Developmental Disorders of the Hypothalamo-Pituitary Axis

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Introduction

Short stature is defined by a height that lies <-2SDS below the mean. Recent molecular advances have led to the identification of the underlying aetiology in a number of cases that were previously believed to be “idiopathic”. This review will briefly discuss the advances in the field of pituitary development and the GH-IGF-1 axis that have led to the unravelling of the genetic basis to a number of these disorders.

Pituitary Development

The pituitary gland, which is a midline structure, consists of three lobes: the anterior and posterior lobes with a smaller intervening intermediate lobe. The three lobes are derived from ectodermal tissue; however, the embryological origin of the tissues differs. The posterior lobe arises from neural ectoderm whilst the anterior and intermediate lobes originate from oral ectodermal tissue. The gland is a central regulator of growth and development in children. The mature anterior pituitary gland is populated by five neuroendocrine cell types, each defined by the hormone produced: corticotropes (ACTH), thyrotropes (TSH), gonadotropes (LH, FSH), somatotropes (GH) and lactotropes (PRL). The posterior pituitary secretes oxytocin and anti-diuretic hormone (ADH). Recent work provides evidence for an induction model that explains the tissue interaction between the neural and oral ectoderm, a pre-requisite for the initial formation of the pituitary gland and subsequent differentiation into its five cell types. Development of this small gland has been shown to follow a similar pattern in a number of different species, but has been best studied in rodents such as the mouse and rat. As a result of the various naturally-occurring mutations in mice along with the information generated by murine knock-out models, it has been possible to begin the task of identifying the genes crucial to the development of this gland and to attempt to start placing these genes into some kind of genetic pathway which ultimately determines pituitary development. Various intrinsic and extrinsic transcription factors and signalling molecules have been implicated in normal anterior pituitary development. The transcription factors include homeobox genes that encode homeodomain factors. These contain a DNA-binding region called the homeodomain which can then bind to target DNA and regulate its expression, either switching on (activating) or repressing the expression of these genes.

Human disorders associated with GHD

Growth hormone deficiency may be isolated (IGHD) or combined with other pituitary hormone deficiencies (Combined Pituitary Hormone Deficiency; CPHD). It may also be a component of a number of syndromes such as septo-optic dysplasia (SOD). IGHD is reported to occur with a frequency of 1/3000-1/4000, whilst CPHD and syndromic GHD are much less common.
A genetic basis for CPHD was first demonstrated in 1992, when mutations in the pituitary-specific transcription factor *POU1F1/PIT1* were first described. *POU1F1* is a member of the POU family of transcription factors, characterised by the presence of a highly conserved bi-partite DNA binding domain, comprising the POU specific domain (POU-S) and the POU homeodomain (POU-HD). *POU1F1* is expressed relatively late during anterior pituitary development. Expression is restricted to the thyrotrope, somatotrope and lactotrope cell lineages, consistent with functional studies of this protein which show that expression of the GH, PRL, TSH-β subunit and GHRHR genes is regulated by *POU1F1*. The first proband described with *POU1F1* mutations presented with GH and PRL deficiencies and with severe central hypothyroidism. The mutation in question was a nonsense homozygous mutation within the POU-S domain leading to a severely truncated protein lacking 171 amino acids, including half of the POU-S and the whole of the POU-HD regions. Such a protein would be unable to bind to downstream targets such as the GH, PRL and TSH promoters. In addition to recessive mutations, a number of dominant mutations have also been described. Of these, the R271W substitution in the carboxy-terminus of the homeodomain of *POU1F1* is the most frequently described mutation. So far, 23 mutations have been described in the *POU1F1* gene. The mutations inherited recessively affect the structure of the POU-S and HD regions and the phenotypes are characterised by severe GH and PRL deficiency with a variable TSH deficiency, whereas the dominant mutations are generally found outside the DNA-binding domains. We have recently reported a 21 year old with a *POU1F1* mutation with no TSH deficiency to date.

