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Molecular basis of pituitary dysfunction in mouse and human

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The pituitary gland functions as an intermediary between the brain and the peripheral endocrine organs of the body (Tortora and Grabowski 1996). In the context of a complex system of feedback control, the pituitary gland relays signals from the hypothalamus to its target organs by secreting various hormones. These hormone signals, which are transmitted throughout the endocrine system, reflect the current homeostatic conditions of the body. Mechanisms of compensation, including the regulation of gene transcription, hormone secretion, and cell proliferation, allow the pituitary to respond to the continually changing needs of the organism. It is in this way that the pituitary aids in the regulation of many vital processes, including growth, metabolism, reproduction, and stress response.

The mammalian pituitary is comprised of three lobes: the posterior lobe, the intermediate lobe, and the anterior lobe. The anterior lobe of the pituitary is composed of functionally distinct cell types that are the primary site of endocrine regulation. During embryogenesis, the anterior and intermediate lobes of the pituitary develop from an invagination in the oral ectoderm known as Rathke's pouch (Dubois and ElAmraoui 1995). The pouch eventually separates from the oral ectoderm and undergoes a period of intense proliferation. This is followed by differentiation into the five anterior pituitary cell types on the rostral side of the pouch and the intermediate lobe melanotropes on the caudal side of the pouch (Dubois and Hemming 1991). By birth, the anterior pituitary is composed of functioning corticotropes, thyrotropes, somatotropes, gonadotropes, and lactotropes that produce adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), growth hormone (GH), gonadotropins (FSH and LH), and prolactin (PRL), respectively. Expression of each hormone gene, which defines the terminal differentiation of each cell type, occurs in a spatially and temporally specific manner during pituitary organogenesis (Japon et al. 1994; Burrows et al. 1999). The mechanisms that control this process involve the secretion of signals from surrounding structures and the expression of a cascade of homeodomain transcription factors. Studies in both mouse and human reveal that control of signaling events and homeobox gene expression is essential for proper pituitary gland development (Watkins-Chow and Camper 1998; Dasen and Rosenfeld 1999). Deregulation of these events can lead to various forms of pituitary gland dysfunction, including hypopituitarism and adenomas.

Transcription factor genes in anterior pituitary development

Studies involving spontaneous mouse mutants and engineered mouse models have helped elucidate the role transcription factor genes play in pituitary gland ontogeny (Watkins-Chow and Camper 1998). The function of numerous transcription factors in the process of pituitary gland organogenesis has been described in recent years (Table 1). Homeobox genes critical for the development of the anterior pituitary include the LIM homeodomain transcription factor *Lhx3*, the 'paired'-like homeodomain transcription factors *Hesx1* (also known as Rathke's pouch homeobox or *Rpx*) and *Prop1*, and the POU homeodomain transcription factor *Pit1* (Li et al. 1990; Sheng et al. 1996; Sornson et al. 1996; Dattani et al. 1998). The nuclear receptor steroidogenic factor 1 (SF1) also plays a significant role in pituitary gland development and function (Ingraham et al. 1994; Luo et al. 1994; Zhao et al. 2000). These genes, along with a number of other transcription factor genes expressed in the developing pituitary, comprise a genetic hierarchy essential for proper pituitary gland ontogeny (Fig. 1).

In the mouse, the development of Rathke's pouch begins at approximately embryonic day 8.5 (e8.5; Dubois and ElAmraoui 1995). *Lhx3* is one of the genes that play an important role in this process (Sheng et al. 1996). The formation of a definitive pouch during pituitary organogenesis requires the expression of *Lhx3*, or its family member *Lhx4* (Sheng et al. 1997). In the mouse, the expression of *Lhx3* is restricted to neural and neuroendocrine cells (Dawid et al. 1995; Sheng et al. 1997). Its expression is detected in the pituitary gland, motor neurons of the spinal cord, motor neurons in the hindbrain, the retina, and the pineal gland. *Lhx3* expression is first evident in the developing pouch at e9.5 and persists in the pituitary throughout adulthood. In *Lhx3*-/-mice, pituitary development is arrested after initial pouch commitment (Sheng et al. 1996). Rathke's pouch forms in these mice, but fails to grow and differentiate. *Lhx3* expression is also required for the differentiation and proliferation of four anterior pituitary cell types. The pituitary glands of *Lhx3*-deficient mice lack somatotropes, lactotropes, thyrotropes, and gonadotropes (Sheng et al. 1996). While corticotrope differentiation is initiated in these animals, they fail to expand and/or survive, leading to an absence of corticotropes at birth. In addition, loss of *Lhx3* results in a failure to maintain *Hesx1* expression and activate *Pit1* expression. These findings are consistent with the transactivation capabilities of LHX3. In vitro experiments have revealed that LHX3 can transactivate the promoters of several pituitary hormone markers, including PRL, TSHβ, and αGSU (the α subunit of the glycoprotein hormones LH, FSH, and TSH, also known as *Cga*) (Sloop et al. 1999). LHX3 can also activate transcription of the *Pit1* gene. As a result of their developmental abnormalities, *Lhx3*-/-mice die at or shortly after birth (Sheng et al. 1996). *Lhx4*-/-mice exhibit commitment and differentiation of all five cell types, but in substantially reduced numbers (Sheng et al. 1997). In contrast to both *Lhx3*-/-mice and *Lhx4*-/-mice, pituitary gland development in mice lacking both *Lhx3* and *Lhx4* is arrested prior to the formation of a definitive pouch (Sheng et al. 1997). These data indicate that either *Lhx3* or *Lhx4* is required for the formation of a definitive pouch. In addition, *Lhx3* expression is essential for pouch expansion and differentiation of most anterior pituitary cell types.

