Hypothalamic and pituitary development: novel insights into the aetiology

Daniel Kelberman and Mehul Tulsidas Dattani

Developmental Endocrine Research Group, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

(Correspondence should be addressed to M T Dattani; Email: m.dattani@ich.ucl.ac.uk)

Abstract

The anterior pituitary gland is a central regulator of growth, reproduction and homeostasis, and is the end-product of a carefully orchestrated pattern of expression of signalling molecules and transcription factors leading to the development of this complex organ secreting six hormones from five different cell types. Naturally occurring and transgenic murine models have demonstrated a role for many of these molecules in the aetiology of combined pituitary hormone deficiency (CPHD). These include the transcription factors HESX1, PROP1, POU1F1, LHX3, LHX4, TBX19, SOX2 and SOX3. The expression pattern of these transcription factors dictates the phenotype that results when the gene encoding the relevant transcription factor is mutated. The highly variable phenotype may consist of isolated hypopituitarism, or more complex disorders such as septo-optic dysplasia and holoprosencephaly. Since mutations in any one transcription factor are uncommon, and since the overall incidence of mutations in known transcription factors is low in patients with CPHD, it is clear that many genes remain to be identified, and the characterization of these will further elucidate the pathogenesis of these complex conditions and also shed light on normal pituitary development.

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Normal forebrain and pituitary development

The development of the pituitary gland is highly complex, occurring at a very early stage of embryogenesis, and is closely linked to that of the forebrain. The pituitary is derived from the midline of the anterior neural ridge and the development of the pituitary gland is similar in all vertebrates studied, with the three lobes of the mature gland (anterior, intermediate and posterior) having a dual embryonic origin. The anterior and intermediate lobes of the pituitary are derived from the oral ectoderm, whilst the posterior pituitary originates from neural ectoderm. Development of the anterior pituitary occurs in distinct stages of differentiation and has been extensively studied in the mouse. The juxtaposition of the oral ectoderm forming Rathke's pouch and the neural ectoderm of the diencephalon is maintained throughout the early stages of pituitary organogenesis, and inductive tissue interactions and extrinsic signalling from the neuroectoderm of the infundibulum are critical for the initial development of the pituitary gland (1). A cascade of signalling molecules and transcription factors play crucial roles

in organ commitment, cell proliferation, cell patterning and terminal differentiation events within the developing pituitary (extensively reviewed elsewhere (2–4)).

In comparison with the rodent, relatively little is known about pituitary development in humans. However, it would appear that several transcription factors involved in the embryological development of the murine pituitary also appear to be involved in human pituitary organogenesis. Spontaneous or artificially induced mutations and gene knockouts in the mouse have led to significant insights into human pituitary disease, with the identification of human mutations in a number of genes that give rise to hypopituitary phenotypes in their respective murine orthologues. Many have been implicated in the aetiology of both murine and human hypopituitarism, including *Hesx1*, *Lhx3*, *Lhx4*, *Prophet of Pit1 (Prop1)*, *Pou1f1* (previously called *Pit-1*), *Pitx2*, *T-Pit (Tbx19)*, *Sox2* and *Sox3* (Table 1). This review will deal primarily with transcription factors implicated in the aetiology of hypopituitarism in humans as a result of the identification and characterization of mutations within these genes in patients and their respective murine orthologues.

HESX1

Given the closely linked development of the pituitary gland and forebrain during normal embryogenesis, it is not

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Table 1 Human mutations causing abnormal hypothalamo–pituitary development and function.

Gene	Phenotype	Inheritance
Combined pituitary hormone deficiency (<i>CPHD</i>)		
<i>POU1F1</i>	GH, TSH, prolactin deficiencies; usually severe; small or normal AP	Recessive, dominant
<i>PROP1</i>	GH, TSH, LH, FSH, prolactin deficiencies; evolving ACTH deficiency; small, normal or enlarged AP	Recessive
Specific syndrome		
<i>HESX1</i>	IGHD, <i>CPHD</i> , septo-optic dysplasia; APH, EPP, absent infundibulum, ACC	Recessive, dominant
<i>LHX3</i>	<i>CPHD</i> (GH, TSH, LH, FSH, prolactin deficiencies), short neck, limited rotation; small, normal or enlarged AP, short cervical spine	Recessive
<i>LHX4</i>	<i>CPHD</i> (GH, TSH, ACTH deficiencies); small AP, EPP, cerebellar abnormalities	Dominant
<i>SOX3</i>	IGHD and mental retardation, panhypopituitarism; APH, infundibular hypoplasia, EPP	X Linked
<i>SOX2</i>	Hypogonadotrophic hypogonadism; APH, bilateral anophthalmia/microphthalmia, abnormal corpus callosum, learning difficulties, oesophageal atresia, sensorineural hearing loss	<i>De novo</i>
<i>TBX19</i>	Neonatal ACTH deficiency	Recessive

