. Appropriate samples were pooled based on genotype, total RNA isolated, and cDNA prepared using the cap-trapper method to enhance the representation of full-length clones and normalization to enrich for rare sequences [93,107]. Two subtracted libraries were prepared to enhance the representation of *Prop1* targets [57]. One subtracted library was enriched for genes activated at e14.5 but not e12.5, and the other was enriched for genes expressed in normal pituitaries but not in *Prop1* mutants at e14.5. Single pass DNA sequencing was conducted on 56,716 clones using an algorithm to maximize gene discovery and minimize re-sequencing cDNAs representing the same genes. The cDNA sequences were analyzed by comparison with publicly available databases using an expect value of 10^{-5} to favor putative identifications that could be validated by more complete sequence analysis. We placed the matches of single pass sequence with public database entries into a local database that is searchable by gene name, RefSeq ID, Unigene ID, gene ontology terms (GO), and DNA sequence and identified 12,009 different expressed genes. To obtain database access contact Drs. Camper or Lyons at scamper@umich.edu or boblyons@umich.edu.

Analysis of the database revealed numerous homeobox genes not previously known to be expressed in the pituitary gland, members of signaling pathways, and genes involved in cell proliferation, apoptosis, cell migration, and cell adhesion [57,94]. Here we highlight transcription factors identified by this approach that are members of the PITX, and LHX gene families, as well as the forkhead group, families of basic helix-loop-helix (bHLH), high mobility group (HMG), and the T-box family. Members of each of these families have been implicated in pituitary development and function, and there are several precedents for overlapping and critical functions of related genes within a single family for pituitary development [19,65].

Initial validation studies reveal that the single pass sequences obtained from the 3’ end are not as reliable as those from the 5’ end, probably due to the difficulty of accurate amplification through stretches of polyadenine. In rare cases we identified chimeric clones or unexplained differences between the single pass sequence and the validation by complete sequencing, but in most cases the predictions are accurate [57]. Another important type of verification is demonstration that the cDNA is expressed in the pituitary gland because the embryonic pituitary dissection includes some surrounding mesenchyme, oral ectoderm, and neural ectoderm. Complete sequencing and expression studies have not yet been done on all of the genes in the basic helix-loop-helix (bHLH) and high mobility group (HMG) families

reported here or for another study based on gene ontology, biological process terms [94]. Thus, this report represents a foundation for comprehensive validation and future studies.

The PITX gene family

The PITX gene family illustrates how multiple members within the same gene family can have unique and overlapping functions in organ development. PITX2 mutations are one cause of Rieger syndrome, a genetically heterogeneous, dominant disorder characterized by defects in development of the eyes, teeth, and umbilical cord [108]. Some individuals have additional abnormalities including heart defects, and rarely, growth hormone insufficiency. Molecular studies show that missense mutations can cause loss of function, gain of function, or dominant negative effects, but loss of function mutations appear to be the most common, consistent with a haploinsufficiency disorder [109].

Mice heterozygous for loss of function mutations typically have no obvious abnormalities, and eye and tooth defects occur with extremely low penetrance. Multiple genetic backgrounds studied do not increase the penetrance (Camper and Gage, unpublished observations). This suggests that reduced levels of PITX2 are better tolerated in mice than humans. Homozygous mutants die at approximately e12.5 due to severe heart and abdominal body wall defects [67]. Multiple organs are severely affected, including the eyes, teeth, and lungs, which exhibit isomerization. The pituitary gland exhibits hypoplasia, partly caused by enhanced cell death at the ventral aspect that is suggestive of defective sonic hedgehog and/or FGF signaling [65].

Mice homozygous for a partial loss of function allele, *Pitx2*^{neo}, have milder pituitary defects, with little reduction in organ size, but reduction in the *Pou1f1* lineage, resulting in reduced expression of both GH and TSH [66]. The reduced differentiation of this lineage could explain the reduction in GH secretion that characterizes some patients with *PITX2* lesions. Homozygotes for this hypomorphic allele lack gonadotrophs, as there is virtually no detectable *Nr5a1, Lhb* or *Fshb* expression. *Gata2* expression is profoundly reduced as well. There are no reports of hypogonadism in Rieger patients, but it is possible that feedback regulation from the ovaries and testes normalizes gonadotropin production after birth. Indeed, there are some animal models that exhibit transient hypogonadism and delayed puberty but eventually become fertile [110-112].

No *PITX1* mutations have been described in humans to date. *Pitx1* loss of function is recessive lethal in mice [113,114]. Mutants have multiple defects including reduction in the mandible and hind limbs, but the pituitary is mildly affected, exhibiting a reduction in the number of gonadotropes at birth. Despite the fact that *Pitx1* is dispensable for development of a normally sized pituitary gland and differentiation of the major cell types of the anterior lobe at birth, *Pitx1* and *Pitx2* have overlapping functions early in pituitary development and are required for activation of *Lhx3* [65,66,115].

