REVIEW

Novel mutations associated with combined pituitary hormone deficiency

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Abstract

The pituitary gland produces hormones that play important roles in both the development and the homeostasis of the body. A deficiency of two or several of these pituitary hormones, known as combined pituitary hormone deficiency, may present in infants or children due to an unknown etiology and is considered congenital or idiopathic. Advancements in our understanding of pituitary development have provided a genetic basis to explain the pathophysiological basis of pituitary hormone disease. Nevertheless, there are several challenges to the precise characterization of abnormal genotypes; these exist secondary to the complexities of several of the hypothalamic/pituitary developmental factors and signals, which ultimately integrate in a temporal and spatial dependent manner to produce a mature gland. Furthermore, the clinical presentation of pituitary hormone disease may be dynamic as subsequent hormone deficiencies may develop over time. The characterization of patients with mutations in genes responsible for pituitary development provides an opportunity to discover potential novel mechanisms responsible for pituitary pathophysiology. The focus of this review is to report the most recent mutations in genes responsible for pituitary development in patients with hypopituitarism and emphasize the importance to physicians and researchers for characterizing these patients. Continuing efforts toward understanding the molecular basis of pituitary development as well as genetic screening of patients with pituitary disease will offer new insights into both diagnostic and potential therapeutic options that will decrease the morbidity and mortality in patients with hypopituitarism.

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Introduction

The development of the human pituitary has been mapped to the expression of transcription factors in a coordinated temporal and spatial sequence that ultimately produces five distinct pituitary cell types. These cell types (along with the hormone product) include the somatotroph (GH), thyrotroph (TSH), lactotroph (prolactin (PRL)), gonadotroph (FSH and LH), and corticotroph (ACTH). Our knowledge regarding pituitary embryogenesis has been gained from experimental animal models, which have helped to categorize developmental genes necessary for pituitary development and function (Dasen & Rosenfeld 2001, Scully & Rosenfeld 2002, Kelberman & Dattani 2006). These transcription factors, in addition to signaling molecules, initiate the formation of Rathke's pouch and direct the proliferation of cells into distinct cell lineages. Any disruption of this cascade adversely affects the development or survival of single or multiple cell types, thus ultimately leading to deficiencies in hormone products.

In humans, combined pituitary hormone deficiency (CPHD) is diagnosed when the production of two or more of these hormones is insufficient or absent. The etiology of CPHD is typically multifactorial and may be secondary to a neurological insult. However, a subset of patients, who present during infancy or childhood with CPHD, is classified as having idiopathic disease. These patients may be considered to have a genetic etiology for their deficiencies, associated with mutations in several of these transcription factors responsible for pituitary development. Although the incidence of reported mutations in CPHD patients remains low, current research continues to delineate the complexities of pituitary development and more clearly define the molecular basis of hormone deficiency in these patients.

Pituitary developmental factors

The identification and characterization of pituitary developmental factors *in vivo* and *in vitro* has helped to

establish a genetic basis for CPHD in humans who harbor similar mutations. These factors include *HESX1*, *LHX3*, *LHX4*, *POU1F1*, *PROP1*, *SIX6*, *OTX2*, *PTX2*, *GLI2*, and *SOX3*, all of which have been shown to play a role in the development and maturation of the pituitary gland. For example, the *Hesx1* mutant mouse model demonstrates forebrain and pituitary malformation along with abnormalities in the septum pellucidum and eye. This phenotype is similar to the human phenotype of septo-optic dysplasia (SOD; Dattani *et al.* 1998). Despite the identification of several *HESX1* mutations in patients with SOD, reports state that <1% of patients with SOD demonstrate coding region mutations (Cohen *et al.* 1999a, Thomas *et al.* 2001, Tajima *et al.* 2003, Sobrier *et al.* 2006, Coya *et al.* 2007, McNay *et al.* 2007). Consequently, the incidence of identifying a mutation in humans remains low, but this may simply reflect that other factors or signaling molecules remain to be identified (Reynaud *et al.* 2006). Figure 1 illustrates a modified overview of pituitary development that has been adapted from previous embryological studies of mice. This cascade of events emphasizes the importance of both the temporal and the spatial expression of both developmental factors and signals required for the

appropriate differentiation and proliferation of the mature pituitary cell types (Fig. 1).

The focus of this review is to inform the reader of recently reported mutations in the most commonly studied transcription factors since ~2001. Although some of the factors have been implicated in hypopituitarism for more than a decade, this review is not intended to present the reader with a comprehensive list of DNA abnormalities in these patients. We hope to highlight the current advancements in molecular genetics that have allowed researchers to not simply identify mutations or deletion of genes but more importantly provide potential mechanisms of pathology through the use of functional studies.

CPHD has a wide spectrum of presentations and quite often hypopituitarism is a developing entity as patients may acquire hormone deficiencies overtime. The range of clinical presentations emphasizes the complexity of pituitary development and suggests that both compensatory mechanisms, as well as interplay between transcription factors, ultimately determine the fate of pituitary function.

HESX1

The human *HESX1* gene, located on chromosome 3p14.3, contains a 185 amino acid open reading frame with four exons and acts as a promoter-specific transcriptional repressor (Dattani *et al.* 1998, Brickman *et al.* 2001). In early development, *Hesx1* transcripts are expressed in the anterior visceral endoderm and neural ectoderm but are eventually restricted to the Rathke's pouch (Hermesz *et al.* 1996, Thomas & Beddington 1996, Andoniadou *et al.* 2007). Its role as a repressor has been demonstrated by its downregulation, which precedes a rise in *PROP1* at mouse embryonic day 12 (e12). Pituitary development continues as the DNA binding motif of *HESX1* in the presence of *PROP1* changes to preferentially bind with *PROP1* as a heterodimer (Kato *et al.* 2010). More recently, the complexities of *HESX1*'s role in pituitary development have been demonstrated through its association with other pituitary factors during development. Table 1 lists several reported interactions between *HESX1* and other factors that when perturbed can affect pituitary development. The discovery and characterization of these interactions for *HESX1* and other developmental factors are important steps in not only delineating pituitary development but also understanding clear mechanisms of pathology in hypopituitarism (Gaston-Massuet *et al.* 2008, Sajedi *et al.* 2008a, Carvalho *et al.* 2010).