AP(H), anterior pituitary (hypoplasia); EPP, ectopic posterior pituitary; ACC, agenesis of corpus callosum.

surprising that abnormalities of the two structures can be linked in human disease. Septo-optic dysplasia (SOD), often referred to as de Morsier syndrome (5), is a rare, highly heterogeneous condition initially described by Reeves in a 7-month-old baby with absence of the septum pellucidum and optic nerve abnormalities (6). The condition is defined loosely by any combination of the triad of optic nerve hypoplasia (ONH), midline neuroradiological abnormalities (such as agenesis of the corpus callosum and absence of the septum pellucidum) and pituitary hypoplasia with consequent panhypopituitarism (5–9). The reported incidence of SOD is 1/10 000 live births (10), and it is thought to be equally prevalent in males and females. Although the condition is generally sporadic, familial cases have been described.

The phenotype is highly variable, and a diagnosis of SOD usually made if two or more of the triad of ONH, hypopituitarism or midline brain defects are present. According to Morishima & Aranoff (11), ~30% of SOD cases have complete manifestations, 62% have the complication of hypopituitarism and 60% have an absent septum pellucidum. The condition is thought to be more frequent in children born to younger mothers (mean maternal age 22 years) (8, 12), although this has been disputed (13), and in some studies, there is a preponderance of primigravida mothers (13, 14). Recently, cases of both isolated ONH and SOD have been shown to cluster in high population density, inner city areas with high rates of unemployment and teenage pregnancy (10).

It is believed that the condition is due to a developmental insult that would have to occur during the critical period of morphogenesis for these structures, corresponding to between 4 and 6 weeks of gestation in humans. Several aetiologies have been postulated to account for the sporadic occurrence of SOD, such as viral infections, environmental teratogens and vascular or degenerative damage (15, 16). However, the precise aetiology of the condition remains unknown and is most likely to be multifactorial, with a combination of genetic and environmental factors. Familial cases of SOD are

rare, but implicate a genetic defect underlying the developmental mechanisms involved, and are more frequently associated with an autosomal recessive manner of inheritance (17–19) although dominant inheritance has also been reported (12, 20–22).

The first gene to be implicated in SOD is *HESX1*, a member of the paired-like class of homeobox genes (23) which functions as a transcriptional repressor, with repression domains within the N-terminal region and the DNA-binding homeodomain (24, 25). It is one of the earliest markers of murine pituitary development, being initially expressed during gastrulation in a region fated to form the forebrain, and then being restricted to the ventral diencephalon by embryonic day (E) 9.0, and also in the thickened layer of oral ectoderm that will give rise to Rathke's pouch, the primordium of the anterior pituitary. *Hesx1* continues to be expressed in the developing anterior pituitary until E12, when expression is attenuated corresponding to progressive pituitary cell differentiation, finally becoming undetectable by E13.5.

Homozygous disruption of *Hesx1* in mice is associated with a phenotype closely resembling that of SOD. Abnormalities are fully penetrant, although variable, in *Hesx1* null mice and features include a reduction in prospective forebrain tissue, absence of developing optic vesicles, markedly decreased head size, craniofacial dysplasia with a short nose, severely reduced forebrains with no sign of telencephalic vesicle or infundibulum development, absence of olfactory placodes, hypothalamic abnormalities and aberrant morphogenesis of Rathke's pouch (26). Further analysis of neonatal and adult mutants revealed hypoplastic nasal cavities, hypoplastic olfactory bulbs, microphthalmia and anophthalmia, with abnormalities of the septum pellucidum and corpus callosum. A more detailed analysis of the *Hesx1*-null mutants revealed that a proportion of mice (5%) exhibited a more severe phenotype in which no anterior pituitary gland was formed, although thickening of the oral ectoderm was detected (24). The majority of these mice showed

multiple invaginations of the oral ectoderm, and aberrant morphogenesis of Rathke's pouch, which displayed abnormal bifurcations resulting in the apparent formation of multiple pituitary glands.

In light of the similarity between the phenotype of *Hesx1* null mice and SOD, we investigated the role of the human homologue of *HESX1* (OMIM 601802) in patients with SOD. A homozygous missense mutation (R160C) in the homeobox of *HESX1* was initially identified in a highly consanguineous family, in which two affected siblings presented with optic nerve hypoplasia, hypoplastic corpus callosum and hypoplasia of the anterior pituitary gland with an undescended/ectopic posterior pituitary and consequent panhypopituitarism (17, 26). This substitution leads to a loss of DNA binding. The parents were heterozygous for the mutation and phenotypically normal, consistent with an autosomal recessive mode of inheritance. Four additional homozygous mutations have subsequently been identified (Table 1), with phenotypes ranging from evolving hypopituitarism in the absence of midline and eye defects through SOD and pituitary aplasia (27, 28). To date, reports of screening patients with sporadic SOD have yielded eight novel heterozygous mutations within *HESX1* (Table 2). These heterozygous mutations are generally associated with milder phenotypes when compared with the homozygous mutations, leading to growth hormone deficiency (GHD) with or without an undescended posterior pituitary (20), although optic nerve hypoplasia as well as midline forebrain abnormalities may be associated (21). The penetrance may be variable, and the presence of a mutation is not always associated with a phenotype. We have now screened over 800 patients with SOD and hypopituitarism, and identified mutations in <1% of

