The molecular basis of congenital hypopituitarism and related disorders

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Abstract

Context: Congenital hypopituitarism (CH) is characterized by the presence of deficiencies in one or more of the six anterior pituitary (AP) hormones secreted from the five different specialized cell types of the AP. During human embryogenesis, hypothalamo-pituitary (HP) development is controlled by a complex spatio-temporal genetic cascade of transcription factors and signaling molecules within the hypothalamus and Rathke's pouch, the primordium of the AP.

Evidence Acquisition: This mini-review discusses the genes and pathways involved in HP development and how mutations of these give rise to CH. This may present in the neonatal period or later on in childhood, and may be associated with craniofacial midline structural abnormalities such as cleft lip/palate, visual impairment due to eye abnormalities such as optic nerve hypoplasia and microphthalmia or anophthalmia, or midline forebrain neuroradiological defects including agenesis of the septum pellucidum or corpus callosum or the more severe holoprosencephaly.

Evidence Synthesis: Mutations give rise to an array of highly variable disorders ranging in severity. There are many known causative genes in HP developmental pathways that are routinely screened in CH patients; however, over the last 5 years this list has rapidly increased due to the identification of variants in new genes and pathways of interest by next generation sequencing.

Conclusion: The majority of patients with these disorders do not have an identified molecular basis, often making management challenging. This mini-review aims to guide clinicians in making a genetic diagnosis based on patient phenotype, which in turn may impact on clinical management.

Keywords

Pituitary development, Hypothalamus development, Endocrine, Congenital Hypopituitarism

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Embryonic development of the hypothalamo-pituitary region

The three lobes that constitute the pituitary gland are located within the sella turcica in the sphenoid bone just above the brain stem. The lobes are derived from two adjacent ectodermal layers: the anterior and intermediate lobes from the oral ectoderm, and the posterior lobe from the overlying neural ectoderm (1, 2). The mature gland is a central regulator of growth, homeostasis, metabolism, development and reproduction, through control of other endocrine glands throughout the body (1). Hypothalamo-pituitary (HP) development is reliant on the communication between the oral and neural ectoderm, which occurs through the intertwined genetic cascade of transcription factors and signaling molecules that may be either intrinsic or extrinsic to the developing Rathke's pouch (3) (Figure 1). A succession of precise molecular steps in cell proliferation and differentiation gives rise to the five specialized AP cell types that secrete six hormones: somatotrophs [growth hormone (GH)], thyrotrophs [thyroid-stimulating hormone (TSH)], gonadotrophs [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)], lactotrophs [prolactin (PRL)] and corticotrophs [adrenocorticotropic hormone (ACTH)] (4). Specific ligands travel from the hypothalamus to their respective receptors on the anterior pituitary (AP) cells via the hypophyseal portal blood vessels (Figure 1). This then results in the synthesis of the six AP hormones. These in turn are secreted into the bloodstream to regulate their targets throughout the body.

This mini-review aims to summarize the key players in embryonic development of the HP region (Figure 1) and classify the signaling pathways and their components implicated in the etiology of specific disorders of congenital hypopituitarism (CH) (Table 1). Congenital hypopituitarism is a highly variable condition that encompasses severe midline developmental disorders such as holoprosencephaly (HPE) and septo-optic dysplasia (SOD), hypopituitarism in isolation or combined with other congenital abnormalities such as a short stiff neck, cerebellar abnormalities, sensorineural hearing loss, and polydactyly, and isolated hormonal deficiencies such as isolated GH, ACTH, TSH and gonadotrophin deficiencies.

This review aims to assist both adult and pediatric endocrinologists in making more

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precise diagnoses based on genotypic profiles by attempting to define genotype-phenotype correlations. However, a number of confounding factors make this a challenging process. Recent data suggest that, as is the case with Kallmann syndrome/Hypogonadotrophic hypogonadism, oligogenicity may contribute to the complex and highly variable phenotypes and penetrance observed in CH patients. Rare and unique forms of CH with accompanying midline abnormalities may be caused by mutations in two or more genes involved in the complex signaling pathways that are critical for HP embryonic development (5). The limited number of patients reported in cohort studies, the variable frequency of mutations in different ethnic backgrounds, and the marked phenotypic variability associated with different genes, all make genotype-phenotype correlations difficult to establish (6). Additionally, very few studies have examined all of the patients within a cohort for mutations in all of the known genes implicated in CH. Hence there may be a bias in the reported frequencies of identified mutations.