The LHX family

Several LIM homeodomain transcription factors are expressed in the pituitary gland: *Lhx2, Lhx3, Lhx4* and *Isl1*. The first to be implicated in the pituitary gland was *Lhx2*, which was identified by cloning the transcription factor that bound to a critical cis-acting sequence in the gene that encodes the alpha subunit of the pituitary glycoprotein hormones, *Cga*, in the gonadotrope- and thyrotrope-like cell lines, T3-1 and TSH, respectively [116]. Additional studies demonstrated that LHX2 interacts with a LIM domain binding protein (LBD1) to regulate *Cga* expression, and the single-stranded DNA binding protein, SSBP3, regulates the abundance of this complex [117]. We identified *Lhx2* expression in a cDNA library made by subtracting e12.5 Rathke's pouch cDNA from e14.5, suggesting that *Lhx2* could have a role

in pituitary development [57]. To explore the spatial expression of *Lhx2* in early pituitary development we carried out immunohistochemical staining (Figure 2). The majority of the stain appears in the neural ectoderm, dorsal to the prospective posterior lobe and signaling center for BMP and FGF. Staining is also readily detectable in the caudal hindbrain, but little or no staining is detected in the developing pituitary gland at these stages. These immunohistochemistry results are consistent with the failure to detect *Lhx2* transcripts in Rathke's pouch or its derivatives at e10.5 and e14.5 [118-120]. This suggests that LHX2 regulation of *Cga* expression initiates later in development or requires amounts of the protein that are difficult to detect. In some tissues *Lhx2* is expressed after stem cells become committed progenitors but before terminal differentiation occurs [121]. If *Lhx2* plays a role in pituitary gland, it might explain our difficulties in detecting immunoreactivity. *Lhx2* mutants die during gestation from defects in erythropoiesis and exhibit anophthalmia and brain defects [122]. Additional studies are necessary to define the role of *Lhx2* in pituitary gland development.

LHX4 is a LIM homeodomain transcription factor involved in pituitary organogenesis, and crucial for the genesis and development of Rathke's pouch. As a LIM homeodomain transcription factor, LHX4 shows significant structural similarity with LHX3, suggesting a possible overlap between each factor during Rathke's pouch formation [123]. This point is suggested by the observation of similar activities of LHX4 and LHX3 in assays using pituitary hormone promoter genes, and by the role of each factor in ventral motor neuron differentiation [124].

LHX4 mutations produce a wide intra- and inter-family range of phenotypes in humans, both in terms of hypopituitarism and of pituitary/cerebral MRI images of the morphology. The gland may exhibit hypo- or hyperplasia, variable ectopic posterior lobe, and assorted intracranial abnormalities including Chiari syndrome and corpus callosum hypoplasia, and poorly developed sella turcica. To date, 5 heterozygous mutations, including 1 intronic lesion, are reported, suggesting that the mechanism underlying the functional defect is haplo-insufficiency [20,21,125,126].

LHX3 mutations generally lead to MPHD with variablity in corticotrope axis function, abnormal neck rotation, mild to severe hearing impairments, and/or mental retardation. The pituitary can either be hypo- or hyperplastic, or even associated with a microadenoma. Only 9 *LHX3* mutations have been reported, and all are inherited in an autosomal recessive manner [22,127-130].

Mice homozygous for an *Lhx4* disruption induced by homologous recombination ($Lhx4^{-/-}$) have an abnormal pituitary phenotype and die soon after birth from lung defects, whereas heterozygous animals ($Lhx4^{+/-}$) seem unaffected [19,131]. It is possible that homozygous *LHX4* mutation causes lethality in humans. It is noteworthy that mice heterozygous for *Lhx4* loss of function do not appear affected, just as *PITX2* haploinsufficiency is evident in humans but not mice. This species difference in the tolerance for reduced LHX4 and PITX2 levels illustrates a limitation of the comparison between human and mice, though the accessibility of tissues in mice throughout development is invaluable for understanding the mechanisms that underlie human pituitary developmental defects.

Mice homozygous for *Lhx3* disruption ($Lhx3^{-/-}$) exhibit a severe phenotype with death within 24 hours after birth [64]. In contrast, $Lhx3^{+/-}$ mice appear normal. Embryonic $Lhx3^{-/-}$ mice show normal rudimentary Rathke's pouch formation but lack further pituitary development from e10.5 onward and undergo apoptosis [69,132]. Dorsal-ventral patterning is modified with dorsal location of some progenitors normally located in the ventral aspect

of the gland [69]. At birth, *Lhx3*$^{-/-}$ mice lack the anterior and intermediate lobes of pituitary gland.

Lhx3 and *Lhx4* are expressed throughout the pouch at e9.5 [19]. At e12.5 *Lhx3* continues to be expressed throughout the pouch in a gradient with higher protein levels at the dorsal aspect of the pouch [131], while *Lhx4* expression becomes restricted to the developing anterior lobe. At e15.5, *Lhx4* decreases, while *Lhx3* continues to be expressed at high levels. The overlap in gene expression suggests the possibility of functional overlap, which is borne out by analysis of double mutant mice.

Lhx3$^{-/-}$ and *Lhx4*$^{-/-}$ single mutants form a definitive pouch [19,64]. The pouch fails to expand in *Lhx3* mutants due to increased apoptosis resulting in severe pituitary hypoplasia [69,132]. *Lhx3* nulls exhibit reduced ACTH immunostaining and deficiency of all other hormones normally produced in the anterior lobe. The *Lhx4*$^{-/-}$ phenotype is less severe. Specification of five hormone-producing cell types occurs, but expansion of these lineages is greatly reduced, and increased apoptosis is evident [131]. One wild type *Lhx3* or *Lhx4* allele is sufficient for formation of a definitive pouch, as evidenced by analysis of *Lhx3*$^{-/-}$, *Lhx4*$^{+/-}$ and *Lhx3*$^{+/-}$, *Lhx4*$^{-/-}$ mutants. Loss of all alleles for *Lhx3* and *Lhx4* results in formation of a rudimentary pouch, which fails to grow into a definitive pouch and remains under the sphenoid cartilage [19]. The fact that a definitive pouch is formed in the absence of *Lhx3*, but only a rudimentary pouch is formed when both *Lhx3* and *Lhx4* are deleted, suggests that *Lhx4* can substitute for the function of *Lhx3* to support the formation of a definitive pouch. The fact that deletion of *Lhx3* alone results in loss of most of the anterior lobe cell types suggests that *Lhx4* can not substitute for the function of *Lhx3* to activate a pituitary-specific transcription program [133].