individuals confirming the rarity of *HESX1* mutations (12). As a result of this screening, we have identified a number of sequence variants, including a change of unknown functional importance in a highly conserved base in a known *cis*-regulatory region upstream of *HESX1*. Whether these variants contribute to the pathogenesis remains to be proven. The overall frequency of *HESX1* mutations is low, suggesting that mutations in other known or unknown genes contribute to this complex disorder, together with a likely contribution from environmental factors (10, 29).

SOX3

SOX3 (OMIM 313430) is a member of the SOX (SRY-related high mobility group (HMG) box) family of transcription factors, which were initially identified based on homology to the conserved binding motif of the HMG class, present in the mammalian sex-determining gene, *SRY* (30). Approximately 20 different SOX genes have been identified in mammals, and variation in homology exhibited within the HMG box between different members allows them to be grouped into different subfamilies (31). SOX3 was among the first of the SOX genes to be cloned, and together with SOX1 and SOX2, belongs to the SOXB1 subfamily exhibiting the highest degree of similarity to *SRY* (30). SOX3 is encoded by a single exon producing a transcript with a coding region of ~1.3 kb, mapping to chromosome Xq27. The SOX3 protein consists of a short 66 amino acid N-terminal domain of unknown function, a 79 amino acid DNA-binding HMG domain and a longer C-terminal domain, containing four polyalanine

Table 2 Reported mutations in *HESX1*.

Mutation	Inheritance	Endocrine phenotype	Neuroradiological findings	Reference
Q6H	Dominant	GH, TSH, LH, FSH deficiency	AP hypoplasia, ectopic PP	(20)
I26T	Recessive	GH, LH, FSH deficiency; evolving ACTH, TSH deficiency	AP hypoplasia, ectopic PP, normal ON	(113)
c.306_307insAG	Dominant	GH, LH, FSH deficiency; hypothyroidism	AP hypoplasia, ON hypoplasia	(22)
Q117P	Dominant	GH, TSH, ACTH, LH, FSH deficiency	AP hypoplasia, ectopic PP	(114)
c.357+2T>C	Recessive	GH, TSH, ACTH, PRL deficiency	AP aplasia, normal PP, normal ON	(28)
Alu insertion (exon 3)	Recessive	Panhypopituitarism	AP aplasia, hypoplastic sella, normal PP and infundibulum	(27)
E149K	Dominant	GH deficiency	AP hypoplasia, ectopic PP, infundibular hypoplasia	(12)
c.449_450delCA	Recessive	GH, TSH, ACTH deficiency	AP aplasia, normal PP, normal ON, thin CC, hydrocephalus	(28)
R160C	Recessive	GH, TSH, ACTH, LH, FSH deficiency	AP hypoplasia, ectopic PP, ON hypoplasia, ACC	(26)
S170L	Dominant	GH deficiency	Normal AP, ON hypoplasia, ectopic PP, partial ACC	(20)
K176T	Dominant	GH deficiency, evolving ACTH, TSH deficiency	Ectopic PP	(114)
g.1684delG	Dominant	GH deficiency	AP hypoplasia, ON hypoplasia, ACC, absent PP bright spot	(21)
T181A	Dominant	GH deficiency	AP hypoplasia, normal ON, absent PP bright spot	(20)

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

stretches, shown to be involved in transcriptional activation (30, 32).

Members of the *SOXB1* subfamily of genes are expressed throughout the developing central nervous system (CNS) and are some of the earliest neural markers that are believed to play a role in neuronal determination (33). High levels of expression have also been noted in the ventral diencephalon, including the infundibulum and presumptive hypothalamus (34). Targeted disruption of *Sox3* in mice results in mutants that have a variable and complex phenotype, including craniofacial abnormalities, midline CNS defects and a reduction in size and fertility (34, 35). *Sox3* mutant mice of both sexes are born with expected frequency, showing no evidence for embryonic lethality, and approximately one-third of mutant mice are viable and fertile with no gross abnormalities. Heterozygous females are mosaic with respect to the mutation due to X inactivation and generally appear normal, although some display a mild craniofacial phenotype. However, ~43% of *Sox3* null mice do not survive to weaning, and the most severely affected mice exhibit profound growth insufficiency and general weakness with craniofacial defects including overgrowth and misalignment of the front teeth and abnormality of the shape of the pinna, which was completely absent in some animals (34).