In humans, clinical manifestations of mutations in the *HESX1* gene are variable and contribute to CPHD or as part of SOD with an inheritance pattern classified as either autosomal recessive or autosomal dominant

Figure 1 Expression of developmental factors and signals required for anterior pituitary development. A modified overview of murine pituitary development adapted from previous embryological studies illustrates the sequence of events that determine the proliferation and differentiation of mature pituitary cell types. Initially, contact of the oral ectoderm with the neural ectoderm followed by a cascade of events consisting of both signaling molecules and transcription factors expressed in a specific temporal and spatial fashion leads to the development of Rathke's pouch (RP). At embryological day 9.5 (e9-5), expression of BMP4 and Nkx2.1 along with sonic hedgehog (Shh) initiates the development of the primordial RP. The expression of the factors *Gli1*, *Gli2*, *Lhx3*, *Pitx1*, and *Pitx2* result in the development of progenitor pituitary cell types. These events are followed by the expression of *HESX1*, *ISL1*, *PAX6*, *SIX3*, and *SIX6*, which are important for cellular development, proliferation, and migration. The attenuation of *Hesx1* (dotted arrows at ~e12.5) is required for the expression of *Prop1*. By e12.5, RP has formed and by e17.5, differentiation of specific pituitary cell types has been completed. The mature pituitary gland contains the differentiated cell types: somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, and corticotrophs (Dasen & Rosenfeld 2001, Scully & Rosenfeld 2002, Kelberman & Dattani 2006, Zhu *et al.* 2007).

Table 1 Factors reported to interact with HESX1

Transcription factor	Role	Mechanism of interaction	Functional studies	References
SIX3	Homeobox transcription factor important for eye and pituitary development	Regulates through the WNT/ β -catenin signaling pathway to allow normal forebrain development	Six3 +/– Hesx1Cre/+ compound heterozygote mice demonstrate postnatal dwarfism, low thyroid function, and poor testicular development; abnormal pituitary phenotype secondary to disrupted signaling	Gaston-Massuet <i>et al.</i> (2008)
DNA methyltransferase 1 (DNMT1)	Nuclear protein for CpG DNA-methylation and gene regulation coexpressed with HESX1 and acts as binding protein	May induce permanent gene silencing by the specific methylation of CpG residues of HESX1 target genes	Transfected 293T cells demonstrate that HESX1 and DNMT1 form complexes; <i>in situ</i> hybridization with wild-type mouse embryos suggest that HESX1 may repress transcription through methylation of targets	Sajedi <i>et al.</i> (2008a,b)
TLE1/TLE3	Groucho-related co-repressors that bind to transcription factors	Augment repressor role of HESX1	Transfected 293T cells demonstrate TLE1 and TLE3 augment HESX1 repression of PROP1; transgenic mice with ectopic TLE3 and HESX1 expression disrupt differentiation of thyro-trophs and gonadotrophs	Carvalho <i>et al.</i> (2010)
PROP1	Pituitary-specific homeo-box transcription factor	The presence of PROP1 leads to HESX1 motif change: TAATT to TAAT forming heterodimer with HESX1; such interaction allows PROP1 to advance pituitary development	Used random oligonucleotides and an electrophoretic mobility shift assay to examine binding preference of HESX1	Kato <i>et al.</i> (2010)

with incomplete penetrance. This correlates with murine models bearing *Hesx1* mutations, which bear midline cranial defects along with pituitary and eye abnormalities similar to SOD (Dattani *et al.* 1998). A diagram summarizing the most recent *HESX1* mutations and their position in reference to their exon are shown in Fig. 2. A novel p.I26T mutation in exon 1 was reported in a patient with early GH deficiency (GHD), FSH/LH deficiency, and evolving TSH and cortisol deficiency, along with pituitary structural abnormalities (Carvalho *et al.* 2003). Although mutations in *HESX1* associated with pituitary disease appear to modulate the DNA-binding affinity of HESX1, this mutation appeared to impair transcriptional repression (Brickman *et al.* 2001, Carvalho *et al.* 2003).

A group of investigators further studied this mutation through the generation of a mouse model with *Hesx1* mutations p.I26T and p.R160C in order to demonstrate the effects of HESX1 on embryonic development. Both mutations had complete penetrance of pituitary defects; however, the effect on ocular and forebrain development varied with the I26T/I26T less severely

affected than I26T/(null). The authors suggested that the pituitary may be more sensitive to dosage reductions of HESX1 than the eye and forebrain (Sajedi *et al.* 2008b).

More recently, a report of a homozygous frameshift mutation, c.449_450delAC (E150delX167), and a splice defect, c.357+2Tb>C, was demonstrated to interfere with the translation of the homeodomain (Sobrier *et al.* 2006). Functional studies of the mutated HESX1 demonstrated failure to repress PROP1 activity versus wild-type HESX1, which repressed gene activation. Each patient exhibited severe CPHD and magnetic resonance imaging (MRI) abnormalities, but normal optic nerves, and anterior pituitary (AP) aplasia with a flat sella turcica.

LHX3

The LHX3 transcription factor maps to the subtelomeric region of chromosome 9 at band 9q34.3 and contains seven exons and six introns (Sloop *et al.* 2001). The protein possesses two LIM domains involved in

Figure 2 Reported *HESX1* gene mutations associated with human CPHD since 2001. The coding exons are illustrated in the top line, whereas the *HESX1* mRNA structure is shown below with the homeodomain (HD) region in yellow color. The location of reported mutations is indicated by the arrows.

protein–protein interactions, a DNA binding homeodomain and a carboxyl terminus that contains the major transactivation domain. In humans, the identification and study of two isoforms, LHX3a and LHX3b, demonstrates that these factors differentially activate genes, although LHX3a appears to be the most active isoform (Sloop *et al.* 1999, West *et al.* 2004, McGillivray *et al.* 2005). In humans, homozygous loss-of-function mutations in *LHX3* have been identified in patients with hypopituitarism including GH, TSH, PRL, LH, and FSH deficiencies and AP defects and cervical abnormalities with or without restricted neck rotation. ACTH deficiency has also been diagnosed in a subset of patients. Figure 3 illustrates the coding exons of *LHX3a* along with the location of reported human mutations in this gene.