Isl1 expression is detectable throughout Rathke's pouch at e9.5 and by e12.5 it is restricted to the ventral, differentiating cells that express *Cga* and *Foxl2* [69,131]. *Lhx3* and *Lhx4* mutants have different effects on *Isl1* expression in the pituitary gland, causing a gain and loss of expression, respectively [69]. *Isl1* is implicated as a lineage specific transcription factor in cell fate choice in progenitors of the retina, heart, forebrain, and motor neurons [134-138]. *Isl1* deficiency causes an arrest in pituitary gland development at an early stage, and the embryos die at e11.5 [134]. Thus, it is an essential regulator of the early steps, but its role, if any, in later stages is unknown.

Forkhead factors are essential for diverse developmental processes

There are several common functional themes among forkhead factors. First, they are responsible for numerous autosomal dominant human developmental disorders. For example, mutations in four different forkhead genes affect ocular development. Mutations in *FOXC1* result in Axenfeld-Rieger anomaly that is characterized by facial, teeth and eye malformations [139-142]. *FOXC2* mutations result in lymphedema and distichiasis, a double row of eyelashes [143,144]. Mutations in *FOXE3* result in malformations in the anterior segment of the eye referred to as Peter's anomaly [145,146]. *FOXL2* mutations cause eyelid malformations and premature ovarian failure [147,148]. Mutations in the orthologous mouse genes cause similar phenotypes [149-161].

Secondly, many forkhead proteins are important for cell cycle regulation and act as tumor suppressors. Overexpression of the forkhead transcription factors *FoxO3a*, *AFX*, or *FoxO1a* cause growth suppression in a number of cell lines, including a Ras-transformed cell line and a cell line lacking a known tumor suppressor [162]. Amplification of the forkhead gene, *FoxA1*, occurs in lung tumors and esophageal adenocarcinomas implicating this gene in tumorigenesis [163].

Finally, approximately half of the known null mutations in forkhead genes result in death before or shortly after birth [164]. This raises three important points: 1) this family of genes is very important for normal development, 2) members of this family generally do not exhibit functional redundancy, 3) mouse models of forkhead gene deficiency are good predictors of the human phenotype. Null mouse models have been described for approximately 31 forkhead genes so far and all except for *Foxo4* result in an abnormal phenotype. Moreover, a number of these knockouts involved members of the same subfamily. For example, *Foxa1* knockout mice die postnatally with severe growth retardation [165], and *Foxa2* knockout mice do not develop beyond embryonic day 8.5 (e8.5) and lack the node, notochord and foregut [166-169]. These data suggest that FOXA1 and FOXA2 cannot compensate for each other, in contrast to *Foxc1* and *Foxc2*. Loss of *Foxc1* results in multiple abnormalities including hydrocephalus, skeletal, ocular, renal and cardiovascular defects, and *Foxc2* deficiency causes skeletal, cardiovascular and ocular defects, indicating that each gene is required independently for development of several organ systems including the skeleton. Loss of both *Foxc1* and *Foxc2* disrupts somitogenesis, revealing an early overlapping function.

Forkhead factors in pituitary development

Foxl2 (*Pfrk*) is expressed in the prospective anterior lobe of the developing pituitary gland starting at e11.5 and continuing into adulthood in gonadotrope and thyrotrope cells of the anterior pituitary [56,170]. In addition, FOXL2 is part of a transcription complex that binds the gonadotropin-releasing hormone receptor gene in gonadotrope cells [171]. Finally, *Foxl2* stimulates expression of *Cga* (αGSU) in cell culture studies and when overexpressed in transgenic mice [170].

Human patients heterozygous for FOXL2 mutations have dominant blepharophimosis ptosis epicanthus inversus syndrome (BPES, eyelid abnormalities) and premature ovarian failure [172]. Mice homozygous for loss of function alleles are mostly nonviable, but those that survive have craniofacial and ovarian abnormalities [160]. While the effects of this deficiency on the pituitaries of most mutants has not been reported, *Foxl2* transgene expression is sufficient to drive expression of *Cga* at ectopic sites within the pituitary primordium, and it has permanent expression in thyrotropes and gonadotropes, suggesting a role in gonadotrope differentiation and function [170]. Consistent with a role in gonadotrope differentiation, FOXL2 regulates the expression of *Gnrhr*, *Fshb*, and follistatin in gonadotropes [171,173,174]. During pituitary development, FOXL2 protein is localized to quiescent cells, suggesting that FOXL2 may be important for inhibiting cell proliferation [170]. This idea is supported by the discovery that mutations in *FOXL2* are associated with aggressive ovarian granulosa cell tumors in children [175].

Several other forkhead genes are expressed in the pituitary gland: *Foxe1*, *Foxf1*, *Foxa1*, and *Foxd1*. *Foxe1* expression is first detected at e9.5 in the ectoderm that will give rise to the anterior pituitary [176]. *Foxf1* is expressed in the mesenchyme of the developing pituitary gland by e9.5 [177]. FOXA1 (a.k.a. HNF-3α) represses growth hormone expression in mouse and human pituitary tissue by binding to the P sequence element C of the human *GH* gene [178]. FOXD1 (or brain factor 2, BF2) is expressed in the diencephalon, retina, and kidney. Mutations in *Foxd1* affect the retina, optic chiasm and kidney [179]. The kidneys are small with decreased ureteric branching, and the mice die within 24 hours after birth due to renal failure. Ectopic BMP signaling is thought to be responsible for the dysmorphology and loss of kidney function [180]. *Foxd1* expression is detectable in the pituitary gland after birth, and during development it is evident in the diencephalon and the mesenchyme surrounding the pituitary at e10.5. Interestingly, these regions are essential for the production of BMPs, which are required for normal pituitary development, and thus FOXD1

regulation of BMP production in these tissues likely contributes to normal pituitary development.