The mutants had a variable endocrine deficit, the extent of which was correlated with body weight. Pituitary levels of growth hormone (GH), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyrotrophin (TSH) were all lower in mutant mice when compared with wild-type mice at 2 months of age (34). Histological analysis of the pituitary gland at this stage revealed a hypoplastic anterior lobe with the presence of an additional abnormal cleft disrupting the boundary between the anterior and intermediate lobes. Further examination of *Sox3* mutant embryos revealed that Rathke's pouch displayed an abnormally expanded and bifurcated appearance in mutant embryos, which possibly results in the additional cleft observed at later stages of development and in the adult pituitary. *Sox3* is not expressed in Rathke's pouch; however, it is expressed at high levels in the ventral diencephalon including the infundibulum, which provides necessary inductive signals for the formation of the anterior pituitary (4). In *Sox3* mutants, the evagination of the infundibulum was less pronounced than observed in wild-type mice and the presumptive hypothalamus thinner and shorter (34). This suggests that the hypopituitary phenotype observed in mutant mice arises as a secondary consequence of the absence of *Sox3* in the ventral diencephalon.

In humans, tandem duplications involving chromosome Xq26-27 have been identified in several pedigrees with mental retardation and hypopituitarism (36-39). Using array comparative genomic hybridization, Solomon *et al.* (39) defined a critical duplication region of 3.9 Mb between Xq26.1 and Xq27.3 containing 18

annotated transcripts including *SOX3*. The phenotypes of affected males with X-linked hypopituitarism involving duplications within this region are variable. All affected males manifest GH deficiency and varying degrees of developmental delay or mental retardation. Some individuals have been reported to have varying combinations of deficiencies of other hormones including adrenocorticotrophin (ACTH), TSH or gonadotrophins, and complete panhypopituitarism has been documented in some cases. Unaffected carrier females in these pedigrees show preferential inactivation of the duplicated X chromosome; however, a rare family with five affected females presenting with short stature secondary to hypopituitarism, speech and language problems, hearing impairment and facial dysmorphism has also been reported with a 7.5 Mb duplication of chromosome Xq26.2-q27.1 (40). The authors suggested that the duplication may disrupt *SOX3* resulting in hemizygosity in affected females, although this was not confirmed at the molecular level. Woods *et al.* (41) described a pedigree with two half brothers manifesting evidence of X-linked hypopituitarism, in the absence of developmental delay, and harbouring a submicroscopic duplication on chromosome Xq27.1, further refining the critical interval to ~690 kb. The first child manifested GHD and borderline low FT4 concentrations, with hypoplasia of the lower half of the infundibulum and an abnormal corpus callosum, which contained a cyst within the splenium. The second sibling manifested a more severe phenotype of combined pituitary hormone deficiency (CPHD), with complete absence of the infundibulum and hypoplastic genitalia; however, his corpus callosum appeared normal. Both patients had anterior pituitary hypoplasia and an undescended posterior pituitary as revealed by magnetic resonance imaging (MRI). The duplication identified in this family is the smallest described to date encompassing *SOX3* and two additional transcripts of unknown function, neither of which is expressed in the developing infundibulum (41), suggesting that the phenotype in these patients is due to the presence of an additional copy of *SOX3*.

Further implication of *SOX3* in X-linked hypopituitarism comes from the identification of patients harbouring an expansion of one of the polyalanine tracts within the gene (41, 42). Laumonnier *et al.* (42) identified an in-frame duplication of 33 bp occurring between nucleotides 711 and 743 and co-segregating in affected males in a large family with X-linked mental retardation and GH deficiency. This mutation encodes an additional 11 alanine residues and is predicted to cause expansion of the normal polyalanine tract from 15 to 26 residues. Additionally, a second novel expansion of seven alanine residues within the same tract has been identified in three siblings of a consanguineous pedigree presenting with profound and complete panhypopituitarism in association with anterior pituitary hypoplasia, an absent or hypoplastic infundibulum and an

undescended posterior pituitary. There was no evidence of mental retardation or craniofacial dysmorphism in these individuals.

In vitro analysis of the SOX3+7 alanine expansion identified by Woods *et al.* (2005) revealed that the expansion leads to partial loss of function possibly due to impairment of nuclear localization, as the mutant protein was largely excluded from the nucleus when compared with wild-type SOX3 which is predominantly localized within the nucleus of the cell (41). Similar findings have also been shown for the mutant SOX3 protein containing the +11 alanine expansion, which forms aggregates within the cytoplasm (43).

In summary, both duplications of Xq27 encompassing SOX3 and loss-of-function polyalanine expansion mutations are essentially associated with similar phenotypes, predominantly infundibular hypoplasia, suggesting that gene dosage of SOX3 is critical for normal development of the diencephalon and infundibulum, and consequently the anterior pituitary.