A recent published report of six patients diagnosed with CPHD, restricted neck rotation, scoliosis, and congenital hearing impairment was found to have a novel splice acceptor site mutation in intron 3 with recessive inheritance (Kristrom *et al.* 2009). In another group of patients with CPHD, severe AP hypoplasia, skeletal abnormalities, and bilateral sensorineural hearing loss and also in one family hyperextensible joints and loose skin and two homozygous recessive mutations were reported: an intragenic deletion of 3008 bp resulting in complete loss of exons 2–5 and in an unrelated family a homozygous transversion c.267A>T that led to a nonsense mutation in exon 2 (Rajab *et al.* 2008). The authors of this study also demonstrated an association of LHX3 and the transcription factor SOX2, in which SOX2 may play a role in the regulation of LHX3 expression and therefore function in pituitary development (Rajab *et al.* 2008). A group of investigators reported four novel recessive mutations in four consanguineous pedigrees after screening 366 patients diagnosed with isolated GHD (IGHD) or CPHD. These included two homozygous

mutations, p.E173ter and p.W224ter in exons 3 and 5 respectively, that lead to truncated proteins, a mutation in the homeodomain (p.A210V), and a homozygous gene deletion. All patients had CPHD; however, a hypoplastic pituitary or limited neck rotation was not universal (Pfaeffle *et al.* 2007). In an earlier study, a patient was identified to have a p.159delT (exon 2 delT) *LHX3* mutation causing a frameshift and premature termination codon. The patient was diagnosed with deficiencies of GH, PRL, LH, FSH, and TSH, a rigid cervical spine with limited head rotation, mental retardation, and MRI findings of a hypodense lesion in the pituitary consistent with a microadenoma. The authors suggest that mutations in *LHX3* may also lead to abnormal pituitary cell proliferation (Bhangoo *et al.* 2006).

LHX4

The human *LHX4* gene, located on chromosomal position 1q25 with six exons encoding 390 amino acids, is another member of the LIM homeodomain of transcription factors required for the development of Rathke's pouch (Machinis *et al.* 2001, Liu *et al.* 2002, Reynaud *et al.* 2004, Pfaeffle *et al.* 2008). The *Lhx4* (–/–) mutant mouse demonstrated all five AP cell lineages; however, there are reduced numbers leading to a hypoplastic lobe (Sheng *et al.* 1997). Further studies of LHX4 in conjunction with LHX3 demonstrate that LHX4 is required for the proliferation of lineage precursors, whereas LHX3 is necessary to establish the fate of pituitary precursor cells (Sheng *et al.* 1997).

The clinical manifestations of *LHX4* mutations encompass a varied presentation including GHD and variable TSH, gonadotropin, and ACTH deficiencies. Figure 4 illustrates the position, in reference to the exon, of recently reported mutations diagnosed in patients with CPHD. A p.A210P mutation within the homeodomain was found in a father and two daughters,

Figure 3 Reported *LHX3* gene mutations associated with human combined pituitary hormone deficiency. The figure illustrates the coding exons (boxes) and the *LHX3a* mRNA structure with the coding regions for the LIM domains and the homeodomain (HD). The location of reported mutations is indicated by the arrows.

Figure 4 Reported *LHX4* gene mutations associated with human CPHD since 2001. The coding exons are seen in the first line with the translated regions in black color. The *LHX4* mRNA structure is depicted below with the coding regions for the LIM domains and the homeodomain (HD) shown in blue and yellow color respectively. The location of reported mutations is indicated by the arrows.

with one sister diagnosed with CPHD whereas the other sister and father had GHD only (Pfaeffle *et al.* 2008). Another patient with a p.L190R mutation also in the homeodomain was deficient in GH, ACTH, and TSH (Pfaeffle *et al.* 2008). A third patient with GHD, TSH deficiency, and gonadotropin deficiency was found to have a p.R84C mutation located between the LIM domains of the protein. Functional studies of these mutations demonstrated either a reduced or abolished capacity to activate an α GSU reporter. Despite the previous association of *LHX4* mutations with cerebellar abnormalities, the imaging of these three cases demonstrated only a hypoplastic AP with or without an ectopic posterior pituitary (PP). More recently, a novel mutation, p.V101A in the LIM2 domain in exon 3, was found in a patient with CPHD, a small AP, and ectopic PP. This mutation demonstrated an inability to activate the *POU1F1* and FSH β subunit gene promoter, indicating a loss of function mutation (Tajima *et al.* 2009a). This finding highlights previous findings suggesting that *POU1F1* is an *LHX4* target leading to the expression of GH (Machinis & Amselem 2005). Another familial mutation resulting in CPHD and pituitary abnormalities was identified as a C insertion into the third exon of *LHX4*, resulting in a frameshift mutation with an early stop codon (p.Thr99fs; Castinetti *et al.* 2008). Functional and structural studies of the mutant *LHX4* demonstrated a complete loss of transcriptional activity on the *POU1F1* promoter and a lack of DNA binding (Castinetti *et al.* 2008).

Prophet of Pit1

The human Prophet of Pit1 (*PROP1*) gene, located at chromosomal position 5q35, with three exons encoding 226 amino acids, contains both a paired-like homeodomain for DNA binding and a C-terminal transcriptional activator domain (Duquesnoy *et al.* 1998). *PROP1* acts as both a repressor in downregulating

HESX1 and as an activator of *POU1F1* (Dasen & Rosenfeld 2001). A proposed mechanism is through the Wnt/ β -catenin signaling pathway, in which the binding of *PROP1* with β -catenin represses *Hesx1* expression and promotes *POU1F1*-dependent cellular development (Olson *et al.* 2006). The expression of *PROP1* maintains the *POU1F1* lineage and allows for differentiation of lactotrophs, somatotrophs, and thyrotrophs. It has been shown to be dependent on Notch signaling, which is thought to be important for late lineage differentiation (Zhu *et al.* 2006). The Ames mouse, which harbors a mutated *Prop1*, demonstrates severe dwarfism, hypothyroidism, and infertility (Sornson *et al.* 1996). Recently, Ward *et al.* (2007) created a *Prop1* bacterial artificial chromosome transgene, which rescued the mutant mice with the *Prop1* dwarf phenotype when crossed with wild-type mice.

The frequency of human *PROP1* mutations remains low among sporadic cases but accounts for 29.5–50% of familial cases of CPHD (Turton *et al.* 2005a, Kelberman & Dattani 2006). There are at least 24 reported mutations, which have either homozygous recessive or compound heterozygous inheritance (Kelberman & Dattani 2007). Figure 5 illustrates recently reported human mutations associated with CPHD and the mutation location in reference to the exon affected. The clinical phenotypes of human *PROP1* mutations may be variable and dynamic. Typically, patients present with GH, PRL, and TSH deficiencies, although there may be increased risk for cortisol and gonadotropin deficiency with age. Structurally, there is a normal PP and stalk, but the AP may be hypoplastic, normal, or enlarged, which may wax and wane before

Figure 5 Reported *PROP1* gene mutations associated with human CPHD since 2001. The coding exons are illustrated in the first line with the translated regions depicted in black color. The *PROP1* mRNA structure is shown below with homeodomain (HD) and the transactivation domain (TD) in yellow and orange colors respectively. The relative positions of reported mutations are indicated by the arrows.

eventual involution (Turton *et al.* 2005*a*, Kelberman & Dattani 2007).