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Table 1. Transcription factors critical for pituitary development and function.

Gene	Class	Model	Phenotype	Reference
Egr1	Zinc finger	Knockout	Reduced growth, female infertility, no *Lhb* expression, reduction in somatotrope number	Topilko et al. 1998; Lee et al. 1996
Gata2	Zinc finger	Knockout	Embryonic lethal at e10–e11 due to severe anemia	Tsai et al. 1994
Gata2	Zinc finger	Dominant negative Tg	An absence of *Lhb* and *SF-1* (*Nr5a1*) expression; reduced *Tshb* and α*Gsu* expression	Dasen et al. 1999
Lhx3	LIM HD	Knockout	RP forms but fails to expand and differentiate; hypoplastic pituitary lacks GH, TSH, PRL, FSH, LH cells; corticotropes initiate differentiation but fail to expand	Sheng et al. 1996
Lhx4	LIM HD	Knockout	Hypoplastic pituitary with reductions in all cell types	Sheng et al. 1997
Otx1	Bicoid-like HD	Knockout	Transient hypogonadism and dwarfism; delayed production of FSH, LH, and GH; recovered by 4 months of age	Acampora et al. 1998
Pax6	Paired-like HD	Knockout	Ventralization of dorsoventral axis of pituitary gland	Kioussi 1999
Pit1	POU HD	Snell dwarf	Missing thyrotropes, somatotropes, and lactotropes; increased gonadotropes; exhibit dwarfism, hypothyroidism, and infertility	Li et al., 1990
Pitx1	Bicoid-like HD	Knockout	Decreased levels of LH, FSH, and TSH; increased levels of ACTH; die at P1 with cleft palate and limb defects	Szeto et al. 1999; Lanctot et al. 1999
Pitx2	Bicoid-like HD	Knockout	Embryonic lethal at e15; defects of the ventral body wall, heart, eyes, and teeth; pituitary development arrested at e12.5	Gage et al. 1999; Kitamura et al. 1999; Lu et al. 1999; Lin et al. 1999
Prop1	Paired-like HD	Ames dwarf	Near absence of thyrotropes, somatotropes, and lactotropes; reduced production of LH and FSH; exhibit dwarfism, hypothyroidism, and infertility	Sornson et al. 1996; Gage et al. 1996b
Hesx1 (Rpx)	Paired-like HD	Knockout	Anterior CNS defects; bifurcated and hypoplastic RP	Dattani et al. 1998
Nr5a1 (SF1)	Nuclear receptor	Knockout	Lethal by P8 due to adrenal insufficiency; lack gonads; exhibit defects of the VMH; fail to produce LH and FSH	Ingraham et al. 1994; Luo et al. 1994

HD, homeodomain; Tg, transgene; GH, growth hormone; TSH, thyroid stimulating hormone; PRL, prolactin; FSH, follicle stimulating hormone; LH, luteinizing hormone; ACTH, adrenocorticotropic hormone; CNS, central nervous system; RP, Rathke's pouch; VMH, ventromedial hypothalamus.

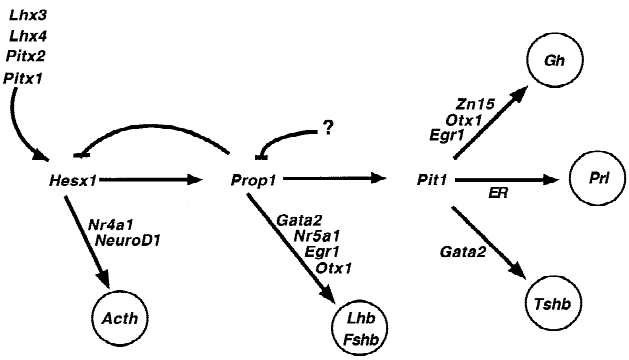

Fig. 1. Transcription factor hierarchy controlling anterior pituitary cell specification. The analysis of patients with pituitary disorders, genetically engineered mouse models, and spontaneous mouse mutants has revealed a cascade of transcription factor gene expression that control pituitary development. *Lhx3, Lhx4, Pitx2,* and *Pitx1* are all involved in the expansion of a committed pouch early in gestation. *Hesx1* expression is not only critical for normal pouch development, it is required for the activation of *Prop1*. Ames dwarf mice, which have a hypomorphic mutation in the *Prop1* gene, and CPHD patients with PROP1 mutations demonstrate that *Prop1* expression is crucial for four pituitary lineages: the somatotropes, thyrotropes, lactotropes, and gonadotropes. It is also needed for the activation of *Pit1* and the repression of *Hesx1*. Analysis of *Pit1*-deficient Snell dwarf animals reveals that *Pit1* expression is necessary for the proliferation and differentiation of the somatotropes, lactotropes, and thyrotropes. Other transcription factor genes [including *Nr5a1, NeuroD1, Gata2, Nr4a1* (also known as *Nurr77*), *Egr1, Otx1, Zn15,* and ER] have more specialized roles in pituitary gland development, supporting the proliferation, differentiation, and/or function of only one to two anterior pituitary cell types (Burrows et al. 1999). Circled transcripts indicate pituitary hormone genes that define the five separate cell types.