SOX2

SOX2 (OMIM 184429) is also a member of the same SOXB1 subfamily as SOX3 and SOX1 (OMIM 602148). In the mouse, initial expression of *Sox2* is detected at 2.5 dpc at the morula stage, and then in the inner cell mass of the blastocyst at 3.5 dpc. Later expression of *Sox2*, following gastrulation, is restricted to the presumptive neuroectoderm, and by 9.5 dpc, it is expressed throughout the brain, CNS, sensory placodes, branchial arches, gut endoderm and the oesophagus and trachea (44, 45). Homozygous loss of *Sox2* results in peri-implantation lethality, whereas *Sox2* heterozygous mice appear relatively normal but show a reduction in size and male fertility (46). Further studies that have resulted in the reduction of *Sox2* expression levels below 40% when compared with the normal levels result in anophthalmia in the affected mutants (47).

Given the observation of growth retardation and reduced fertility, we have recently investigated the role of *Sox2* in murine pituitary development, showing that a proportion of heterozygous animals manifested a variable hypopituitary phenotype, with hypoplasia and abnormal morphology of the anterior pituitary gland with concomitant reduction in levels of GH, LH, ACTH and TSH (48). Similar to its murine counterpart, the human SOX2 gene is composed of a single exon encoding a 317 amino acid protein containing an N-terminal domain of unknown function, a DNA-binding HMG domain and a C-terminal transcriptional activation domain. Twelve heterozygous *de novo* mutations in SOX2 were previously reported in 14 human patients associated with bilateral anophthalmia or severe microphthalmia with additional abnormalities including developmental delay, learning difficulties, oesophageal atresia and genital abnormalities (45,

49–52). All of these mutations occurred *de novo* and included five nonsense, four frameshift, one deletion and two missense mutations. We have subsequently reported six patients harbouring *de novo* heterozygous mutations in SOX2, resulting in loss of function of the mutant protein, four of whom were previously unreported (c.60insG, c.387delC, Y160X and c.479delA). Clinical evaluation revealed that in addition to anophthalmia/microphthalmia, SOX2 mutations were also associated with anterior pituitary hypoplasia and hypogonadotrophic hypogonadism, which resulted in the absence of puberty in all six patients and genital abnormalities in males. All affected individuals exhibited learning difficulties with other variable manifestations including hippocampal abnormalities, defects of the corpus callosum, oesophageal atresia and sensorineural hearing loss (48). The mutations were associated with significant loss of function that included loss of DNA binding, nuclear localization and transcriptional activation, suggesting that these phenotypes arise as a result of haploinsufficiency of SOX2 in development. More recently, Sato *et al.* (53) have reported an additional patient with a missense mutation in the HMG domain (L75Q), resulting in decreased DNA-binding affinity of the mutant protein. The affected individual manifested unilateral right-sided anophthalmia and isolated hypogonadotrophic hypogonadism, with a normal anterior pituitary and normal mental development, further supporting a critical role for SOX2 in the regulation of correct gonadotrophin production in addition to eye development.

LHX3/LHX4

Lhx3 is a member of the LIM family of homeobox genes that are characterized by the presence of a unique cysteine/histidine-rich zinc-binding LIM domain. The protein contains two such tandemly repeated LIM motifs between the N-terminus and the homeodomain, which are likely to be involved in protein–protein interactions (54, 55). *Lhx3* is one of the earliest transcription factors expressed within the developing pituitary, initially detectable with strong uniform expression in Rathke's pouch. Expression is maintained in the pouch and is subsequently restricted to fields fated to form the anterior and intermediate lobes. By 16.5 dpc, the gene is expressed in all regions of the developing anterior and intermediate pituitary, but not the posterior gland, and continued expression is essential for the establishment of hormone-producing cell types. Expression persists throughout development and is also detected in the adult pituitary, suggesting a role in maintenance of one or more of the mature anterior pituitary cell types (54, 56). In addition to the pituitary, *Lhx3* is also detected transiently in regions of the developing spinal cord, prior to neural tube closure, and later within the restricted regions of the hindbrain, in addition to the cells in immediate proximity to

the otic vesicles. Mice with a targeted homozygous disruption of *Lhx3* die shortly after birth, although the cause of death is unknown, and exhibit pituitary aplasia, suggesting an essential role of *Lhx3* in differentiation and proliferation of anterior pituitary cell lineages. Although Rathke's pouch is initially formed in *Lhx3* null mice, a failure of proliferation and growth results in a lack of the anterior and intermediate lobes of the pituitary with depletion of all hormone-producing cell types except corticotrophs, which differentiate and express proopiomelanocortin (POMC) but fail to proliferate (57, 58). The posterior pituitary appeared grossly unaffected, as did the hindbrain and spinal cord, and heterozygous mice were apparently normal and fertile (57).

Homozygous mutations in *LHX3* (OMIM 600577) have currently been identified in 12 patients from seven unrelated consanguineous families, all of which result in loss of *LHX3* function (59–63). The patients presented with an endocrine phenotype similar to that observed in individuals with *PRO1* mutations with a deficit in all anterior pituitary hormones except ACTH. This was additionally associated in 9 out of 12 patients with a short rigid, cervical spine with limited head rotation and trunk movement. As with *PRO1*-deficient patients, pituitary morphology is variable between patients with *LHX3* mutations, with two patients from one family exhibiting small anterior pituitaries with a normal posterior pituitary and midline structures on MRI. However, an additional individual from an unrelated family demonstrated a markedly enlarged anterior pituitary that was not evident in a previous MR scan performed 10 years previously (59). Additionally, Bhargoo *et al.* (2006) recently reported a further patient with a hypointense lesion in the anterior pituitary consistent with a microadenoma (61).