The majority of CH cases are sporadic, with a small number of familial cases where the pathogenic causative gene mutation is inherited from one or both parents. As one would expect, there is a higher frequency of recessive mutations in families from consanguineous backgrounds and of certain ethnicities; for example, most *GHRHR* mutations have been identified in South East Asian communities. Furthermore, a founder effect, which is essentially the loss of variation amongst certain populations, is also apparent in certain cases, whereby a specific mutation within a gene, or different genes on the same chromosome, are present in a particular population, and are passed down the generations together. These may be disease-causing in compound heterozygosity or homozygosity.

Congenital hypopituitarism and midline abnormalities

The association of midline forebrain abnormalities with CH has long been established, suggesting a common developmental origin of the hypothalamus and pituitary and the midline structures within the brain (7) (Figure 2). The highly heterogeneous and complex disorder, septo-optic dysplasia (SOD; de Morsier Syndrome), has a prevalence of

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1/10,000 live births. Phenotypic features include 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 endocrine deficits (8, 9). Around 40% of SOD patients have normal endocrinology, and 75–80% of patients exhibit unilateral (12%) or bilateral (88%) ONH, which is usually the first presenting feature followed by subsequent endocrine dysfunction (10). The considerable phenotypic variability of the disorder and its increased frequency in babies born to younger mothers remain largely unexplained to date. Maternal drug and alcohol abuse have been proposed as risk factors that underlie the increased occurrence at a younger maternal age, when compared with mothers of children with isolated HP defects (11-13). Other variably associated features include developmental delay, seizures, visual impairment, sleep disturbance, precocious puberty, obesity, anosmia, sensorineural hearing loss and cardiac anomalies (11). More severe eye abnormalities such as microphthalmia or anophthalmia often occur in SOD cases (14). To date, most of the genes implicated in the etiology of SOD are transcription factors, such as *HESX1*, *SOX2*, *SOX3* and *OTX2*. More recently, mutations in genes implicated in Kallmann syndrome (KS), such as *KAL1*, *FGFR1*, *PROKR2* and *FGF8*, have also been identified in patients with SOD (15-18). The latter gene was also the first gene to be implicated in recessively inherited semilobar holoprosencephaly (HPE) with diabetes insipidus, and TSH and ACTH insufficiencies (17).

Transcription factors implicated during early HP development

The homozygous p.R53C substitution in HESX1 was identified in a consanguineous Pakistani family in which affected members of the family manifested panhypopituitarism with an abnormal corpus callosum and optic nerve hypoplasia (19, 20). The human phenotype strongly resembled the phenotype observed in homozygous *Hesx1* null mutant mice, which manifested variable anophthalmia/microphthalmia and midline forebrain deficits, with a small proportion (~5%) lacking an AP (21). Other phenotypic features in the mutant mice included a decrease in forebrain tissue, craniofacial abnormalities with a short nose, absent optic vesicles, and morphological abnormalities of the hypothalamus, infundibulum or Rathke's pouch (20). In rare cases, multiple pituitary glands were noted

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in mutant mice due to abnormal pituitary bifurcations (19-21). Further variations on the phenotype, although rare, have been observed in human patients, and include, for example, aplasia of the AP with complete hypopituitarism and retinal coloboma (HESX1 insertion mutation c.385-386ins315) (22). Patients with *HESX1* mutations (<1% of all cases) manifest variably penetrant phenotypes, ranging from IGHD, evolving hypopituitarism without midline and eye defects, to SOD and pituitary aplasia (6, 13, 23). The variability in phenotype and penetrance in human patients with *HESX1* mutations remains largely unexplained (3).

OTX2 regulates the expression of *HESX1* and *POU1F1* during AP development (24) and is required for the formation of anterior structures and maintenance of the forebrain, with mutations being described in ~3% of CH patients with anophthalmia/microphthalmia (19, 24). Mice heterozygous for *OTX2* loss of function can have pituitary hypoplasia, missing or misplaced pituitary glands, and/or pituitary dysmorphology (25). *OTX2* is expressed strongly in the developing posterior pituitary (PP) lobe, hypothalamus and other specific regions of the brain, but expression is modest and transient in Rathke's pouch. Using conditional transgenesis, Mortensen *et al* (26) have shown that disruption of OTX2 in early head development causes a variable dysmorphic pituitary gland phenotype, whereas loss of OTX2 in Rathke's pouch has no effect on cell specification. OTX2 deficiency in the ventral diencephalon has a profound effect on the initial development of the posterior lobe and pituitary stalk. This is associated with reduced and delayed FGF signaling, which secondarily causes anterior lobe hypoplasia.