The majority of reported mutations involves the DNA-binding homeodomain and in part the transcription domain (as depicted in Fig. 5). There was a recent description of a mutation, however, in the initiation codon in c.2T→C in two sisters who were diagnosed with pituitary hypoplasia and CPHD in addition to late onset of adrenal insufficiency. This first described exon 1 mutation resulted in loss of translation of the PROPI protein (Lemos *et al.* 2006). Another novel homozygous mutation upstream of the homeodomain, microdeletion c.112_124, was described in three family members with CPHD from a large consanguineous family (Agarwal *et al.* 2000). This mutation leads to a premature termination codon that resulted in a protein lacking the homeodomain and transactivation domain. Deletions of the entire *PROPI* gene have also been reported in patients with CPHD, which is similar to those harboring a small deletion, thus suggesting that even a small deletion could result in the loss of activity *in vivo* (Abrao *et al.* 2006, Kelberman *et al.* 2008a,b).

POU1F1 (PIT1)

The human *POU1F1* gene, located on chromosome 3p11, is composed of six exons and has two protein domains: a POU-specific domain and a POU homeo-domain (Rosenfeld 1991, Tatsumi *et al.* 1992). Both domains are important in high-affinity DNA binding of genes encoding GH and PRL as well as in the regulation of TSH β , cAMP, and PRL production (Xu *et al.* 1998, Cohen *et al.* 1999a). Furthermore, there is evidence to suggest that additional regulation of *POU1F1* expression includes retinoic acid induction of the *POU1F1* gene distal enhancer during pituitary development (Cohen *et al.* 1999b). A more recent advancement in our understanding of *POU1F1* expression and regulation includes a novel pituitary regulator known as Atbf1, which is a transcription factor belonging to the ‘giant, multiple-homeodomain, and zinc finger family’ (Qi *et al.* 2008). *Atbf1* mutant mice demonstrated a decrease of *POU1F1* mRNA expression along with diminished expression of GH and TSH β , although products of non-Pit1-dependent cell types such as corticotrophs and melanotrophs were unaffected.

The clinical manifestations of *POU1F1* mutations have been well described in patients and include GH, TSH, and PRL deficiency with inconsistent MRI findings of AP hypoplasia. At least 27 different mutations have been described, with both autosomal recessive and dominant negative inheritance (Kelberman *et al.* 2009). Recently reported *POU1F1* mutations in patients diagnosed with CPHD are illustrated in Fig. 6. A study of 129 patients with CPHD found *POU1F1* mutations in ten patients, of whom five had the R271W mutation, a site considered

Figure 6 Reported *POU1F1* gene mutations associated with human CPHD since 2001. The coding exons are illustrated in the first line with the translated regions depicted in black color. The *POU1F1* mRNA structure is shown below with POU-specific domain (SD) and the POU-homeodomain (HD) in orange and yellow colors respectively. The relative positions of reported mutations are indicated by the arrows.

a mutational 'hotspot' (Turton *et al.* 2005b). Despite a majority of mutations reported as missense/nonsense, a homozygous c.682 + 1G > A intronic splice site mutation affecting the exon 4 splice donor site was described in a patient with GH, TSH, and PRL deficiency and a hypoplastic AP (Snabboon *et al.* 2008). A more recent novel mutation, an IVS2-3insA mutation adjacent to the splicing site, was reported to generate a premature stop codon that resulted in loss of the homeodomain and transactivation domain (Carlomagno *et al.* 2009). The function of POU1F1, furthermore, also shares an important relationship with cyclic AMP response element binding protein (CBP). A 20-year-old man with congenital CPHD and a hypoplastic AP gland was reported to have a mutation in exon 4, p.S179R, identified in the POU-specific domain. Functional studies revealed decreased transactivation of GH1, PRL, POU1F1, and TSH β promoters (Miyata *et al.* 2006). The authors demonstrated that both DNA binding and interaction with co-activators such as CBP are critical to POU1F1's function (Miyata *et al.* 2006).

OTX2

OTX2, a member of the orthodenticle family located on chromosome 14q23.1, is expressed early in anterior neuroectoderm development and is known for its critical role in retinal photoreceptor, rostral brain, and midbrain development (Acampora *et al.* 2000, Kurokawa *et al.* 2004, Henderson *et al.* 2009). Although mutations in *OTX2* are commonly associated with eye abnormalities, several reports demonstrate its importance in pituitary function. A recent short report that reviewed reported *OTX2* mutations states that abnormal pituitary structure and/or function occurred in ~30% of patients with mutations (Schilter *et al.* 2010). Figure 7 illustrates several of the most recently reported

Figure 7 Reported *OTX2* gene mutations associated with human CPHD. *OTX2* is organized into five exons of which only three are translated (black boxes) as shown in the first line. The *OTX2* mRNA structure is below with the coding regions for the homeodomain (HD) and the Otx1 transcription factor (TF) regions colored in yellow and green respectively. The location of reported mutations in patients diagnosed with hormone deficiencies +/- eye abnormalities is indicated by the arrows.

OTX2 mutations in patients whose clinical presentation included abnormal pituitary function. In four patients, three novel heterozygous mutations were reported, which include frameshift and nonsense mutations along with a microdeletion of approximately a 2.9 Mb deletion that includes *OTX2* (Dateki *et al.* 2009). Interestingly, two patients were diagnosed with IGHD, one patient with CPHD and two cases with normal pituitary function, yet all patients had ocular abnormalities. The authors demonstrated either reduced or lost transactivation of four promoters including *GNRH1*, *HESX1*, *POU1F1*, and *IRBP* promoters by these mutations compared with the wild-type gene. This same group also reported a *de novo* heterozygous frameshift mutation in a patient with IGHD and bilateral anophthalmia that led to a loss of the *OTX2* transactivation region (Dateki *et al.* 2008). Brain structural abnormalities along with CPHD and anophthalmia were reported in a boy who was found to have a heterozygous 2 base insertion in the third translated exon of *OTX2* [p.S136fsX178 (c.576-577insCT)] (Tajima *et al.* 2009b). The mutant *OTX2* demonstrated an inability to activate the promoters in the *HESX1* and *POU1F1* genes.