Lhx4 is closely related to *Lhx3* and is expressed in specific fields of the developing brain and spinal cord. Similar to *Lhx3*, *Lhx4* is initially expressed throughout the invaginating Rathke's pouch; however, subsequent expression is transient and restricted to the future anterior lobe, whereas *Lhx3* expression is maintained throughout the whole pouch. A similar pattern of transient expression of *LHX4* (OMIM 602146) in the developing pituitary and spinal cord, with continuous expression of *LHX3*, has also been observed in human development (62). Null mutations of *Lhx4* do not prevent pouch formation; however, the pouch is defective with reduced numbers of the various anterior pituitary cell types. *Lhx3*^{-/-}, *Lhx4*^{-/-} double mutant mice show a more severe phenotype than either single mutant with an early arrest of pituitary development; additionally, a single normal copy of either *Lhx3* or *Lhx4* in murine development is sufficient for the formation of a definitive pouch, thereby suggesting that these two genes may act in a redundant manner during early pituitary development (64).

To date, only a single mutation within *LHX4* has been reported in a three-generation family, in which the mutation, which abolishes normal splicing, segregates in a dominant and fully penetrant manner. The probands presented with short stature and were found to be GH, TSH and ACTH deficient (65). Anterior pituitary hypoplasia was revealed by MRI with an ectopic posterior pituitary and absent pituitary stalk. However, other affected family members presented with short stature associated with isolated GH deficiency and a normal posterior pituitary. Additional manifestations included a poorly formed sella turcica and pointed cerebellar tonsils, suggesting that *LHX4* may be a factor that tightly coordinates brain development and skull shaping.

TBX19

TBX19 (OMIM 604614; also referred to as *TPIT*) is a member of the T-box family of transcription factors that contain a homologous DNA-binding domain (the T-box) first identified in the mouse Brachyury (*T*) gene. *Tbx19* is not expressed within Rathke's pouch, but by 11.5 dpc, expression appears within the developing anterior pituitary gland, as well as a region of the ventral diencephalon. Later expression in the developing and adult murine pituitary is detected in only two cell types, the POMC (OMIM 176830)-expressing corticotrophs in the anterior lobe and melanotrophs forming the intermediate lobe. It is essential for cell-specific expression of the POMC gene and terminal differentiation of the pituitary corticotroph lineage (66–68).

Consistent with the exclusive expression in pituitary POMC cells, mutations in *TBX19* are associated at high frequency with neonatal isolated ACTH deficiency, but never in cases of juvenile-onset deficiency. Twelve independent mutations have been identified, including nonsense, missense, frameshift and splicing mutations in addition to a large genomic deletion of 5.2 kb encompassing exons 2 and 3 of the gene (69–72). All of these mutations have been shown or are predicted to result in loss of *TBX19* function, and all patients appear to be homozygous or compound heterozygous for *TBX19* mutations, with unaffected heterozygous parents, implying a recessive mode of inheritance. Vallette-Kasic *et al.* have reported the largest series to date and demonstrated *TBX19* mutations in 17 out of 27 patients from 21 unrelated families, suggesting that mutations in the gene are the principal molecular cause of congenital neonatal isolated ACTH deficiency. Moreover, three patients were identified to carry only one mutant *TBX19* allele, although family history implied recessive inheritance, suggesting that additional, as yet unidentified, mutations may be present in the regulatory regions of the gene in some cases (70). Patients with *TBX19* mutations present with a homogeneous clinical phenotype with an endocrine profile consisting of very low basal plasma ACTH and cortisol levels, with no

significant ACTH response to corticotrophin-releasing hormone; however, plasma cortisol levels may respond to repeated ACTH administration, with normal function of all other pituitary axes. Severe hypoglycaemia, associated with seizures in some cases, and prolonged cholestatic jaundice are classically associated with ACTH deficiency presenting in the neonatal period (70). Furthermore, Vallette-Kasic *et al.* noted that in their series, about 25% of families with segregating *TBX19* mutations (5 out of 21) suffered a neonatal death, suggesting that isolated ACTH deficiency may be an underestimated cause of neonatal death. *Tbx19* null mice constitute a model of isolated ACTH deficiency similar to the phenotype observed in human patients with *TBX19* mutations (69).