Another gene essential for proper formation of Rathke's pouch is the Rathke's pouch homeobox gene (*Rpx* or *Hesx1;* Dattani et al. 1998). During early embryogenesis, *Hesx1* expression is detected in the anterior midline endoderm and prechordal plate precursor cells (Hermesz et al. 1996). As development progresses, the expression of *Hesx1* resolves to the ventral diencephalon. Ultimately, *Hesx1* expression is restricted to the pituitary primordium, Rathke's pouch. Within the developing pituitary, *Hesx1* transcripts begin to disappear in a spatially and temporally specific sequence that corresponds to progressive pituitary cell differentiation. Targeted deletion of the *Hesx1* gene results in variable anterior central nervous system defects, including reduced prosencephalon, eye anomalies, and defects in olfactory development (Dattani et al. 1998). In the pituitary gland, an absence of functional HESX1 leads to abnormal bifurcations in Rathke's pouch as well as pituitary hypoplasia. The presence of a definitive pouch, albeit defective, suggests that *Hesx1* is not essential for initial pouch commitment. It is, however, required for the normal growth and differentiation of the pouch.

Expression of *Prop1,* which is restricted to the developing pituitary in the mouse, is essential for normal pituitary gland development and function (Gage et al. 1996a, 1996b; Sornson et al. 1996). *Prop1* is expressed in the presumptive anterior lobe shortly after *Lhx3* expression begins. Its expression peaks at e12.5 and decreases after e14.5. No *Prop1* transcripts are detectable at birth or beyond. A mutation in the *Prop1* gene is responsible for the hypopituitarism observed in the Ames dwarf mouse ($Prop1^{df/df}$). As a result of a serine-to-proline substitution at residue 83, a conserved residue in the first alpha helix of the homeodomain, there is a profound decrease in the ability of PROP1 to bind DNA (Sornson et al. 1996). As a result of the drastic reduction in PROP1 function, the pituitaries of Ames dwarf animals are severely hypoplastic. A 50% reduction in pituitary size is evident as early as e14, only two days after *Prop1* expression peaks (Gage et al. 1996b). The anterior pituitaries of adults contain less than 0.1% of the normal number of thyrotropes, somatotropes, and lactotropes. In addition, the pituitaries of $Prop1^{df/df}$ animals produce reduced amounts of gonadotropins (Tang et al. 1993). As a result of their hormone deficiencies, Ames dwarf mice exhibit growth insufficiency, hypothyroidism, and infertility (Bartke 1979). Gene expression analysis in the pituitaries of $Prop1^{df/df}$ animals also revealed that *Prop1* is essential for both the activation *Pit1* and the repression of *Hesx1* (Anderson et al. 1995; Gage et al. 1996a). The reduction in pouch expansion and lack of three anterior pituitary cell types in Ames dwarf mice implicates *Prop1* in pituitary cell proliferation at a time prior to the activation of most hormone marker genes.

Pit1 was the first transcription factor gene demonstrated to play an important role in pituitary development (Li et al. 1990).

The expression of *Pit1* begins at e14.5 and is restricted to the anterior pituitary. As mentioned previously, *Pit1* is activated as a result of *Prop1* and *Lhx3* expression. *Pit1* remains activated in the somatotropes, lactotropes, and thyrotropes throughout the life of the animal. Consistent with its cell-specific expression pattern, PIT1 is able to transactivate the promoters of the GH, PRL, TSHβ, growth hormone-releasing hormone receptor (GHRH-R), and thyroid hormone receptor beta type 2 genes (Rhodes and Rosenfield, 1996). PIT1 also regulates its own promoter, providing a mechanism for the maintenance of *Pit1* gene expression. Analysis of two mutant *Pit1* alleles in mice demonstrated the need for PIT1 function during pituitary gland development (Rhodes et al. 1994). In $Pit1^{dw/dw}$ mice, a point mutation in a highly conserved residue in the homeodomain leads to a PIT1 protein that is incapable of binding DNA (Li et al. 1990). The resulting absence of PIT1 function leads to a complete absence of somatotropes, lactotropes, and thyrotropes. The gonadotropes and corticotropes are unaffected. $Pit1^{dwJ/dwJ}$ animals have an identical phenotype, which results from a rearrangement within the *Pit1* gene (Camper et al. 1990; Li et al. 1990). Both are putative null alleles. Similar to the Ames dwarf mice, homozygotes for the $Pit1^{dw}$ or $Pit1^{dwJ}$ alleles exhibit growth failure, hypothyroidism, and infertility as a result of GH, TSH, and PRL deficiencies (Rhodes and Rosenfeld 1996). The phenotypes of *Pit1*-deficient mice indicate that PIT1 is essential for the differentiation and expansion of the somatotrope, lactotrope, and thyrotrope lineages in the anterior pituitary.

Steroidogenic factor 1 (*Sf1* or *Nr5a1*) encodes an orphan nuclear receptor that plays an essential role in the development and function of pituitary gonadotropes, adrenal glands, gonads, and ventromedial hypothalamus (VMH; Ingraham et al. 1994; Luo et al. 1994; Zhao et al. 2000). Mice that have a systemic deletion of *Nr5a1* display agenesis of the adrenal glands and gonads, ablation of the VMH, and decreased expression of the gonadotropin genes. As a result of adrenal insufficiency, homozygous *Nr5a1* knockout mice die by postnatal day 8. Heterozygous *Nr5a1* knockout mice, which are viable, display a more subtle phenotype. *Nr5a1* +/− animals exhibit defects in adrenal gland development and organization that lead to a milder form of adrenal insufficiency (Bland et al. 2000). As a result of this deficiency, *Nr5a1* +/− mice are not able to mount an adequate stress response, suggesting that normal gene dosage of *Nr5a1* is essential for this process. Mice with a pituitary-specific deletion of *Nr5a1* are viable and have histologically normal adrenal glands and VMH (Zhao et al. 2000). In addition, the gonads of the pituitary-specific *Nr5a1* knockout mice are gonadotropin responsive. The pituitary glands of these mice, however, fail to express the gonadotrope markers *Lhb, Fshb,* and *Gnrhr,* indicating an absence of the gonadotrope cells. Consistent with this finding, in vitro analyses have demonstrated that NR5A1 directly regulates the *Lhb* (Halvorson et al. 1996; Keri and Nilson 1996), *Fshb* (Brown and McNeilly 1997), and *Gnrhr* (Duval et al. 1997; Ngan et al. 1999) promoters. The results of these studies demonstrate that *Nr5a1* expression in the anterior pituitary is required for normal development and function of the gonadotrope lineage.