Furthermore, two unrelated patients diagnosed with CPHD and pituitary gland structural abnormalities, but without ocular pathology, were described to harbor a heterozygous *OTX2* mutation in the third translated exon (p.N233S) (Diaczok *et al.* 2008). Despite preserved binding to target genes, the mutant was shown to act as a dominant negative inhibitor of *HESX1* gene expression. Finally, Henderson *et al.* (2009) performed a screening for *OTX2* mutations in 142 patients with eye abnormalities. The authors described a *de novo* heterozygous p.S138X stop mutation in a seven-year-old male who was also noted to have GHD with a normal MRI. Although *OTX2* mutations are rare and often

associated with eye abnormalities, these studies highlight the role of *OTX2* in pituitary pathology as well as the broad spectrum of phenotypes associated with mutations in this gene.

Concluding remarks

Over the past two decades, the pursuit of establishing a genetic basis for the development of idiopathic hypopituitarism has yielded great insight into the mechanisms involved in pituitary development. As more physicians become aware of centers that offer DNA screening in patients with CPHD, we observe increasing number of reports of novel mutations in pituitary developmental factors, which may provide an etiology for a patient's pituitary pathology. The reader should recognize that this review is not comprehensive and, additional reports in patients with CPHD who were found to have mutations in factors such as *GLI2*, *SOX2*, *SOX3*, and others have been reported (Roessler *et al.* 2003, Woods *et al.* 2005, Kelberman *et al.* 2006, 2008a,b, Solomon *et al.* 2007). Furthermore, the characterization of several developmental factors using functional studies has demonstrated that they are interactive and interdependent to ultimately determine cellular function as well as cell survival. This is most likely why hypopituitarism has a heterogeneous clinical phenotype and often a continuum of evolving pathology. Nonetheless, the discovery of single-gene mutations since the 1990s has helped further the advances in mutation detection and offered insights into genotype/phenotype correlation. Understanding the genetics of pituitary pathology offers a chance to improve both the morbidity and the mortality often associated with CPHD. More importantly, the advancing science of gene therapy will someday relieve many patients from the burden of hormone replacement and provide the opportunity for a potential cure.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Abrao MG, Leite MV, Carvalho LR, Billerbeck AE, Nishi MY, Barbosa AS, Martin RM, Arnhold IJ & Mendonca BB 2006 Combined