Basic helix-loop-helix family is highly represented in the developing pituitary gland

Members of the basic helix-loop-helix (bHLH) family of transcriptions factors are found in all eukaryotes, and they bind DNA in complexes of homo- or heterodimers through a conserved helix-loop-helix domain [181]. These transcription factors play diverse roles in many developmental pathways and tissues. *Myod*, *Myog*, and *Myf5* are bHLH proteins that are instrumental in the differentiation of skeletal muscle (Reviewed in [182]), while *Ascl1*, *Neurog1*, and *Neurod1* participate in neuronal differentiation [183-189]. Several members of the bHLH family are known to be expressed in pituitary development: *Ascl1*, *Neurod1*, *Neurod4*, and *Hes1*. Roles of these genes in corticotrope, somatotrope, and melanotrope development will be reviewed below.

Neurod1 (also known as BETA2, BHF-1, bHLHa3, NeuroD) is expressed in the embryonic pituitary at e12.5, where it precedes the appearance of POMC in the corticotrope lineage, and it is down regulated at e15.5 [190,191]. *Neurod1* has a role in corticotrope development and function, and recent evidence suggests that it may affect gene expression in gonadotropes as well [192]. Loss of *Neurod1* delays the terminal differentiation of corticotropes from e12.5 to e16.5, indicating that it is a critical factor for promoting corticotrope differentiation, although it is not required [86,193]. NEUROD1 and TPIT (a T box gene officially named TBX19, discussed below) both bind to the POMC promoter to drive expression. However, neither *Neurod1* nor *Tpit* is required for POMC expression, nor does loss of one prevent the binding of the other to the POMC promoter, suggesting that these transcription factors act independently of each other to drive POMC expression [194,195].

Neurod4 (also known as Math3, Atoh3, bHLHa4) is a downstream target of *Pou1f1* that is necessary for maintenance of the somatotrope cells [53].

The bHLH factor, *Hes1*, is necessary for POMC expression in melanotropes in the intermediate lobe [49]. The Hes transcription factors transduce signals from the Notch signaling pathway, along with the Hey class of bHLH transcription factors. Loss of *Hes1* results in a cell fate switch such that intermediate lobe cells differentiate as GH hormone secreting somatotropes instead of POMC expressing melanotropes [49]. *Hes1* plays an additional role in maintaining anterior lobe precursor cells such that they do not differentiate prematurely [52,53,78]. Recent studies demonstrate that this is accomplished through control of cell cycle regulators [196].

The expression patterns of bHLH transcriptions factors *Hey1* and *Hes6* suggest possible roles in the developing pituitary gland, but more studies are necessary to assess their functional significance. The *Hey1* expression domain overlaps with *Hes1* in presumptive precursor cells in the pouch, suggesting that these genes may have overlapping functions in regulating the progression of cells from proliferation to differentiation [49,50]. In contrast, *Hes6* is expressed in the differentiating cells of the anterior lobe, positioning it for maintaining quiescence and/or cell specification [48]. The contributions of these bHLH transcription factors to pituitary gland development are still speculative.

We searched our cDNA encyclopedia for members of the bHLH family and identified 33 genes including expected factors such as *Neurod1*, *Hes6* and *Hey1* as well as additional bHLH family members whose embryonic pituitary expression was not previously known (Table 2). Among these new bHLH family members are the *Id* genes, which act downstream

of BMP signaling [57]. Identification of *Id3* in the library allowed for its use as a reporter of BMP signaling in the ventral diencephalon overlying Rathke's pouch [46].

Interestingly, the bHLH member, *aryl-hydrocarbon receptor* (*Ahr*), and the *aryl-hydrocarbon interacting protein* (*Aip*, a tetratrico peptide repeat containing protein) are contained in the developmental library. AIP is associated with increased risk of pituitary adenomas that secrete GH in some populations; and the molecular mechanism appears to be loss of tumor suppression [197,198]. The overall contribution of mutations in AIP to sporadic adenoma risk world wide appears to be low [199], but the presence of this *Ahr, Aip* complex during the development of the pituitary suggests that an early developmental mechanism for growth regulation may be recapitulated during adenoma formation.

High mobility group genes expressed in the developing pituitary

The high mobility group or HMG class of DNA binding proteins bind DNA through a conserved domain that consists of three alpha helices arranged in an "L-shape." These proteins are known to bend the DNA to which they are bound [200]. The Sox genes, which are related to SRY, the mammalian male sex determination gene, are members of the HMG family, and they have received increased attention recently because of their role in stem cell maintenance. *Sox2* is expressed in embryonic stem cells and stem cells from a variety of tissues, including a potential stem cell population in the pituitary [201,202]. The presence of a pituitary stem cell population that gives rise to all the cell types of the anterior lobe is an exciting development as it has been a proposed mechanism for explaining the plasticity of the pituitary gland, which can change its cellular make up in response to changing physiological demands.

Sox2 and *Sox3* are also critical factors in embryonic pituitary development. Both are expressed in the ventral diencephalon overlying Rathke's pouch [100,202,203]. *Sox3* homozygous null mice have disruptions in the patterning of the ventral diencephalon such that both *Bmp4* and *Fgf8* expression domains, which are critical for Rathke's pouch induction and proliferation, are expanded, resulting in a dysmorphic Rathke's pouch. *Sox3* null mice also have hypopituitarism with decreased levels of GH, LH, FSH, and TSH [97]. *Sox2* heterozygous mice have a dysmorphic Rathke's pouch, which likely results from a similar mechanism as *Sox3* in the ventral diencephalon. Unlike *Sox3, Sox2* is also expressed in Rathke's pouch so that the hypopituitarism observed in *Sox2* heterozygous mice may result from a direct affect of *Sox2* in the anterior lobe [106]. Both *SOX2* and *SOX3* are associated with human disorders; *SOX3* causes X-linked panhypopituitarism [100], while *SOX2* mutations cause anterior pituitary hypoplasia and hypogonadotropic hypogonadism [203].