Transcription factor genes and hypopituitarism in humans

Short stature due to pituitary hormone deficiency is a relatively common occurrence. Approximately 1 in 4000 live births exhibits growth failure as a result of deficits in one or more pituitary hormones (Vimpani et al. 1977; Procter et al. 1998). Pituitary-related growth insufficiency is associated with two main disorders: isolated growth hormone deficiency (IGHD) and combined pituitary hormone deficiency (CPHD).

IGHD is generally sporadic, resulting from trauma, infections, radiation, chromosomal anomalies, or pituitary tumors. To date, mutations in three genes have been identified in families with IGHD. Mutations in the genes that encode growth hormone (GH1; Procter et al. 1998), growth hormone receptor (GH-R; Clayton et al. 1999), and growth hormone-releasing hormone receptor (GHRH-R; Wajnrajch et al. 1996) lead to both dominant and recessive forms of IGHD. Consistent with the role of GH in promoting postnatal growth, the clinical manifestations of IGHD include a reduction in linear growth velocity and a delay in skeletal maturation (Procter et al. 1998). In addition, the onset of puberty in IGHD patients may be delayed, but their fertility is generally normal.

CPHD is defined as GH deficiency with a lack of at least one other pituitary hormone (Procter et al. 1998). The clinical presentation of patients with CPHD, which varies with which hormones are deficient, includes growth failure, hypothyroidism, and delayed or incomplete secondary sexual development. Similar to IGHD, most cases of CPHD are sporadic. However, recent studies have identified mutations in several homeobox genes that lead to inherited disorders involving multiple hormone deficiencies. These include LHX3, RPX, PROP1, and PIT1 (Table 2; Parks et al. 1999; Dattani and Robinson 2000; Parker et al. 2000).

As mentioned previously, *Lhx3*-deficient mice exhibit a failure in the differentiation of somatotropes, thyrotropes, lactotropes, and gonadotropes (Sheng et al. 1996). On the basis of this finding, Netchine et al. screened two unrelated consanguinous families with deficiencies in GH, TSH, PRL, FSH, and LH for mutations in LHX3 (Table 2). In addition to the CPHD, affected individuals in both families displayed a rigid cervical spine that restricted their head rotation. In the first family, all affected individuals were found to be homozygous for a Tyr116Cys mutation (Netchine et al. 2000). This mutation alters a highly conserved residue in the LIM2 domain of LHX3, a region essential for protein-protein interactions. Affected individuals in the second family are homozygous for a 23-bp deletion beginning at residue 156 (Netchine et al. 2000). This recessive mutation leads to aberrant splicing and premature truncation of LHX3. The resulting protein lacks all of the homeodomain, rendering it incapable of binding DNA. No LHX3 mutations were identified in individuals with CPHD, posterior pituitary ectopia (abnormal positioning of the posterior pituitary), and cervical spines with normal flexibility (Sloop et al. 2000). The identification of LHX3 mutations in two unrelated families with CPHD and rigid cervical spines confirms the role of LHX3 in hypopituitarism and suggests that neck rotation may be a unique feature that will facilitate differential diagnosis at the molecular level.

Hesx1-deficient mice have anterior midline defects, hypopituitarism, and ocular abnormalities (Dattani et al. 1998). This phenotype suggested the possibility that patients with familial or sporadic cases of septo-optic dysplasia (SOD), pituitary hypoplasia, holoprosencephaly, or pituitary hormone deficiency could have mutations in the HESX1 gene. A mutation in HESX1 was identified in two siblings that had SOD with agenesis of the corpus callosum and panhypopituitarism (Table 2; Dattani et al. 1998). Both individuals were homozygous for an Arg53Cys mutation that substantially decreases the ability of HESX1 to bind DNA. The consanguinous parents of these individuals, who were both unaffected, were heterozygous for this mutation. Two additional HESX1 mutations were identified in individuals with IGHD (Table 2; Dattani et al. 1999). Two siblings were found to be heterozygous for a Ser170Leu mutation. HESX1 protein with this dominant mutation has a decreased affinity for DNA binding but does not appear to inhibit the function of the normal protein present. This suggests that these siblings exhibit IGHD as a result of haploinsufficiency. Heterozygosity for an Asn125Ser mutation was demonstrated in a patient diagnosed with IGHD and pituitary hypoplasia. The effect of this mutation on HESX1 function is unclear at this time. These mutation analyses suggest that HESX1 plays a role in both dominant and recessive forms of hypopituitarism, including SOD.

As discussed above, *Prop1*-deficient Ames dwarf mice exhibit

Table 2. Transcription factor gene mutations in human pituitary hormone deficiency disorders.