- pituitary hormone deficiency (CPHD) due to a complete PROP1 deletion. *Clinical Endocrinology* **65** 294–300. (doi:10.1111/j.1365-2265.2006.02592.x)
- Acampora D, Gulisano M & Simeone A 2000 Genetic and molecular roles of otx homeodomain proteins in head development. *Gene* **246** 23–35. (doi:10.1016/S0378-1119(00)00070-6)
- Agarwal G, Bhatia V, Cook S & Thomas PQ 2000 Adrenocorticotropin deficiency in combined pituitary hormone deficiency patients homozygous for a novel PROP1 deletion. *Journal of Clinical Endocrinology and Metabolism* **85** 4556–4561. (doi:10.1210/jc.85.12.4556)
- Andoniadou CL, Signore M, Sajedi E, Gaston-Massuet C, Kelberman D, Burns AJ, Itasaki N, Dattani M & Martínez-Barbera JP 2007 Lack of the murine homeobox gene *Hesx1* leads to a posterior transformation of the anterior forebrain. *Development* **134** 1499–1508. (doi:10.1242/dev.02829)
- Bhangoo AP, Hunter CS, Savage JJ, Anhalt H, Pavlakis S, Walvoord EC, Ten S & Rhodes SJ 2006 Clinical case seminar: a novel LHX3 mutation presenting as combined pituitary hormonal deficiency. *Journal of Clinical Endocrinology and Metabolism* **91** 747–753. (doi:10.1210/jc.2005-2360)
- Brickman JM, Clements M, Tyrell R, McNay D, Woods K, Warner J, Stewart A, Beddington RS & Dattani M 2001 Molecular effects of novel mutations in *Hesx1/HESX1* associated with human pituitary disorders. *Development* **128** 5189–5199.
- Carlomagno Y, Salerno M, Vivenza D, Capalbo D, Godi M, Mellone S, Tiradani L, Corneli G, Momigliano-Richiardi P, Bona G *et al.* 2009 A novel recessive splicing mutation in the *POU1F1* gene causing combined pituitary hormone deficiency. *Journal of Endocrinological Investigation* **32** 653–658.
- Carvalho LR, Woods KS, Mendonça BB, Marcal N, Zamparini AL, Stifani S, Brickman JM, Arnhold IJ & Dattani MT 2003 A homozygous mutation in *HESX1* is associated with evolving hypopituitarism due to impaired repressor–corepressor interaction. *Journal of Clinical Investigation* **112** 1192–1201. (doi:10.1172/JCI18589)
- Carvalho LR, Brinkmeier ML, Castinetti F, Ellsworth BS & Camper SA 2010 Corepressors TLE1 and TLE3 interact with *HESX1* and *PROP1*. *Molecular Endocrinology* **24** 754–765. (doi:10.1210/me.2008-0359)
- Castinetti F, Saveanu A, Reynaud R, Quentien MH, Buffin A, Brauner R, Kaffel N, Albarel F, Guedj AM, El Kholy M *et al.* 2008 A novel dysfunctional LHX4 mutation with high phenotypical variability in patients with hypopituitarism. *Journal of Clinical Endocrinology and Metabolism* **93** 2790–2799. (doi:10.1210/jc.2007-2389)
- Cohen LE, Radovick S & Wondisford FE 1999a Transcription factors and hypopituitarism. *Trends in Endocrinology & Metabolism* **10** 326–332. (doi:10.1016/S1043-2760(99)00180-0)
- Cohen LE, Zanger K, Brue T, Wondisford FE & Radovick S 1999b Defective retinoic acid regulation of the pit-1 gene enhancer: a novel mechanism of combined pituitary hormone deficiency. *Molecular Endocrinology* **13** 476–484. (doi:10.1210/me.13.3.476)
- Coya R, Vela A, Perez de Nancrales G, Rica I, Castano L, Busturia MA, Martul P & GEDPIT group 2007 Panhypopituitarism: genetic versus acquired etiologic factors. *Journal of Pediatric Endocrinology & Metabolism* **20** 27–36.
- Dasen JS & Rosenfeld MG 2001 Signaling and transcriptional mechanisms in pituitary development. *Annual Review of Neuroscience* **24** 327–355. (doi:10.1146/annurev.neuro.24.1.327)
- Dateki S, Fukami M, Sato N, Muroya K, Adachi M & Ogata T 2008 OTX2 mutation in a patient with anophthalmia, short stature, and partial growth hormone deficiency: functional studies using the IRBP, *HESX1*, and *POU1F1* promoters. *Journal of Clinical Endocrinology and Metabolism* **93** 3697–3702. (doi:10.1210/jc.2008-0720)
- Dateki S, Kosaka K, Hasegawa K, Tanaka H, Azuma N, Yokoya S, Muroya K, Adachi M, Tajima T, Motomura K *et al.* 2009 Heterozygous orthodenticle homeobox 2 mutations are associated with variable pituitary phenotype. *Journal of Clinical Endocrinology and Metabolism* **95** 756–764. (doi:10.1210/jc.2009-1334)
- Dattani MT, Martínez-Barbera JP, Thomas PQ, Brickman JM, Gupta R, Martensson IL, Toresson H, Fox M, Wales JK, Hindmarsh PC *et al.* 1998 Mutations in the homeobox gene *HESX1/Hesx1* associated with septo-optic dysplasia in human and mouse. *Nature Genetics* **19** 125–133. (doi:10.1038/477)
- Diazok D, Romero C, Zunich J, Marshall I & Radovick S 2008 A novel dominant negative mutation of OTX2 associated with combined pituitary hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* **93** 4351–4359. (doi:10.1210/jc.2008-1189)
- Duquesnoy P, Roy A, Dastot F, Ghali I, Teinturier C, Netchine I, Cacheux V, Hafez M, Salah N, Chaussain JL *et al.* 1998 Human prop-1: cloning, mapping, genomic structure. Mutations in familial combined pituitary hormone deficiency. *FEBS Letters* **437** 216–220. (doi:10.1016/S0014-5793(98)01234-4)
- Gaston-Massuet C, Andoniadou CL, Signore M, Sajedi E, Bird S, Turner JM & Martínez-Barbera JP 2008 Genetic interaction between the homeobox transcription factors *HESX1* and *SIX3* is required for normal pituitary development. *Developmental Biology* **324** 322–333. (doi:10.1016/j.ydbio.2008.08.008)
- Henderson RH, Williamson KA, Kennedy JS, Webster AR, Holder GE, Robson AG, FitzPatrick DR, van Heyningen V & Moore AT 2009 A rare *de novo* nonsense mutation in OTX2 causes early onset retinal dystrophy and pituitary dysfunction. *Molecular Vision* **15** 2442–2447.
- Hermesz E, Mackem S & Mahon KA 1996 Rpx: a novel anterior-restricted homeobox gene progressively activated in the prechordal plate, anterior neural plate and Rathke's pouch of the mouse embryo. *Development* **122** 41–52.
- Kato Y, Kimoto F, Susa T, Nakayama M, Ishikawa A & Kato T 2010 Pituitary homeodomain transcription factors *HESX1* and *PROP1* form a heterodimer on the inverted TAAT motif. *Molecular and Cellular Endocrinology* **315** 168–173. (doi:10.1016/j.mce.2009.10.006)
- Kelberman D & Dattani MT 2006 The role of transcription factors implicated in anterior pituitary development in the aetiology of congenital hypopituitarism. *Annals of Medicine* **38** 560–577. (doi:10.1080/07853890600994963)
- Kelberman D & Dattani MT 2007 Hypopituitarism oddities: congenital causes. *Hormone Research* **68** (Supplement 5) 138–144. (doi:10.1159/000110610)
- Kelberman D, Rizzoti K, Avilion A, Bitner-Glindzicz M, Cianfarani S, Collins J, Chong WK, Kirk JM, Achermann JC, Ross R *et al.* 2006 Mutations within *Sox2/SOX2* are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. *Journal of Clinical Investigation* **116** 2442–2455. (doi:10.1172/JCI28658)
- Kelberman D, Turton J, Woods K, Mehta A, Al-Khawari M, Greening J, Swift P, Otonkoski T, Rhodes S & Dattani M 2008a Molecular analysis of novel *PROP1* mutations associated with combined pituitary hormone deficiency. *Clinical Endocrinology* **70** 96–103. (doi:10.1111/j.1365-2265.2008.03326.x)
- Kelberman D, de Castro SC, Huang S, Crolla JA, Palmer R, Gregory JW, Taylor D, Cavallo L, Faienza MF, Fischetto R *et al.* 2008b *SOX2* plays a critical role in the pituitary, forebrain, and eye during human embryonic development. *Journal of Clinical Endocrinology and Metabolism* **93** 1865–1873. (doi:10.1210/jc.2007-2337)
- Kelberman D, Rizzoti K, Lovell-Badge R, Robinson IC & Dattani MT 2009 Genetic regulation of pituitary gland development in human and mouse. *Endocrine Reviews* **30** 790–829. (doi:10.1210/er.2009-0008)
- Kristrom B, Zdunek AM, Rydh A, Jonsson H, Sehlin P & Escher SA 2009 A novel mutation in the *LHX3* gene is responsible for combined pituitary hormone deficiency, hearing impairment, and vertebral malformations. *Journal of Clinical Endocrinology and Metabolism* **94** 1154–1161. (doi:10.1210/jc.2008-0325)
- Kurokawa D, Kiyonari H, Nakayama R, Kimura-Yoshida C, Matsuo I & Aizawa S 2004 Regulation of *Otx2* expression and its functions in mouse forebrain and midbrain. *Development* **131** 3319–3331. (doi:10.1242/dev.01220)