*Prop1* (Prophet of Pit1) was positionally cloned using the mouse mutant, the Ames dwarf mouse (*df*), and is a pituitary specific homeodomain transcription factor of the *paired-like* class. In the mouse, expression is first observed in the dorsal region of Rathke’s pouch at E10.5, peaks at E12 and is completely undetectable by E15.5. Human mutations in *PROP1* are associated with CPHD (GH, PRL and TSH deficiency with additional LH and FSH deficiency). Patients with *PROP1* mutations were either unable to enter puberty or puberty is arrested due to gonadotrophin deficiency. Evolving cortisol deficiency has also been described in a small number of patients. To date, 15 different recessive mutations have been identified, making *PROP1* one of the most commonly implicated genes in CPHD. So far, all *PROP1* mutations are inherited in an autosomal recessive manner. An enlarged sella turcica with appearances suggestive of a pituitary tumour is occasionally observed in association with *PROP1* mutations. The exact mechanism underlying the pituitary enlargement remains unclear, with subsequent involution of the mass. This enlargement of the sella turcica is also observed with *LHX3* mutations. There is no clear genotype-phenotype correlation.

**Syndromic GHD**

The paired-like homeobox gene *HESX1* has now been implicated in some cases of septo-optic dysplasia (SOD). The gene is first expressed in the region fated to form the forebrain in the early mouse embryo. Subsequently, the gene is expressed in Rathke’s pouch following which its expression is down-regulated and extinguished. Null mutants for *Hesx1*, in which the entire coding region is deleted, display a phenotype characterized by anopthalmia or microphthalmia, midline neurological deficits eg. absent septum pellucidum, and pituitary hypoplasia, this being highly reminiscent of SOD. Between 1-2% of heterozygote mice also manifested a phenotype.
SOD is a rare condition in man and comprises classically of the triad of optic nerve hypoplasia, midline neuroradiological deficits and pituitary hypoplasia. However the phenotype is highly variable, and only 30% of patients manifest the full phenotype. Several aetiologies have been postulated to account for the sporadic occurrence of SOD, such as viral infections, environmental teratogens, and vascular or degenerative damage. It would appear that the developmental anomaly takes place during a critical period of embryogenesis between 4-6 weeks of gestation in human. Familial cases of SOD are rare, and may be associated with an autosomal recessive inheritance. Given the similarities between the murine Hesx1-null mutant phenotype and SOD, the role of the human homologue of this gene was investigated in patients with SOD and milder pituitary phenotypes. Initially a homozygous missense mutation within the homeobox of this gene was identified in two siblings who had been diagnosed with SOD. These children were born within a highly consanguineous pedigree and presented in the newborn period with hypoglycaemia secondary to cortisol deficiency. Subsequent testing confirmed complete panhypopituitarism. Neuro-radiological imaging revealed agenesis of the corpus callosum, optic nerve hypoplasia, a hypoplastic anterior pituitary gland and an ectopic/undescended posterior pituitary gland. Subsequently, 2 further homozygous and five heterozygous mutations have been described, in association with a range of phenotypes (IGHD with an undescended posterior pituitary, anterior pituitary aplasia, pituitary hypoplasia, SOD). These data suggested that, as with the heterozygous Hesx1-null mutant mice, heterozygous mutations of HESX1 are associated with a milder, variably penetrant phenotype. The presence of heterozygous mutations associated with a phenotype within a condition in which homozygous mutations have been described and where obligate carriers manifest no phenotype would indicate that the inheritance of this disorder is complex and may involve a number of genes ± environmental factors.

Duplications of Xq26-27 have recently been described in association with hypopituitarism. The smallest duplication to date is approximately 3.9MB and suggests that the hypopituitarism is due to over-dosage of a gene that lies in this critical region. Additionally, a polyalanine expansion within SOX3, a gene that lies within the critical region, has been described in a pedigree with X-linked mental retardation and GHD.