Patients with *OTX2* mutations again manifest highly variable phenotypes including IGHD, CPHD or HH, with severe ocular malformations including ONH, retinal dystrophy or coloboma with or without anophthalmia/microphthalmia (27-29).

The transcription factor *RAX* is implicated in eye and forebrain development, with murine null mutants manifesting anophthalmia, cleft palate, and an abnormal hypothalamus resulting in perinatal lethality (30). Bilateral microphthalmia or anophthalmia in some patients has arisen from compound heterozygous or recessive *RAX* mutations, with variable associated clinical features (31-34). Recently, a homozygous truncating *RAX*

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mutation, p.Pro89Argfs*114, has been reported in a patient with anophthalmia, CH (including GH, TSH and ACTH deficiencies with probable gonadotrophin deficiency), diabetes insipidus, bilateral cleft lip and palate, micropenis (likely hypogonadotropic hypogonadism) and an absent anterior and PP gland on MRI (35). This is the most severe phenotype to date, and appears to correspond to the severity of the mutation itself, as opposed to the previously published missense mutations associated with less severe molecular dysfunction.

Phenotypes encompassing bilateral anophthalmia/microphthalmia with developmental delay, learning difficulties, esophageal atresia and male genital abnormalities have been associated with *SOX2* mutations in 10-15% of patients with severe eye defects (36, 37) (33), with some patients manifesting AP hypoplasia and HH (38). Functional studies have suggested that mutations cause haploinsufficiency during development, due to reduced DNA binding, transcriptional activation or nuclear localization. Although the majority of mutations are *de novo*, some are inherited by either somatic or germline mosaicism (39). Human expression of *SOX2* is found in the developing hypothalamus, Rathke's pouch and the eye (14), and conditional deletion of murine *Sox2* in the hypothalamus and pituitary is associated with GH, TSH and gonadotrophin deficiencies (40), thus supporting the role of this gene during HP embryogenesis, particularly with respect to GnRH neuron specification. More recently, *SOX2* mutations have been identified in patients with non-syndromic hypogonadotrophic hypogonadism (41, 42). Importantly, the same *SOX2* mutation can be associated with variable phenotypes within the same family; for instance hypogonadotrophic hypogonadism in a parent with severe eye defects in offspring (41). Furthermore, *SOX2* mutations have been implicated in patients with slow-progressing pituitary tumors (43); this finding has not been explained to date. However, studies have suggested that Sox2 plays a critical role in maintenance of pituitary progenitor/stem cells, and that embryonic and adult Sox2+ pituitary progenitor/stem cells can differentiate into all hormone-producing lineages, highlighting the physiological maintenance of the adult mouse pituitary by Sox2 (44). Furthermore, murine studies have shown the occurrence of such tumors following targeted expression of oncogenic β-catenin in Sox2+ cells (44), indicating that Sox2+ pituitary stem/progenitor cells may be implicated in tumorigenesis *in vivo*. Sox2 is also essential for melanotroph cell fate, aside from its established early

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role in promoting progenitor proliferation (45).

Mutations in *SOX3* are most commonly implicated in infundibular hypoplasia, with patients manifesting an ectopic/undescended PP in combination with AP hypoplasia on MRI. An in-frame expansion of 11 alanines in the polyalanine tract of SOX3 was the first to be described in a patient with IGHD and learning difficulties (46) with subsequent reports of loss of function polyalanine tract expansions in patients with multiple hormone deficiencies thereafter (47). Duplications of varying length (from submicroscopic to 3000Kb) that span the region in which *SOX3* is located had previously been associated with hypopituitarism. Surprisingly, deletions of *SOX3* have also been described in CPHD patients with an abnormal corpus callosum on MRI, and with absence or hypoplasia of the infundibulum (47, 48). In one rare patient with CPHD, a persistent craniopharyngeal canal on MRI was associated with a deletion of *SOX3* within a 2.31Mb deletion (49). Interestingly, contraction of the polyalanine tract has been associated with gain of function, and this may be a situation that mirrors excess gene dosage associated with *SOX3* duplications (50). These data suggest that both loss and gain of function mutations in SOX3 are associated with congenital hypopituitarism; these phenotypes are similar to those observed in *Sox3* null mice (47, 50). This paradigm suggests that reported SOX3 duplications, loss/gain of function mutations and deletions illustrate the importance of optimal gene dosage during the embryonic development of the diencephalon, pituitary stalk and the AP. Hughes *et al.* (51) have shown in the mouse that the polyalanine expansion of 11 alanine residues (*Sox3*-26ala) is associated with reduced functional protein levels in the nucleus, possibly due to efficient clearance of misfolded protein by the cell (51).