- Lemos MC, Gomes L, Bastos M, Leite V, Limbert E, Carvalho D, Bacelar C, Monteiro M, Fonseca F, Agapito A *et al.* 2006 PROP1 gene analysis in Portuguese patients with combined pituitary hormone deficiency. *Clinical Endocrinology* **65** 479–485. (doi:10.1111/j.1365-2265.2006.02617.x)
- Liu Y, Fan M, Yu S, Zhou Y, Wang J, Yuan J & Qiang B 2002 cDNA cloning, chromosomal localization and expression pattern analysis of human LIM-homeobox gene LHX4. *Brain Research* **928** 147–155. (doi:10.1016/S0006-8993(01)03243-7)
- Machinis K & Amselem S 2005 Functional relationship between LHX4 and POU1F1 in light of the LHX4 mutation identified in patients with pituitary defects. *Journal of Clinical Endocrinology and Metabolism* **90** 5456–5462. (doi:10.1210/jc.2004-2332)
- Machinis K, Pantel J, Netchine I, Leger J, Camand OJ, Sobrier ML, Dastot-Le Moal F, Duquesnoy P, Abitbol M, Czernichow P *et al.* 2001 Syndromic short stature in patients with a germline mutation in the LIM homeobox LHX4. *American Journal of Human Genetics* **69** 961–968. (doi:10.1086/323764)
- McGillivray SM, Bailey JS, Ramezani R, Kirkwood BJ & Mellon PL 2005 Mouse GnRH receptor gene expression is mediated by the LHX3 homeodomain protein. *Endocrinology* **146** 2180–2185. (doi:10.1210/en.2004-1566)
- McNay DE, Turton JP, Kelberman D, Woods KS, Brauner R, Papadimitriou A, Keller E, Keller A, Haufs N, Krude H *et al.* 2007 HESX1 mutations are an uncommon cause of septo-optic dysplasia and hypopituitarism. *Journal of Clinical Endocrinology and Metabolism* **92** 691–697. (doi:10.1210/jc.2006-1609)
- Miyata I, Vallette-Kasic S, Saveanu A, Takeuchi M, Yoshikawa H, Tajima A, Tojo K, Reynaud R, Gueydan M, Enjalbert A *et al.* 2006 Identification and functional analysis of the novel S179R POU1F1 mutation associated with combined pituitary hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* **91** 4981–4987. (doi:10.1210/jc.2005-2289)
- Olson LE, Tollkuhn J, Scafoglio C, Krones A, Zhang J, Ohgi KA, Wu W, Taketo MM, Kemler R, Grosschedl R *et al.* 2006 Homeodomain-mediated beta-catenin-dependent switching events dictate cell-lineage determination. *Cell* **125** 593–605. (doi:10.1016/j.cell.2006.02.046)
- Pfaeffle RW, Savage JJ, Hunter CS, Palme C, Ahlmann M, Kumar P, Bellone J, Schoenau E, Korsch E, Bramswig JH *et al.* 2007 Four novel mutations of the LHX3 gene cause combined pituitary hormone deficiencies with or without limited neck rotation. *Journal of Clinical Endocrinology and Metabolism* **92** 1909–1919. (doi:10.1210/jc.2006-2177)
- Pfaeffle RW, Hunter CS, Savage JJ, Duran-Prado M, Mullen RD, Neeb ZP, Eiholzer U, Hesse V, Haddad NG, Stobbe HM *et al.* 2008 Three novel missense mutations within the LHX4 gene are associated with variable pituitary hormone deficiencies. *Journal of Clinical Endocrinology and Metabolism* **93** 1062–1071. (doi:10.1210/jc.2007-1525)
- Qi Y, Ranish JA, Zhu X, Krones A, Zhang J, Aebersold R, Rose DW, Rosenfeld MG & Carriere C 2008 Atbf1 is required for the Pit1 gene early activation. *PNAS* **105** 2481–2486. (doi:10.1073/pnas.0712196105)
- Rajab A, Kelberman D, de Castro SC, Biebermann H, Shaikh H, Pearce K, Hall CM, Shaikh G, Gerrelli D, Grueters A *et al.* 2008 Novel mutations in LHX3 are associated with hypopituitarism and sensorineural hearing loss. *Human Molecular Genetics* **17** 2150–2159. (doi:10.1093/hmg/ddn114)
- Reynaud R, Saveanu A, Barlier A, Enjalbert A & Brue T 2004 Pituitary hormone deficiencies due to transcription factor gene alterations. *Growth Hormone & IGF Research* **14** 442–448. (doi:10.1016/j.ghir.2004.07.001)
- Reynaud R, Gueydan M, Saveanu A, Vallette-Kasic S, Enjalbert A, Brue T & Barlier A 2006 Genetic screening of combined pituitary hormone deficiency: experience in 195 patients. *Journal of Clinical Endocrinology and Metabolism* **91** 3329–3336. (doi:10.1210/jc.2005-2173)
- Roessler E, Du YZ, Mullor JL, Casas E, Allen WP, Gillesen-Kaesbach G, Roeder ER, Ming JE, Ruiz i Altaba A & Muenke M 2003 Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. *PNAS* **100** 13424–13429. (doi:10.1073/pnas.2235734100)
- Rosenfeld MG 1991 POU-domain transcription factors: pou-er-ful developmental regulators. *Genes and Development* **5** 897–907. (doi:10.1101/gad.5.6.897)
- Sajedi E, Gaston-Massuet C, Andoniadou CL, Signore M, Hurd PJ, Dattani M & Martinez-Barbera JP 2008a DNMT1 interacts with the developmental transcriptional repressor HESX1. *Biochimica et Biophysica Acta* **1783** 131–143. (doi:10.1016/j.bbamcr.2007.08.010)
- Sajedi E, Gaston-Massuet C, Signore M, Andoniadou CL, Kelberman D, Castro S, Etchevers HC, Gerrelli D, Dattani MT & Martinez-Barbera JP 2008b Analysis of mouse models carrying the I26T and R160C substitutions in the transcriptional repressor HESX1 as models for septo-optic dysplasia and hypopituitarism. *Disease Models & Mechanisms* **1** 241–254. (doi:10.1242/dmm.000711)
- Schilter KF, Schneider A, Bardakjian T, Soucy JF, Tyler RC, Reis LM & Semina EV 2010 OTX2 microphthalmia syndrome: four novel mutations and delineation of a phenotype. *Clinical Genetics* **79** 158–168. (doi:10.1111/j.1399-0004.2010.01450.x)
- Scully KM & Rosenfeld MG 2002 Pituitary development: regulatory codes in mammalian organogenesis. *Science* **295** 2231–2235. (doi:10.1126/science.1062736)
- Sheng HZ, Moriyama K, Yamashita T, Li H, Potter SS, Mahon KA & Westphal H 1997 Multistep control of pituitary organogenesis. *Science* **278** 1809–1812. (doi:10.1126/science.278.5344.1809)
- Sloop KW, Meier BC, Bridwell JL, Parker GE, Schiller AM & Rhodes SJ 1999 Differential activation of pituitary hormone genes by human Lhx3 isoforms with distinct DNA binding properties. *Molecular Endocrinology* **13** 2212–2225. (doi:10.1210/me.13.12.2212)
- Sloop KW, Dwyer CJ & Rhodes SJ 2001 An isoform-specific inhibitory domain regulates the LHX3 LIM homeodomain factor holoprotein and the production of a functional alternate translation form. *Journal of Biological Chemistry* **276** 36311–36319. (doi:10.1074/jbc.M103888200)
- Snabboon T, Plengpanich W, Buranasupkajorn P, Khwanjaipanich R, Vasinanukorn P, Suwanwalaikorn S, Khovidhunkit W & Shotelersuk V 2008 A novel germline mutation, IVS4 + 1G > A, of the POU1F1 gene underlying combined pituitary hormone deficiency. *Hormone Research* **69** 60–64. (doi:10.1159/000111797)
- Sobrier ML, Maghnie M, Vie-Luton MP, Secco A, di Iorgi N, Lorini R & Amselem S 2006 Novel HESX1 mutations associated with a life-threatening neonatal phenotype, pituitary aplasia, but normally located posterior pituitary and no optic nerve abnormalities. *Journal of Clinical Endocrinology and Metabolism* **91** 4528–4536. (doi:10.1210/jc.2006-0426)
- Solomon NM, Ross SA, Forrest SM, Thomas PQ, Morgan T, Belsky JL, Hol FA, Karnes PS, Hopwood NJ, Myers SE *et al.* 2007 Array comparative genomic hybridisation analysis of boys with X-linked hypopituitarism identifies a 3.9 mb duplicated critical region at Xq27 containing SOX3. *Journal of Medical Genetics* **44** e75. (doi:10.1136/jmg.2007.049049)
- Somson MW, Wu W, Dasen JS, Flynn SE, Norman DJ, O'Connell SM, Gukovsky I, Carriere C, Ryan AK, Miller AP *et al.* 1996 Pituitary differentiation by the prophet of pit-1 homeodomain factor defective in ames dwarfism. *Nature* **384** 327–333. (doi:10.1038/384327a0)
- Tajima T, Hattori T, Nakajima T, Okuhara K, Sato K, Abe S, Nakae J & Fujieda K 2003 Sporadic heterozygous frameshift mutation of HESX1 causing pituitary and optic nerve hypoplasia and combined pituitary hormone deficiency in a Japanese patient. *Journal of Clinical Endocrinology and Metabolism* **88** 45–50. (doi:10.1210/jc.2002-020818)
- Tajima T, Yorifuji T, Ishizu K & Fujieda K 2009a A novel mutation (V101A) of the LHX4 gene in a Japanese patient with combined pituitary hormone deficiency. *Experimental and Clinical Endocrinology & Diabetes* **118** 405–409. (doi:10.1055/s-0029-1225612)