Gene	Mutation	Inheritance	Phenotype	Reference
LHX3	Tyr116Cys	Recessive	CPHD with rigid cervical spine	Netchine et al. 2000
	23 bp deletion at codon 156	Recessive	CPHD with rigid cervical spine	Netchine et al. 2000
HESX1	Arg53Cys	Recessive	SOD with panhypopituitarism	Dattani et al. 1998
	Ser170Leu	Dominant	IGHD	Dattani et al. 1999
	Asn125Ser	Dominant	IGHD with pituitary hypoplasia	Dattani et al. 1999
PROP1	A to T substitution at exon 3 splice acceptor site	Recessive	CPHD	Duquesnoy et al. 1998
	A149G150del	Recessive	CPHD	Fofanova et al. 1998a
	A150del	Recessive	CPHD	Krzisnik et al. 1999a
	Arg73Cys	Recessive	CPHD	Duquesnoy et al. 1998
	Arg73His	Recessive	CPHD	Vallette-Kasic et al. 2000
	Phe88Ser	Recessive	CPHD	Osorio et al. 2000
	Arg99Gin	Recessive	CPHD	Vieira et al. 2000
	Arg99Ter	Recessive	CPHD	Vallette-Kasic et al. 2000
	A301G302	Recessive	CPHD	Wu et al. 1998
	Phe117lle	Recessive	CPHD	Wu et al. 1998
	Arg120Cys	Recessive	CPHD	Wu et al. 1998
PIT1	Pro14Leu	Dominant	CPHD	Fofanova et al. 1998b
	Pro24Leu	Dominant	CPHD	Ohta et al. 1992
	Phe135Cys	Recessive	CPHD	Pelligrini-Bouiller et al. 1996
	Arg143Gln	Recessive	CPHD	Ohta et al. 1992
	Ala158Pro	Recessive	CPHD	Pfäffle et al. 1992
	Arg172Ter	Recessive	CPHD	Tatsumi et al. 1992
	Glu174Gly	Recessive	CPHD	Brown et al. 1998
	Trp193Arg	Recessive	CPHD	Bakker et al. 1997
	Lys216Glu	Dominant	CPHD	Botero et al. 2000
	Pro239Ser	Recessive	CPHD	Pernasetti et al. 1998
	Glu250Ter	Recessive	CPHD	Irie et al. 1995
	Arg271Trp	Dominant	CPHD	Radovick et al. 1992

CPHD, combined pituitary hormone deficiency; SOD, septo-optic dysplasia; IGHD, isolated growth hormone deficiency.

growth insufficiency, hypothyroidism, and infertility as a result of deficits in GH, TSH, and PRL and a reduction in gonadotropin production (Tang et al. 1993; Gage et al. 1996b; Sornson et al. 1996). This phenotype indicates that CPHD, with deficiencies in GH, TSH, PRL, FSH, and LH, could result from loss of function mutations in the PROP1 gene. To date, at least 12 different PROP1 mutations have been identified in patients with CPHD (Table 2; Parker et al. 2000). Six of these mutations are amino acid substitutions that affect highly conserved residues in the PROP1 protein (Duquesnoy et al. 1998; Wu et al. 1998; Osorio et al. 2000; Vallette-Kasic et al. 2000; Vieira et al. 2000). These mutations lead to varying degrees of loss of PROP1 function. One mutation (Arg99Ter) is a nonsense mutation that results in loss of more than half the PROP1 protein (Vallette-Kasic et al. 2000). Another alters the splice acceptor site at exon 3 (Duquesnoy et al. 1998). This change, which results in the generation of a major transcript that retains intron 2, leads to premature truncation and loss of the transactivation domain. The remaining four mutations are deletions that, owing to a frameshift, cause premature truncation during translation (Duquesnoy et al. 1998; Fofanova et al. 1998a; Krzisnik et al. 1999a; Agarwal et al. 2000). This results in a protein that lacks most of the homeodomain and all of the transactivation domain. The A301G302del is the most common mutation, accounting for approximately 55% of all PROP1 mutation-containing alleles (Cogan et al. 1998).

One unique aspect of the CPHD that results from PROP1 mutations is its exceptional phenotypic variability (Flück et al. 1998). It has become apparent, as more studies examine PROP1 in CPHD patients, that there is no genotype-phenotype correlation in these cases. Patients with identical mutations, even within the same family, can exhibit a wide range of disease severity with a variable age of onset. The hormone deficiencies in the majority of patients with the A301G302del are limited to GH, TSH, PRL, FSH, and LH. However, a small subset of patients with this mutation develops ACTH deficiency with age (Pernasetti et al. 2000). In addition, magnetic resonance imaging of CPHD patients with PROP1 mutations has demonstrated that the size of their pituitary glands may be normal, reduced, or enlarged (Fofanova et al. 2000). At this time, there is no explanation for the phenotypic variability observed in PROP1-related CPHD, but it is likely to involve genetic background effects.

Consistent with the absence of somatotropes, thyrotropes, and lactotropes in *Pit1*-deficient mice, CPHD patients with PIT1 mutations generally exhibit deficiencies in GH, TSH, and PRL (Rhodes and Rosenfeld 1996; Parker et al. 2000). Magnetic resonance imaging reveals that patients with PIT1-related CPHD generally exhibit hypoplastic pituitary glands. Twelve different PIT1 mutations have been identified that result in recessive and dominant forms of CPHD (Table 2). The recessive mutations decrease DNA binding and/or transactivation to varying degrees, while the dominant mutations generally exert dominant negative effects (Parks et al. 1999; Parker et al. 2000). Most of these mutations lie within the POU-specific domain or the homeodomain. Two mutations are nonsense mutations that result in premature truncation during translation (Tatsumi et al. 1992; Irie et al. 1995). The most common PIT1 mutation in CPHD patients is an arginine-to-tryptophan substitution at residue 271 (Radovick et al. 1992). This mutation alters the homeodomain such that it acts as a dominant inhibitor of transcription, although it can bind to PIT1 target genes. The mechanism for this dominant inhibition is currently unknown. Another mutation in the PIT1 gene that causes CPHD is a lysine-to-glutamic acid substitution at residue 216 (Botero et al. 2000). This mutation is interesting in that it creates a loss of function by interfering with two aspects of PIT1 function. Both phosphorylation of the PIT1 protein and retinoic acid induction of PIT1 transcription are inhibited. Finally, an Ala158Pro substitution in the POU-specific domain creates an intriguing partial loss of function phenotype. GH and PRL are not produced, but TSH tran-

scription and thyrotrope cell proliferation are intact (Pfäffle et al. 1992). This suggests that the POU-specific domain is more critical than others for transcription of some PIT1 target genes.