NUPR1 (p8) is a high mobility group protein that was discovered as a differentially expressed gene in cell lines representing different stages of gonadotrope development [204]. It is expressed during late gestation in mice, concomitant with the activation of the gonadotropin beta subunit genes. It is essential for timely activation of gonadotropin expression [205]. In addition, it is implicated in pituitary tumorigenesis [206,207].

Given the importance of HMG genes in pituitary development we screened our developmental library for expected and novel HMG family members expressed in the pituitary (Table 2). We identified 19 different HMG genes in our libraries. Five members of the Sox group were identified, including *Sox2*. Mixed-lineage leukemia 3 (*Mll3*) is in this set. Myeloid leukemias have deletions in *MLL3* [208]. Further analysis of these HMG genes will enrich our understanding of pituitary gland development, and perhaps adenoma formation.

Several T-box genes are expressed in the developing pituitary gland

The *T-box* genes are a family of transcription factors that bind DNA through the ~200 amino acid T-box domain. They are highly conserved, found in all metazoans and every known vertebrate genome. *T-box* genes can act as transcriptional regulators, either as activators or repressors, in a context-dependent manner. *T-box* genes are involved in early embryogenesis, extra-embryonic tissue survival, cell fate decisions, embryonic patterning and organogenesis (reviewed in [209]). The first *T-box* gene to be identified in the pituitary was *Tbx19* (*Tpit*) [86]. In the absence of TPIT, the POMC lineages, including intermediate lobe melanotropes and anterior lobe corticotropes, fail to differentiate fully [210]. In addition, TPIT inactivation results in a cell fate change, permitting prospective melanotropes to differentiate into gonadotropes and *Pou1f1*-independent thyrotropes. The phenotype of mutant mice predicted the clinical characteristics of human patients with *TPIT* mutations [195].

We identified *Tpit, Tbx2* and *Tbx3* as genes expressed in the developing pituitary gland between e12.5 and e14.5 using our cDNA encyclopedia. *Tbx2* and *Tbx3* expression was detected by RT-PCR in cDNA prepared from dissected pituitary glands at e12.5, e14.5, e18.5, and in *Prop1*$^{df/df}$ cDNA at e14.5. Localization of *Tbx2* and *Tbx3* transcripts by *in situ* hybridization suggests that there is little or no overlap of either gene expression pattern with that of *Tpit* (Fig. 3) and [211]. Both genes are strongly expressed in the developing ventral diencephalon and the posterior lobe of the pituitary gland. Prominent expression of *Tbx3* overlaps the area of the neural ectoderm where factors that induce pituitary growth, BMP and FGF, are expressed. There are no obvious differences in *Tbx3* expression in normal and *Prop1*$^{df/df}$ mice (Fig. 3). *Tbx3* is expressed in the rostral tip, where *Pou1f1*- independent thyrotropes are located [211]. These transcriptional repressors, *Tbx2* and *Tbx3*, might have functional overlap in regulating posterior lobe development because the expression patterns overlap, but neither gene has overlapping expression with *Tpit*. The function of these genes in pituitary development could best be determined using organ specific inducible loss of function models because of the embryonic lethality and ancillary organ defects characteristic of mice homozygous for systemic null alleles [212,213].

Several other *T-box* family members are expressed in and around the developing pituitary gland, but they have not been studied extensively and were not detected in our cDNA encyclopedia. These include *Tbx15, Tbx18*, and the *T-box brain gene 1, Tbr1* (www.genepaint.org). While *Tbx18* and *Tbr1* do not display overlapping expression patterns with *Tbx2, 3*, or *Tpit*, they appear to be expressed in a subset of anterior lobe cells at e14.5, consistent with expression of *Pou1f1* in that region.

Future directions

The developing pituitary cDNA libraries we made and analyzed reveal that the transcriptome has great depth at the time the cells are differentiating and the organ is undergoing substantial growth. There are already several compelling precedents for functional overlap of transcription factors within a particular gene family, i.e. PITX and LHX families. Thus, the discovery of many members of the Forkhead, HMG, bHLH and Tbox families, suggested that there may be genes with essential functions that overlap important transcription factors in these families like *Foxl2, Nupr1, NeuroD1, Hes1, Sox2* and *Sox3*, and *Tpit*. Expression studies suggest that *Tbx2, 3* have distinct functions from *Tpit*, and *Lhx2* may act differently than the *Lhx3, 4* genes. The sheer number of genes in these families that are expressed at a time when they could have an important impact suggests that the ideal strategy for identifying the genes with essential functions is a high throughput screen. Given the strong parallels between the function of orthologous developmental regulators in fish and mammalian pituitary gland, it is possible that zebrafish

could provide the basis for such a screen (reviewed in: [214]). Alternatively, embryonic stem cells have recently been coaxed to differentiate into pituitary hormone producing cells, suggesting embryonic stem cells might be adaptable for screening studies [215,216]. Success with such a high throughput screening approach would be invaluable for nominating candidates for human mutation screening in cases of hypopituitarism of unknown etiology.

Acknowledgments

NIH R37HD030428, R01HD034283 (SAC); University of Michigan Center for Computational Medicine and Biology, Clinical Translational Science Award (SAC), Reproductive Sciences Training Grant (NIH T32 HD07048 (SWD & BSE), NIH NRSA F32-HD046300 (BSE), Endocrine Society, International Scholar's Program (LC), Novo-Nordisk and University of Michigan Center for Genetics in Health and Medicine (FC).