PROP1

Prop1 is a paired-like homeodomain transcription factor, expressed exclusively within the embryonic pituitary. Expression is first detected in the dorsal portion of the murine Rathke's pouch in a region overlapping the expression domain of *Hesx1*. Maximal expression of *Prop1* is achieved at 12 dpc in the full caudomedial area of the developing anterior pituitary followed by a marked decrease, becoming undetectable by 15.5 dpc (73). The Ames dwarf (*df*) mouse harbours a naturally occurring serine to proline (S83P) substitution within the homeodomain of *Prop1*, resulting in a mutant protein with an eightfold lower DNA-binding affinity than wild-type *Prop1* (73). Homozygous Ames dwarf mice exhibit severe proportional dwarfism, hypothyroidism and infertility, and although early pituitary development is similar to wild-type mice, the emerging anterior pituitary gland is reduced by about 50%, displaying an abnormal looping appearance (74). The adult Ames dwarf mouse exhibits GH, TSH and prolactin (PRL) deficiency resulting from a severe reduction of somatotroph, lactotroph and caudomedial thyrotroph lineages with ~1% of the normal complement of each cell type. Additionally, these mice have reduced gonadotrophin expression correlating with low LH and FSH plasma levels (73–75).

Following the identification of *Prop1* as the gene underlying the Ames dwarf phenotype in mice, Wu *et al.* (76) reported the first mutations in *PROP1* (OMIM 601538) in human patients with GH, TSH and PRL deficiency in addition to reduced gonadotrophins and a failure to enter puberty spontaneously. To date, 22 distinct mutations have been identified in more than 170 patients, suggesting that *PROP1* mutations are the most common cause of CPHD reported, accounting for ~50% of familial cases (77, 78), although the incidence in sporadic cases is much lower (79). All affected individuals exhibit recessive inheritance, and, with one exception, all mutations identified to date involve the DNA-binding homeodomain, which is highly conserved between mouse and human sharing 91% identity at the

nucleotide level (80). The majority of these mutations are predicted to result in complete loss of function of the protein by ablating DNA binding and transcriptional activation. However, *in vitro* analysis has shown that some missense mutations retain partial activity (76, 81, 82). By far, the most common mutation (50–72% of all familial *PROP1* mutations) (77, 78, 83), detected in multiple unrelated families from several different countries, is a 2 bp deletion among three tandem GA repeats (²⁹⁶-GAGAGAG-³⁰²) within exon 2 resulting in a frameshift at codon 101 and the introduction of a termination codon at position 109 (often referred to as S109X), and probably represents a mutational hot spot within the gene, rather than a single common founder mutation (84). Recently, Reynaud *et al.* (85) reported the first *PROP1* mutation downstream of the homeodomain involving a substitution of a tryptophan residue for a stop codon at position 194 (W194X) in the transactivation domain, with the resultant mutant protein showing only 34% activity when compared with wild-type *PROP1*.

Recessive inheritance of mutations in *PROP1* is typically associated with GH, TSH, PRL and gonadotrophin deficiencies, although the time of initiation and severity of pituitary hormone deficiencies is highly variable. Most patients present with early-onset GH deficiency and growth retardation; however, normal growth in early childhood has been reported in a patient who attained a normal final height without GH replacement therapy (86). TSH deficiency is also highly variable and has been reported as the first presenting symptom in some cases, while others show delayed TSH deficiency which may not be present at birth (77, 87–89). Individuals with *PROP1* mutations exhibit normal ACTH/cortisol levels in early life but often demonstrate an evolving cortisol deficiency that is strongly and significantly correlated with increasing age (89–93). However, patients as young as 6–7 years have also been described with cortisol deficiency (93, 94).

Although *Prop1* is essential for the differentiation of gonadotrophs in foetal life, the spectrum of gonadotrophin deficiency is again extremely variable in patients with *PROP1* mutations. Clinical variability can range from hypogonadism with complete lack of pubertal development to reports of spontaneous, albeit often delayed, onset of puberty with subsequent development of gonadotrophin deficiency requiring hormone replacement (77, 87, 88, 91).

The pituitary morphology in patients with *PROP1* mutations is also highly variable; most individual reports have documented a normal pituitary stalk and posterior lobe, with a small or normal size anterior pituitary gland on MRI. However, in some cases, an enlarged anterior pituitary gland has been reported (76, 89, 95). Longitudinal analyses of anterior pituitary size over time have revealed that a significant number of patients demonstrated pituitary enlargement in early childhood with subsequent regression and involution;

thus, ensuing MRI in older patients usually demonstrates anterior pituitary hypoplasia (92, 96). The pituitary enlargement consists of a mass lesion interposed between the anterior and posterior lobes, possibly originating from the intermediate lobe (96). Turton *et al.* (2005) recently demonstrated that the mass can wax and wane in size prior to eventual involution (79). To date, the underlying mechanism for the mass remains unknown. There has been only one report of a biopsy of the 'tumour', and the histology was non-specific with the presence of amorphous material with no signs of apoptosis and no recognizable cell lines (97, 98). Consequent to the highly variable phenotype associated with *PROP1* mutations, no genotype–phenotype correlation has been identified; furthermore, phenotypic differences have been reported in siblings with identical mutations (87). The evolving nature of hormone insufficiencies in patients with *PROP1* mutations suggests a progressive decline in the anterior pituitary axis, indicating a need for continual monitoring of patients for the development of hormone insufficiencies that may not be apparent at initial presentation.