- Tajima T, Ohtake A, Hoshino M, Amemiya S, Sasaki N, Ishizu K & Fujieda K 2009b OTX2 loss of function mutation causes anophthalmia and combined pituitary hormone deficiency with a small anterior and ectopic posterior pituitary. *Journal of Clinical Endocrinology and Metabolism* **94** 314–319. (doi:10.1210/jc.2008-1219)
- Tatsumi K, Miyai K, Notomi T, Kaibe K, Amino N, Mizuno Y & Kohno H 1992 Cretinism with combined hormone deficiency caused by a mutation in the PIT1 gene. *Nature Genetics* **1** 56–58. (doi:10.1038/ng0492-56)
- Thomas P & Beddington R 1996 Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Current Biology* **6** 1487–1496. (doi:10.1016/S0960-9822(96)00753-1)
- Thomas PQ, Dattani MT, Brickman JM, McNay D, Warne G, Zacharin M, Cameron F, Hurst J, Woods K, Dunger D *et al.* 2001 Heterozygous HESX1 mutations associated with isolated congenital pituitary hypoplasia and septo-optic dysplasia. *Human Molecular Genetics* **10** 39–45. (doi:10.1093/hmg/10.1.39)
- Turton JP, Mehta A, Raza J, Woods KS, Tiulpakov A, Cassar J, Chong K, Thomas PQ, Eunice M, Ammini AC *et al.* 2005a Mutations within the transcription factor PROP1 are rare in a cohort of patients with sporadic combined pituitary hormone deficiency (CPHD). *Clinical Endocrinology* **63** 10–18. (doi:10.1111/j.1365-2265.2005.02291.x)
- Turton JP, Reynaud R, Mehta A, Torpiano J, Saveanu A, Woods KS, Tiulpakov A, Zdravkovic V, Hamilton J, Attard-Montalto S *et al.* 2005b Novel mutations within the POU1F1 gene associated with variable combined pituitary hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* **90** 4762–4770. (doi:10.1210/jc.2005-0570)
- Ward RD, Davis SW, Cho M, Esposito C, Lyons RH, Cheng JF, Rubin EM, Rhodes SJ, Raetzman LT, Smith TP *et al.* 2007 Comparative genomics reveals functional transcriptional control sequences in the Prop1 gene. *Mammalian Genome* **18** 521–537. (doi:10.1007/s00335-007-9008-6)
- West BE, Parker GE, Savage JJ, Kiratipranon P, Toomey KS, Beach LR, Colvin SC, Sloop KW & Rhodes SJ 2004 Regulation of the follicle-stimulating hormone beta gene by the LHX3 LIM-homeodomain transcription factor. *Endocrinology* **145** 4866–4879. (doi:10.1210/en.2004-0598)
- Woods KS, Cundall M, Turton J, Rizotti K, Mehta A, Palmer R, Wong J, Chong WK, Al-Zyoud M, El-Ali M *et al.* 2005 Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism. *American Journal of Human Genetics* **76** 833–849. (doi:10.1086/430134)
- Xu L, Lavinsky RM, Dasen JS, Flynn SE, McInerney EM, Mullen TM, Heinzel T, Szeto D, Korzus E, Kurokawa R *et al.* 1998 Signal-specific co-activator domain requirements for pit-1 activation. *Nature* **395** 301–306. (doi:10.1038/26270)
- Zhu X, Zhang J, Tollkuhn J, Ohsawa R, Bresnick EH, Guillemot F, Kageyama R & Rosenfeld MG 2006 Sustained notch signaling in progenitors is required for sequential emergence of distinct cell lineages during organogenesis. *Genes and Development* **20** 2739–2753. (doi:10.1101/gad.1444706)
- Zhu X, Wang J, Ju BG & Rosenfeld MG 2007 Signaling and epigenetic regulation of pituitary development. *Current Opinion in Cell Biology* **19** 605–611. (doi:10.1016/j.ceb.2007.09.011)

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