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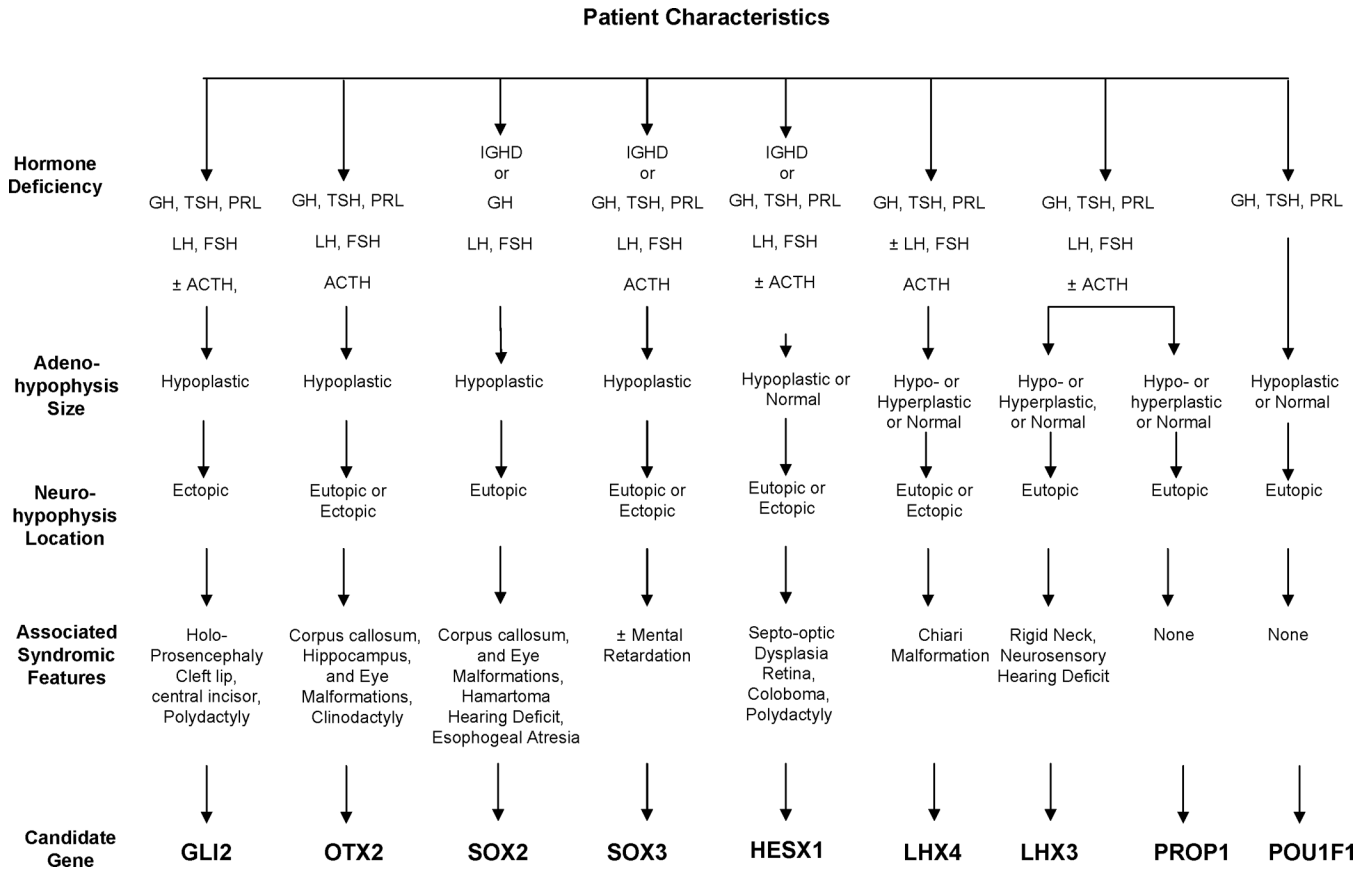

Figure 1. A guide for planning genetic screening for hypopituitary patients based on clinical findings

The patient characteristics identified by hormone screening, imaging studies, and analyses of syndromic features are itemized for each candidate gene based on currently known patient mutations. Note that for most genes there are variable hormone deficiencies, pituitary size and placement, and variable syndromic features.