Although they do not result in a growth insufficiency phenotype, mutations in the *NR5A1* gene have also been documented in human patients with endocrine disorders. From on the adrenal gland and gonad phenotype of the *Nr5a1* knockout mice, a 46X,Y individual with male pseudohermaphroditism and primary adrenal failure was screened for NR5A1 mutations (Achermann et al. 1999; Ito et al. 2000). This individual, who also had streak-like gonads and elevated gonadotropin levels, was found to carry a dominant mutation (G35E) in the DNA-binding domain of NR5A1. The residue altered is the last amino acid in the proximal box of the first zinc finger, a region critical for the recognition of DNA binding sites. More recently, a mutation (R255L) was identified in a genotypically and phenotypically normal female who exhibited adrenal insufficiency (Biason-Lauber and Schoenle 2000). The R255L mutation, which lies in exon 4 of NR5A1, results in a transcriptionally inactive protein with decreased DNA-binding capabilities.

The identification of novel mutations, in these genes and in other genes important in pituitary gland development, is beneficial in several ways. The discovery of new mutations may help us to more clearly understand the structure-function relationships of the various proteins. This will, in turn, further our understanding of pituitary gland organogenesis and function. A clinically relevant benefit to the identification of new mutations is that it may facilitate the diagnosis of endocrine-related growth insufficiency disorders.

The molecular genetics of human pituitary adenomas

Pituitary dysfunction in adults is often attributable to the presence of a pituitary adenoma that can reduce or increase hormone production. Most pituitary adenomas are benign tumors that arise from a single anterior pituitary cell. Only one-third of pituitary adenomas are locally invasive; fewer than 1% are capable of metastasis (Selman et al. 1986). Although malignant tumors are quite rare, the incidence of pituitary neoplasms in general is quite frequent. Estimates from autopsy data suggest that approximately 20% of the general population have an undiagnosed pituitary tumor (Burrow et al. 1981; Elster 1993). The incidence of pituitary adenomas increases to more than 30% when individuals aged fifty to sixty are examined.

Tumors of the pituitary gland can be classified as functioning or nonfunctioning (Asa and Ezzat 1998; Asa 1999). Clinically functioning tumors are hormone secreting and are often diagnosed as a result of some endocrinopathy. Nonfunctioning tumors do not secrete any hormone and often present with symptoms of an intracranial mass, such as headaches and visual field disturbances. The most common type of nonfunctioning tumor is the null cell adenoma, which makes up approximately one quarter of all pituitary adenomas. These tumors lack a serum hormone marker and are unassociated with a clinical syndrome. As a result, individuals with null cell adenomas are generally diagnosed because of mass effects. It is believed that this type of tumor arises from expansion of an undifferentiated precursor cell type.

Each of the hormone-producing cell types of the anterior pituitary is represented by some fraction of human pituitary adenomas. Prolactinomas, which secrete prolactin, account for approximately 30% of pituitary tumors (Wilson and Dempsey 1978). The excessive circulating levels of prolactin lead to galactorrhea and amenorrhea in females and decreased libido and impotence in males (Asa 1999). Growth hormone-secreting tumors comprise approximately 10–15% of pituitary neoplasms (Wilson and Dempsey 1978). In adults, these tumors are associated with the clinical syndrome acromegaly and often present with enlargement of the hands, feet, and lower jaw (Asa 1999). They may also present with clinical manifestations related to the metabolic effects of growth hormone, such as hypertension or glucose intolerance. In children and adolescents, growth hormone-secreting tumors may cause gigantism. Another 10–15% of pituitary neoplasms can be accounted for by ACTH-secreting tumors (Wilson and Dempsey 1978; Asa 1999). These tumors are associated with Cushing disease and often lead to an excess of circulating glucocorticoids. The clinical manifestations that result from glucocorticoid excess include central obesity (e.g., moon-shaped facies, supraclavicular fat pads), hirsutism, easy bruising, menstrual irregularities, and high blood pressure (Asa 1999). TSH-secreting tumors and gonadotropin-secreting tumors have been reported but are infrequent forms of pituitary adenomas (Beck-Peccoz et al. 1989).

Pituitary neoplasms secreting more than one hormone are classified as multihormonal (Lloyd 1993; Asa 1999). The most common multihormonal tumor secretes both GH and PRL and represents approximately 6% of all pituitary adenomas. This type of tumor is thought to result from proliferation of somatomammotropes, a transitional cell type that secretes both GH and PRL. A high proportion of the rare thyrotrope adenomas also secrete GH and PRL, suggesting that, in these tumors, PIT1 precursor cells are undergoing uncontrolled proliferation (Melmed 1995; Asa 1999).

Despite the prevalence of human pituitary adenomas, little is known about their etiology (Melmed 1999; Heaney and Melmed 2000). Several factors can be involved in the pathogenesis of pituitary adenomas. These include imbalances in hormone feedback control, mutations in signal transduction molecules, disruptions in growth factor control, loss of tumor suppressor gene function, and alterations in transcription factor or cytokine expression. An increased propensity for pituitary adenoma formation can result from the inappropriate activation or overexpression of genes involved in promoting cell proliferation or from the reduced function of genes important in cell cycle regulation (Table 3).

