HIGHLIGHT

Combined pituitary hormone deficiency – lessons from the murine models

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The molecular pathogenesis of some forms of combined pituitary hormone deficiency (CPHD, impaired production of growth hormone (GH) and one or more of the other five pituitary hormones) was established with the discovery of mutations in the pituitary transcription factor, *POU1F1* (the human homologue of mouse *Pit1*) several years ago (1). Pit-1 is a 33 kDa pituitary-specific transcription factor present in somatotrophs, lactotrophs and thyrotrophs of the mature pituitary, the embryonal expression of which precedes that of GH and prolactin (PRL), but not thyroid-stimulating hormone (TSH) β . It is a member of the POU-domain protein family, possessing both a POU-specific and a POU-homeodomain region necessary for high-affinity binding to the promoter regions of GH and PRL genes (2). Pit-1 co-operates with E twenty-six specific oncogene-related (Ets) transcription factors to regulate PRL gene expression (3), and with Zn-15 in the regulation of GH gene expression (4). In the thyrotroph, Pit-1 cooperates with activator protein-1-like (or GATA DNA-sequence-specific factor-2 (GATA-2)) factors to regulate TSH β gene expression (5).

As one might have predicted, elucidation of the genetics in murine models of dwarfism enabled linkage of a similar phenotype in man to mutations of Pit1. The Jackson dwarf mouse (*dw/dw*) has a major deletion in the *Pit1* gene (6), whereas Snell dwarf mice possess the *dw/dw* mutant *Pit1* allele that converts a tryptophan to cysteine (Trp-Cys) at amino acid 261 within the POU homeodomain (7). This mutation abolishes binding to a high-affinity *Pit1*-binding site, and is associated with deficiencies of GH, prolactin and TSH. Shortly thereafter, studies in a male patient with mental retardation, short stature, GH, PRL and TSH deficiency, but normal gonadotrophins and serum testosterone, identified a cytosine to thymine (C-T) mutation in *POU1F1* that resulted in substitution of an arginine residue by tryptophan (Arg-Trp) at codon 271 (1, 8). This mutation inhibited transactivation from a GH promoter by the mutant gene product, and thyrotrophin-releasing hormone stimulation showed a blunted TSH response consistent with a pituitary defect. In two other separate families, children with a similar clinical phenotype were investigated. They presented with growth impairment,

GH and PRL deficiency, and either later onset or early postnatal central hypothyroidism. Affected members of one family were homozygous for a cytosine to guanine (C-G) transition of *POU1F1* that altered amino acid 158 from alanine to proline (Ala-Pro, A158P), whereas affected siblings of the second family were compound heterozygotes for A158P and a separate coding sequence deletion mutant (9).

These observations established a central role for mutations in *POU1F1* in the molecular pathogenesis for many of the CPHD families, but left unresolved the nature of the genetic defect in those CPHD kindreds in which deficiency of gonadotrophins was also present. The recent cloning of the *PROP1* (Prophet of Pit-1) gene has shed much light on the area. The human *PROP1* gene (and the mouse homologue, *Prop1*) encodes a tissue-specific, paired homeodomain transcription factor expressed during embryonal pituitary development (10). Paired-class homeodomains bind as dimers to cognate DNA response elements containing TAAT palindromes, and the homeodomain region is highly conserved between human and mouse genes at both nucleotide and amino acid levels. Determination of the genetic defect in the Ames dwarf mouse proved to be very informative. These mice carry the *df/df* allele, which has a serine to proline (Ser-Pro) mutation in the $\alpha 1$ helix of the *PROP1* homeodomain, resulting in functional impairment of *PROP1*, near complete absence of somatotrophs, lactotrophs and thyrotrophs, and failure of activation of the *Pit1* gene (10). In the embryonal pituitary, *PROP1* expression precedes that of *Pit1* and, interestingly, the *df/df* phenotype results from marked loss of Pit1 lineage cells and failure of *Pit1* gene expression.

Interestingly, various different mutations in the human *PROP1* gene have now been identified in patients with CPHD. In a study of non-related CPHD kindreds with GH, PRL and TSH deficiencies in whom *POU1F1* alleles did not cosegregate with the CPHD phenotype, four kindreds with *PROP1* mutations were identified. These were substitution of arginine to cysteine at codon 120 (R120C), a 2-bp deletion (301–302delAG, two kindreds) and compound heterozygosity for the 2-bp mutation and a thymine to adenosine

(T-A) transversion mutation at nucleotide 349 (resulting in substitution of phenylalanine to isoleucine at codon 117, F117I) (11). When tested in DNA-gel shift and reporter studies, these mutant *PROP1* constructs exhibited absent or greatly reduced binding to the paired domain DNA consensus sequence, and either negligible or significantly reduced transactivation capacity (11). Further studies characterised the R120C mutation, in the third DNA-binding helix of the homeodomain, in affected siblings from two unrelated consanguineous families presenting with growth retardation. In the first family, all three affected siblings were homozygous for R120C, and displayed growth retardation around the age of 8 years and entered puberty having received GH treatment. However, gonadotrophin deficiency subsequently evolved to accompany the GH, PRL and TSH deficiencies present. In the second family, both affected siblings presented at or before 1 year of age (the first with central hypothyroidism), developed unequivocal GH, PRL and TSH deficiency, but retained measurable although decreased follicle-stimulating hormone (FSH) and luteinizing hormone (LH) responses (12). Thus the concomitant loss of FSH and LH support action of *PROP1* in earlier pituitary progenitor cells, in addition to the failure of somatotroph, lactotroph and thyrotroph differentiation (13).

The 301–302delAG *PROP1* mutation in exon 2 results in a coding sequence frameshift and premature termination at codon 109, predicting the loss of DNA-binding homeo- and C-terminal transactivation domains. This mutation has been identified in six of 10 probands from independent kindreds (five homozygous, one heterozygous) and three of 21 sporadic CPHD subjects (two and one respectively) (14). As the *PROP1* sequence reads 295-CGAGAGAGTC-304, the 301–302delAG mutation is identical to 296–297delGA, described in children with CPHD including complete GH and complete or partial PRL and TSH deficiencies, together with a 149–150delGA mutation (15). Studies on two other possibly related families with *PROP1* deficiency have been published recently. Two siblings in one and six affected (of nine) siblings in the other family all had normal intelligence, short stature, immature facies and high pitched voices, with no signs of sexual maturation (ages 17.5–39.8 years). Serum GH after clonidine ingestion was extremely low as was insulin-like growth factor-I. PRL, free thyroxine, LH, FSH, testosterone and oestradiol concentrations were substantially reduced. Both pairs of parents were heterozygous and all eight affected individuals were homozygous for a GA or an AG deletion mutation in the sequence 296GAGAGAG in exon 2 of *PROP1*, confirming autosomal recessive inheritance and reinforcing the identity of this mutational 'hot-spot' (16).

It is clear that the clinical phenotype of mutations in *PROP1* shares many features displayed by

patients with mutations in the *POU1F1* gene, as both develop deficiencies of GH, PRL and TSH. The major distinguishing feature of CPHD resulting from disruption of *PROP1* function is a reduction in gonadotrophins, because deficiencies of LH/FSH are not observed in *POU1F1* mutations. Interestingly, the clinical phenotype of CPHD differs between the various *PROP1* gene mutations, and also between the affected siblings with the same mutation in the same family (12). The reasons for these differences remain to be elucidated. However, future studies that characterise the biological factors responsible for development of these differing phenotypes will be of great interest.

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