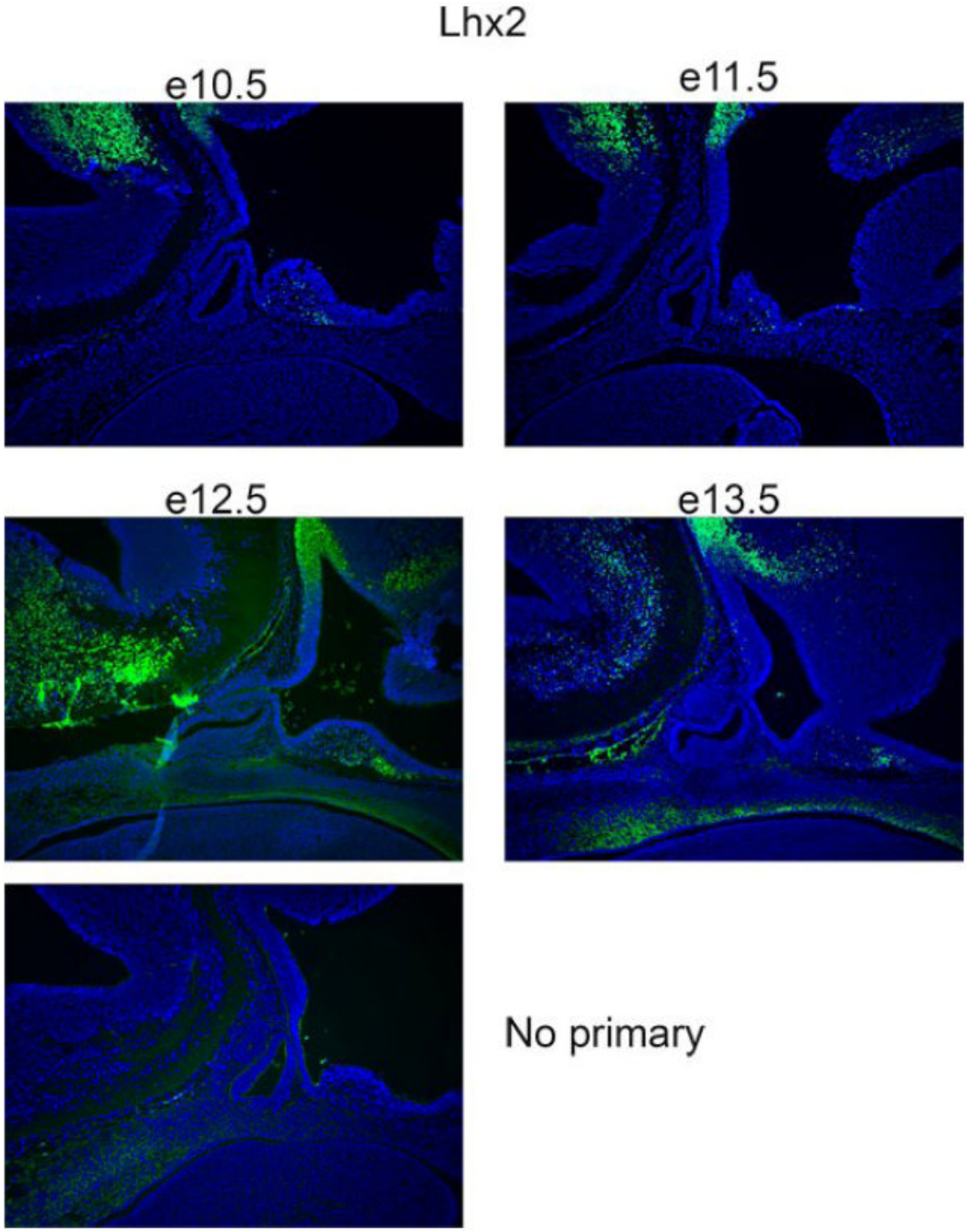

Figure 2. LHX2 expression in the neural ectoderm

LHX2 immunoreactivity (green) is detected in sagittal sections of developing mice at e10.5 through e13.5. DAPI (blue) counterstain reveals nuclei of individual cells.