Although most are sporadic, pituitary adenomas may be encountered as a component of a familial cancer syndrome. Multiple endocrine neoplasia (MEN), type 1, is an autosomal dominant syndrome that results from loss of MEN1 function (Chandrasekharappa et al. 1997). In addition to tumors of the parathyroid and endocrine pancreas, MEN1 patients develop either PRL- or GH-secreting pituitary adenomas (Burgess et al. 1996). The Carney complex, another autosomal dominant endocrine neoplasia syndrome, is characterized by myxomas and spotty skin pigmentation, as well as by tumors of the testis, ovary, adrenal gland, and anterior pituitary (Stratakis et al. 1996a, 1996b). Approximately 20% of individuals with the Carney complex harbor GH-secreting pituitary tumors. The genes responsible for Carney complex have been localized to 2p16 and 17 q24 by linkage analysis (Stratakis et al. 1996a; Casey et al. 1998). Recently, mutations in the gene encoding protein kinase A regulatory subunit 1-α (PRKAR1A), which maps to 17q24, have been identified in patients with Carney complex (Casey et al. 2000; Kirschner et al. 2000). One missense mutation, one splice site mutation, and two deletions resulting in a frameshift and premature truncation during translation have been found in affected individuals.

The first mutations linked to sporadic pituitary adenomas were activating mutations in the GH signaling pathway (Table 3); Spada et al. 1992; Arvanitakis et al. 1998). These lesions, known as *gsp* mutations, are in the GNAS1 gene that encodes the α-subunit of the stimulatory G (Gs) protein (Vallar et al. 1987). Missense mutations (Arg201Cys, Arg201His, Gln227Arg, or Gln227Leu) in this gene lead to a constitutively active Gs protein. This causes an elevation in intracellular cAMP levels and growth hormone hypersecretion. These mutations are present in approximately 30–40% of GH-secreting adenomas. They have also been found, at a much lower frequency, in ACTH-secreting tumors, where they

Table 3. Genetic alterations in human pituitary adenomas.

Gene	Tumor Type(s)	Comments	Reference
GHRH-R	GH	Truncated receptor is activated in the absence of ligand	Hashimoto et al. 1995
GNAS1 (gsp)	GH, ACTH	Mutations lead to constitutive activation of Gs α-subunit	Vallar et al. 1987
H-RAS	Invasive, metastatic	Oncogenic mutations in invasive tumors (may correlate with aggressiveness)	Karga et al. 1992
PTTG	All types	Overexpression increases angiogenesis and invasiveness in rats	Pei and Melmed 1997; Zhang et al. 1999a
HST (FGF-4)	PRL	Overexpressed in large prolactinomas	Shimon et al. 1998
RB1	Invasive, metastatic	LOH at this locus correlates with increased invasiveness and metastasis	Woloschak et al. 1992
CDKN2A (p16)	All types	Decreased or absent expression in tumors	Woloschak et al. 1996
PITX1	ACTH	Expression reduced by 50% in ACTH-secreting tumors	Skelly et al. 2000
PITX2	ACTH, GH, gonadotropin	Absent in ACTH- and GH-secreting tumors; highly expressed in gonadotropic tumors	Heaney and Melmed 2000

GH, growth hormone; ACTH, adrenocorticotropic hormone; PRL, prolactin; LOH, loss of heterozygosity.

presumably mimic constitutive activation of the corticotropin-releasing hormone receptor.

Recently, several genes have been identified that are overexpressed in human pituitary adenomas (Table 3). The pituitary tumor-transforming gene (PTTG) encodes a protein that inhibits sister chromatid separation (Zou et al. 1999). Although it is not normally expressed in the adult pituitary gland (Zhang et al. 1999b), high levels of PTTG expression have been detected in all types of pituitary adenomas (Zhang et al. 1999a). It has also been detected in many other human cancers. It is believed that overexpression of PTTG inhibits sister chromatid separation during cell division, leading to increased genomic instability and the acquisition of additional tumor promoting mutations. The heparin-binding secretory transforming gene (HST), which encodes fibroblast growth factor-4 (FGF-4), is also overexpressed in pituitary tumors (Shimon et al. 1998). In contrast to the broad action of PTTG, elevated levels of FGF-4 expression have been found only in PRL-secreting tumors. Experiments in rats have demonstrated that expression of FGF-4 leads to an increase in angiogenesis and subsequent tumor invasiveness.

Loss of heterozygosity (LOH) studies have identified several chromosomal regions that may contain tumor suppressor genes involved in pituitary adenoma formation (Melmed 1999; Heaney and Melmed 2000). LOH at 11q13, 13q14, 10q26, and 9p suggests that tumor suppressor genes in these regions may play a role in regulating pituitary cell growth and proliferation. Despite their chromosomal locations, the MEN1 (11q13) and RB1 (13q14) genes do not appear to have significant roles in the process of sporadic pituitary tumor initiation (Pei et al. 1995; Prezant et al. 1998). Loss of the RB1 gene does, however, correlate with an increase in tumor invasiveness and metastasis (Heaney and Melmed 2000).

Anterior pituitary cell proliferation and differentiation are highly specific and tightly regulated processes. The expression of numerous homeodomain transcription factors is critical for proper pituitary gland ontogeny. Thus, it is reasonable to suspect that misregulation of the genes that encode these transcription factors may play a role in anterior pituitary tumorigenesis. Recent studies have examined the expression of a number of transcription factor genes, including PITX1 and PITX2, in all types of human pituitary adenomas. Alterations in the expression of both PITX1 and PITX2 have been demonstrated in specific tumor types (Table 3). PITX1 expression has been shown to be reduced by more than 50% in a number of corticotrope tumors (Skelly et al. 2000), while it has been suggested that the expression of PITX2 is increased in some gonadotrope tumors and decreased or absent in both somatotrope and corticotrope tumors (Heaney and Melmed 2000). Additional studies will be necessary to determine whether these subtle changes in homeobox gene expression play a causal role in pituitary adenoma formation.