We have recently identified two siblings with a duplication at Xq27 that spans approximately 690Kb and contains only three transcripts, of which SOX3 is the only one to be expressed in the infundibulum. Both siblings have variable hypopituitarism associated with anterior pituitary hypoplasia, infundibular hypoplasia and an ectopic/undescended posterior pituitary. Additionally, we have identified a novel polyalanine expansion associated with partial loss of function possibly due to impaired nuclear localization in a pedigree with complete panhypopituitarism, anterior pituitary hypoplasia, a hypoplastic infundibulum and an ectopic/undescended posterior pituitary. Hence, both over- and under-dosage of SOX3 are associated with infundibular hypoplasia and variable X-linked hypopituitarism.

We have also identified mutations in a related gene SOX2 that are associated with hypopituitarism, anophthalmia, learning deficits and oesophageal atresia. The inheritance is dominant, and hypoplasia of the corpus callosum may be associated.

LHX3 (P-LIM/LIM-3) is a LIM-containing homeobox gene. Expression of Lhx3 is initially observed throughout the developing brain and spinal cord but is later restricted to the invaginating Rathke's pouch. Mutations within the human homologue of this gene were recently reported in affected individuals within two unrelated consanguineous pedigrees. The affected patients had severe growth retardation and were deficient in all but one of the anterior pituitary hormones (ACTH). Additionally, the patients also presented with a rigid cervical spine leading to limited head rotation.
LHX4, a LIM homeobox gene is closely related to LHX3 and has a very similar expression pattern within the developing pituitary. Whilst the expression pattern of Lhx3 is relatively broad within the pituitary during development, in comparison Lhx4 expression is seen in a restricted pattern within the Lhx3 expression domain. A mutation within this second LIM homeobox gene was described within a large consanguineous family. The probands presented with GH, TSH and ACTH deficiency. Neuroimaging of the affected individuals revealed the presence of a small sella turcica, a persistent craniopharyngeal canal, a hypoplastic anterior pituitary lobe and an ectopic/undescended posterior pituitary. The mutation associated with this phenotype within this family was shown to be a heterozygous G>C transversion at the 3′ end of intron 4. This splice site change affects the invariant AG dinucleotide of the splice acceptor site. Transfection studies revealed that the mutation led to splicing defects whereby mutant isoforms of the protein were generated, due to utilization of two cryptic splice acceptor sites present within exon 5. Both of these variant LHX4 isoforms lead to disruption of the homeodomain of this gene. It is noteworthy that a heterozygous mutation of this gene appears to be sufficient to result in a disease phenotype whereas heterozygous changes within LHX3 do not result in a clinical phenotype, even though on analysis of the murine null mutants, homozygous disruption of Lhx3 led to a more severe phenotype than that observed with Lhx4.

**Isolated GHD**

Clinically, isolated GHD may be inherited as an autosomal recessive (Type 1 GHD), autosomal dominant (Type II GHD) or X-linked recessive (Type 3). Isolated growth hormone deficiency (GHD) can be due to mutations in either the GH1 gene or the GHR receptor (GHRHR) gene. GH1 is the definitive gene involved in the synthesis of pituitary GH. In Type 1A GHD, large homozygous deletions (6.7 – 45 kB) lead to absence of GH. Type II GHD is associated with heterozygous mutations affecting splicing, and is therefore inherited as an autosomal dominant. It is believed that splice site mutations lead to the production of an alternatively spliced products (17.5, 20kDa GH), which can be associated with a dominant negative effect, and can also affect the secretion of other hormones such as ACTH, TSH and gonadotrophins with an evolving hypopituitarism. To date, four mis-sense mutation has been identified in GH1. All four mutations are associated with Type II GHD.

GHRH (Growth hormone releasing hormone) is secreted by the hypothalamus and binds to GHRH receptors on somatotrophs within the anterior pituitary. The receptor belongs to the G-protein family of receptors that are characterized by the presence of seven transmembrane domains. A recessive mutation within the GHRHR has been documented in the little mouse, and several recessive mutations have now been identified in the human homologue of the gene. The mutations are scattered throughout the gene, affecting both the ability of GHRH to bind the GHRHR and also the ability to transactivate the receptor.