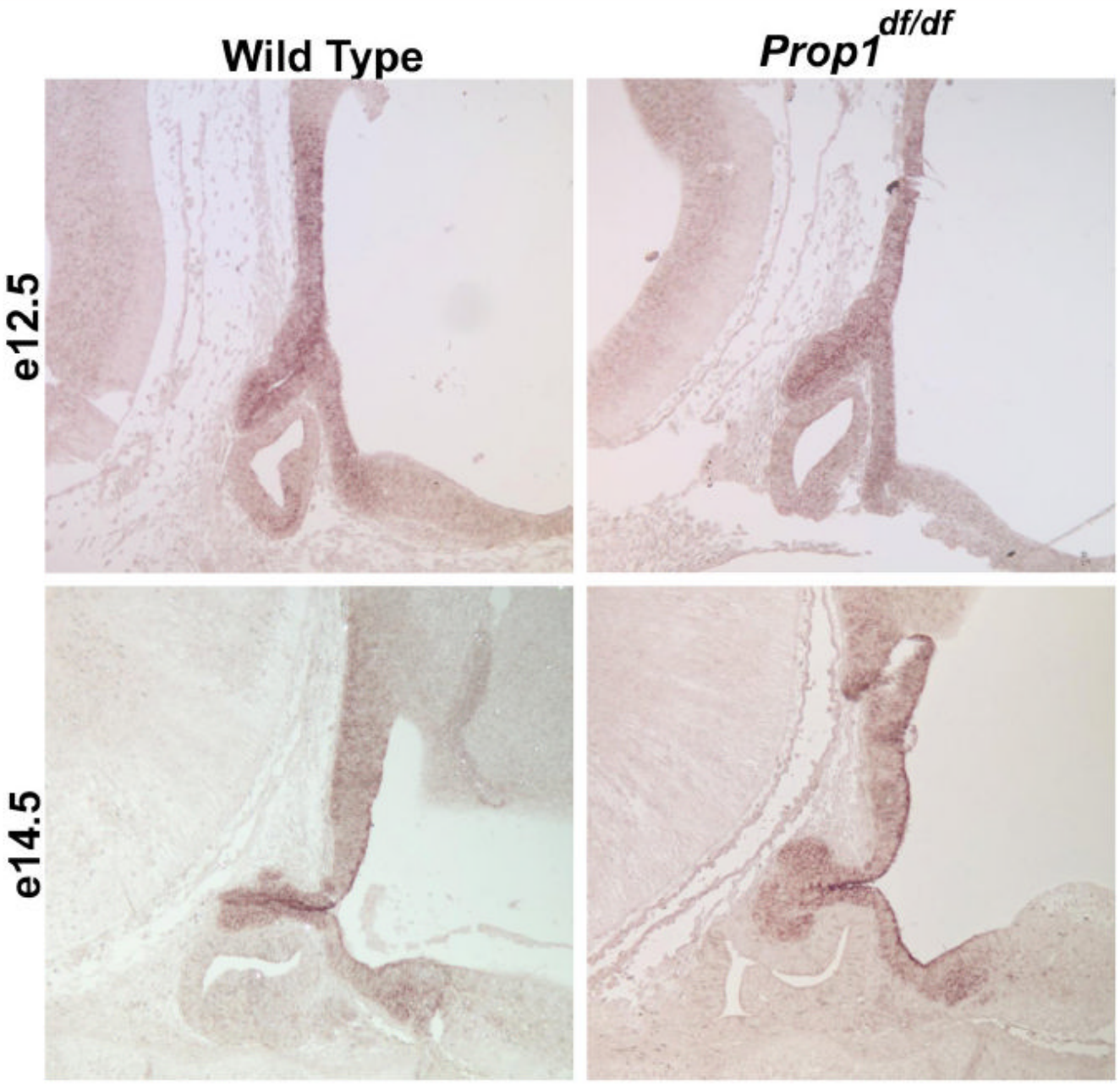

Figure 3. *Tbx3* expression in the ventral diencephalon and prospective posterior lobe of the pituitary gland during development

Tbx3 transcripts are readily detectable in mid-sagittal sections of developing normal and *Prop1*$^{df/df}$ mouse embryos at e12.5 and e14.5 by in situ hybridization.

Table 1

Variety of transcription factor defects affect pituitary function

Gene	DNA binding motif	Clinical features, mouse phenotypes
Syndromic: affecting pituitary development and other head structures		
PITX2	Paired/bicoid homeo	Rieger syndrome: eyes, teeth, umbilical defects Rarely, isolated GH deficiency, haploinsufficient in humans but not obviously so in mice
OTX2	POU homeo	Anophthalmia, microphthalmia, hypopituitarism
LHX3	LIM homeo	GH, TSH, PRL, LH, FSH, ACTH, variable including rigid cervical spine, sensorineural deafness
LHX4	LIM homeo	GH, TSH, PRL, LH, FSH, ACTH, cerebellar and skull defects
SOX2	HMG box	Hypogonadotrophic hypogonadism, rare isolated GH deficiency
SOX3	HMG box	MPHD, mental retardation
HESX1	Paired homeo	Variable including septo-optic dysplasia and severe or mild pituitary hypoplasia or aplasia; GH, TSH, PRL, LH, FSH, ACTH, or IGHD
GLI2	Kruppel family	Holoprosencephaly, cleft lip, central incisor, hypopituitarism
Nonsyndromic: affecting pituitary development		
PROP1	Paired homeo	Progressive hypopituitarism, GH, TSH, PRL, LH, FSH, ACTH
POU1F1	POU homeo	GH, TSH, PRL
TPIT	T box	ACTH
OTX1	POU homeo	No human mutations described, mice have delayed growth, puberty
Syndromic: affecting pituitary development and other peripheral organs		
NR5A1	Nuclear receptor	LH, FSH, 46, XY disorder of sexual development, hypogonadism, premature ovarian failure, adrenal failure

Table 2

Developing pituitary transcriptome contains many transcription factors of the bHLH, HMG and Tbox families

bHLH = basic helix-loop-helix			
Gene symbol	**Full gene name**	**Gene symbol**	**Full gene name**
Ahr	aryl-hydrocarbon receptor	*Mlx*	MAX-like protein X
Arnt	aryl hydrocarbon receptor nuclear translocator	*Mnt*	max binding protein
Arntl	aryl hydrocarbon receptor nuclear	*Msc*	musculin
Ascl1	translocator-like achaete-scute complex homolog-like 1 (*Mash1)*	*Mxi1*	Max interacting protein 1
Bhlhb9	basic helix-loop-helix domain containing, class B9	*Mycl1*	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived
Ebf2	early B-cell factor 2	*Mycn*	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived
Ebf4	early B-cell factor 4	*Neurod1*	neurogenic differentiation 1
Hes6	hairy and enhancer of split 6	*Npas3*	neuronal PAS domain protein 3, transcript variant 2
Hey1	hairy/enhancer-of-split related with YRPW motif 1	*Srebf1*	sterol regulatory element binding factor 1
Hif1a	hypoxia inducible factor 1, alpha subunit	*Tcf23*	transcription factor 23
Hif3a	hypoxia inducible factor 3, alpha subunit	*Tcf25*	transcription factor 25
Id1	inhibitor of DNA binding 1	*Tcf4*	transcription factor 4
Id2	inhibitor of DNA binding 2	*Tcfe2a*	transcription factor E2a
Id3	inhibitor of DNA binding 3	*Tcfe3*	transcription factor E3
Id4	inhibitor of DNA binding 4	*Tcfeb*	transcription factor EB
Max	Max protein	*Usf2*	upstream transcription factor 2
Mesp2	mesoderm posterior 2		
HMG = High mobility group			
Bbx	bobby sox homolog	*Nsbp1*	nucleosome binding protein 1
Cic	capicua homolog	*Sox2*	SRY-box containing gene 2
Hmga1	high mobility group AT-hook 1	*Sox9*	SRY-box containing gene 9
Hmga2	high mobility group AT-hook 2	*Sox11*	SRY-box containing gene 11
Hmgb1	high mobility group box 1	*Sox12*	SRY-box containing gene 12
Hmgb2	high mobility group box 2	*Sox30*	SRY-box containing gene 30
Hmgb3	high mobility group box 3	*Ssrp1*	structure specific recognition protein 1
Hmgn1	high mobility group nucleosomal binding domain 1	*Taf1*	TAF1 RNA polymerase II, TATA box binding protein (TBP)- associated factor
Hmgn3	high mobility group nucleosomal binding domain 3	*Tfam*	transcription factor A, mitochondrial
Mll3	myeloid/lymphoid or mixed-lineage leukemia 3		
T-box			
Tbx19	T-box19 (*Tpit*)	*Tbx3*	T-box 3
Tbx2	T-box 2		