Table 4. Mouse models of pituitary adenomas.

Mouse Model	Phenotype	Reference
Dopamine receptor D2 knockout	Prolactinomas	Asa et al. 1999
αGSU-LIF transgene	Cushing disease, Rathke's cysts	Yano et al. 1998
rGH-LIF transgene	Rathke's cysts	Akita et al. 1997
hGHRH transgene	GH-secreting tumors	Hammer et al. 1985
αGSU knockout	Nonfunctional TSHβ tumors	Kendall et al. 1995
PRL-TGFα transgene	Prolactinomas	McAndrew et al. 1995
hαGSU-SV40 T Ag transgene	Gonadotropin-secreting tumors	Windle et al. 1990
TSHβ-SV40 T Ag transgene	Null cell adenomas	Maki et al. 1994
hFSHβ-SV40 T Ag transgene	Null cell adenomas	Kumar et al. 1998
Polyoma early region promoter-Polyoma T Ag transgene	Cushing disease	Helseth et al. 1992

αGSU, alpha glycoprotein subunit; LIF, leukemia inhibitory factor; rGH, rat growth hormone; hGHRH, human growth hormone releasing hormone; PRL, prolactin; TGFα, transforming growth factor alpha; T Ag, T antigen.

Animal models of pituitary adenomas

The creation and examination of animal models of pituitary adenomas may help us better understand the molecular mechanisms of pituitary tumorigenesis. A number of mouse models of pituitary cell proliferation disorders have been generated (Table 4). Targeted deletion and overexpression of genes expressed in the anterior pituitary have been used to create physiologically relevant models of several types of pituitary adenomas and of Rathke's cleft cysts. For example, deletion of the gene that encodes the D2 dopamine receptor leads to unregulated production of prolactin and subsequent prolactinoma formation (Asa et al. 1999). Overexpression of the hypothalamic factor growth hormone releasing hormone (GHRH) stimulates the production and secretion of growth hormone, leading to the overproliferation of somatotropes and subsequent somatotrope tumor formation (Hammer et al. 1985). These two mouse models illustrate the critical roles of these genes in regulating pituitary hormone production. In the context of normal feedback regulation, dopamine acts to inhibit prolactin secretion, whereas GHRH promotes the production of growth hormone. Disruption of these processes in humans could potentially result in pituitary adenoma formation. The creation of these types of tumor models will further our understanding of the processes of tumor initiation and progression and may facilitate the identification of additional genes involved in human pituitary tumorigenesis.

In contrast, mice with targeted deletion of either *Rb* or *p27*, which develop pituitary tumors of intermediate lobe origin, are unlikely to correlate with human pituitary adenoma formation (Jacks et al. 1992; Fero et al. 1996).

Models of pituitary adenomas have also been generated by targeting the expression of oncogenic transgenes to anterior pituitary cells by using pituitary-specific promoters. In these models, the type of tumor formed generally correlates with the promoter used. For example, an SV40 large T antigen (T Ag) transgene driven by a prolactin gene promoter leads to the formation of prolactin-secreting tumors (McAndrew et al. 1995). Mice that express an SV40 T Ag transgene under the control of a *Tshb* promoter, however, do not develop TSH-secreting adenomas (Maki et al. 1994). Interestingly, the expression of this transgene leads to the overproliferation of undifferentiated anterior pituitary cells. The tumors formed in these mice do not produce any hormone and likely represent null cell adenomas. Although these oncogene-induced models of pituitary tumors may not provide insight into the mechanisms of tumor initiation in humans, they will help further our understanding of the mechanisms involved in tumor progression.

In addition to the previously mentioned mouse models, the rat pituitary provides an excellent system for the examination of estrogen-dependent pituitary tumor formation. Recent studies have shown that chronic estrogen treatment of rats can induce uncontrolled lactotrope proliferation. However, the relative sensitivity to the tumor-promoting actions of estrogen is highly strain-specific. Taking advantage of this finding, Wendell and Gorski have utilized two strains of rats (Fischer 344 and Brown Norway) to identify quantitative trait loci (QTL) for estrogen-dependent pituitary mass (Wendell and Gorski 1997; Wendell et al. 2000). The presence of a Fischer 344 allele at four separate QTL corresponds to an increased pituitary mass. A second group, using the genetically related AxC-Irish and Copenhagen rat strains, has determined that the twofold greater response of AxC-Irish rats to estrogen treatment results from the actions at a single locus (Spady et al. 1999). These studies reveal the complexity of the regulation of lactotrope proliferation. Although these models have a lot of promise, the identification of QTL genes genes is a very laborious and difficult process.

Summary

Homeodomain transcription factors have critical roles in both pituitary gland development and function (Watkins-Chow and Camper 1998). Deregulation of homeobox genes expressed in the anterior pituitary has been shown to result in various forms of pituitary gland dysfunction. Spontaneous and engineered mouse models with loss of function mutations in these genes have helped elucidate the specific role(s) each transcription factor plays in pituitary gland ontogeny. In addition, the examination of these genes in humans with pituitary hormone deficiency disorders and pituitary adenomas has contributed to our understanding of pituitary gland development (Heaney and Melmed 2000; Parker et al. 2000). The identification of novel homeobox gene mutations involved in human pituitary disorders, the examination of mouse models with increased transcription factor function, and the analysis of pituitary-specific deletions of transcription factor genes will further our understanding of pituitary gland organogenesis in both mice and humans.

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