POU1F1

POU1F1 (OMIM 173110; previously known as *PIT1*) is a pituitary-specific transcription factor belonging to the POU homeodomain family of transcription factors (named after the genes *PIT1*, *OCT1* and *unc-86*) characterized by a highly conserved DNA-binding domain consisting of a POU-specific domain and a POU homeodomain. In the mouse, *Pou1f1* is expressed relatively late during pituitary development (14.5 dpc), and expression persists throughout post-natal life and adulthood, restricted to the anterior pituitary lobe (99). *Pou1f1* is essential for the development of somatotroph, lactotroph and thyrotroph cell lineages in the anterior pituitary (100), and for the subsequent expression of the *GH-1* (OMIM 139250), *PRL* (OMIM 176760) and *TSH- β* (OMIM 188540) genes between 15.5 and 17 dpc (101). Two naturally occurring murine models have shed light on the role of *Pou1f1* in normal pituitary development. In the Snell dwarf (*dw*) mouse, a recessive point mutation (W261C) results in the absence of somatotrophs, lactotrophs and thyrotrophs (102). A similar phenotype results in the Jackson dwarf mouse (*dwf*) that harbours a recessive null mutation due to rearrangement of *Pou1f1*. *Pou1f1*-binding sites have also been found in the *GHRHR* (OMIM 139191) and the *Pou1f1* gene itself (100, 103), and autoregulation is required to sustain gene expression once the *Pou1f1* protein has reached a critical threshold (104).

The first mutation within *POU1F1* was identified by Tatsumi *et al.* (1992) in a child with GH, PRL and profound TSH deficiency caused by homozygosity for a nonsense mutation within the gene (105). The majority of mutations identified in *POU1F1* to date are recessive;

however, in addition, a number of heterozygous point mutations have been reported (106). Of these, the amino acid substitution R271W appears to be a 'hot spot' for *POU1F1* mutations (106) and has been identified in several unrelated patients of different ethnic backgrounds. When co-transfected with wild-type *POU1F1*, this mutant protein prevented transcriptional activation by the wild-type protein acting as a dominant negative (107), although this has been recently disputed (108).

The spectrum of hormone deficiency can vary in patients with *POU1F1* mutations; GH and PRL deficiencies generally present early in life; however, TSH deficiency can be highly variable with presentation later in childhood (109, 110). We have recently described a *POU1F1* mutation in a 21-year-old patient with GH and PRL deficiency who has normal thyroid function to date (111). Magnetic resonance imaging demonstrates a small or normal anterior pituitary with a normal posterior pituitary and infundibulum, and no midline abnormalities. Since the first report, a total of 27 *POU1F1* mutations have been described including 22 recessive and 5 dominant mutations in over 60 patients originating from 19 different countries, all of which have been associated with a broadly similar phenotype of GH, TSH and PRL deficiency (112).

Conclusions

Over the past decade, there has been an explosion in the knowledge of the genetic cascade that orchestrates hypothalamo–pituitary development. Several transcription factors and signalling molecules are critical for cell differentiation and proliferation at a very early stage of gestation. The mouse has served as an excellent model for understanding the genetic basis of congenital hypopituitarism in humans, although the correlation between mouse and human disease phenotypes is variable. This candidate gene approach, based on mouse studies, had led to the identification of several human mutations that disrupt hypothalamo–pituitary development resulting in specific patterns of hormone dysfunction.

Establishing the genotype can aid the management of individual patients with hypopituitarism. For example, a patient with an identified *PROP1* mutation exhibiting an enlarged anterior pituitary may be at risk of visual impairment due to anterior pituitary hyperplasia; however, a number of reports in individuals with *PROP1* mutations have shown the enlarged anterior pituitary to undergo spontaneous involution. Careful monitoring of the anterior pituitary in such cases may prevent the patient undergoing further invasive procedures. Additionally, identification of a mutation within *POU1F1* predicts that cortisol and gonadotrophin secretion will remain normal in the patient. Identification of the genotype can also aid in genetic

counselling and early diagnosis, particularly in autosomal dominant *POU1F1* mutations.

However, no genetic aetiology has been established to date in most patients with hypopituitarism. Given that a number of these patients may represent familial cases, it is clear that many genes implicated in hypopituitarism remain to be identified. Mapping and identification of the underlying causative mutations in these rare familial cases will help to identify novel genes for mutational screening in the more common sporadic forms of the condition. Additionally, naturally occurring and genetically engineered mouse models of hypopituitary phenotypes have proved to be extremely fruitful in the past in identifying genes involved in pituitary development in humans. The identification and characterization of novel genes, together with their downstream targets and interacting partners, will in future enable a greater understanding of the disorder, in addition to unravelling the processes inherent in the normal development of the pituitary gland.

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