The multifunctional LIM homeobox proteins LHX3 and LHX4 appear to be expressed at a later stage of pituitary development than the above genes and are involved in transactivation and protein-protein interactions (52-54). Mutations have been reported in <1% of all patients with CPHD in cohort studies (6, 55, 56). Following the identification of the first *LHX4* variant (intronic) reported in a pedigree in which the affected patients manifested hypopituitarism (57), a number of patients with heterozygous *LHX4* mutations and variably penetrant CPHD have been described, probably as a result of *LHX4*

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haploinsufficiency (18, 52). In the majority of cases, the PP is ectopic (EPP), although it may be normally sited (58). The variable penetrance remains unexplained; for example an unaffected parent may harbor the same mutation/deletion of *LHX4* and yet manifest no pituitary phenotype. Interestingly, the first novel and only reported case of a recessive lethal *LHX4* mutation was reported in three siblings with severe CPHD, an EPP and mid-facial hypoplasia. The patients died due to respiratory distress syndrome associated with their severe hypopituitary phenotype (59). A recent study examining a large cohort (N=417) for *LHX4* mutations identified mutations in 1.4% (60).

Patients with *LHX3* mutations (recessive) usually have panhypopituitarism, including ACTH deficiency that may appear later, and have the characteristic hallmark of a short stiff neck caused by the absence of neck rotation (61). Sensorineural hearing loss and skeletal anomalies are being increasingly recognized as components of the LHX3 mutant phenotype. Sobrier *et al* (62) described compound heterozygosity for two mutations in *LHX3* in a pedigree in which the proband had CPHD associated with scoliosis. One of the mutations was found to have a dominant negative effect, with a mild phenotype of limited neck rotation in the heterozygote parent and grandparent. The latter c.252-3C>G mutation, which disrupts an acceptor splice site, would lead to a severely truncated protein containing a single LIM domain, accounting for the dominant negative effect (62). MRI in patients with *LHX3* mutations usually reveals a small AP with a eutopic PP, although an enlarged AP gland has rarely been reported (63).

Lhx3 and Lhx4 are also characterised by the presence of the unique cysteine/histidine-rich zinc-binding LIM domain. After induction by Fgf8, *Lhx3* is expressed strongly and uniformly in Rathke's pouch from E9.5, in the ventral hindbrain and in spinal cord (64). Early in pituitary development (E9.5-E10.5), there is overlap in the expression pattern of *LHX3* and *ISL1*, but their expression becomes mutually exclusive at the later stages of development (65). By E16.5, *Lhx3* is expressed in the developing anterior and intermediate pituitary, but not in the posterior gland, and its expression persists into adulthood suggesting that *Lhx3* has a role in the establishment of hormone-producing cell-types and in the maintenance of at least some cell types in the mature AP (64, 66).

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Lhx3 null mice (*Lhx3*$^{-/-}$) show early lethality with lack of the anterior and intermediate pituitary lobes and, although Rathke's pouch is initially formed, development of the pituitary gland is arrested with defects in the differentiation of all hormone-secreting cell types, as there is failure to maintain expression of *Hesx1* and induce *Pou1f1* (67).

Murine *Lhx3* and *Lhx4* are expressed at embryonic day 9.5 (E9.5) in Rathke's pouch, *Lhx4* is then confined to the tissue that will become the AP gland by E12.5 but yet has lower transcript levels than *Lhx3* in the mature gland, whilst *Lhx3* maintains expression throughout the whole pouch (68). *LHX4* is expressed in the developing hindbrain, cerebral cortex, pituitary gland and spinal cord in both humans and rodents (69). Both LHX3/4 work in conjunction with one another to establish the specialized mammalian pituitary cell lineages (70), with *Lhx4* being required for the correct temporal expression of regulatory genes including *Lhx3* (52). Thus, patients with *LHX4* mutations may have a partial loss of LHX3 function. The crucial role of these transcription factors is demonstrated through *in vivo* studies that show arrested pituitary development at the rudimentary pouch stage in mice lacking both of these genes. Furthermore, mice lacking Lhx4 specifically exhibit incomplete pituitary gland development. Homozygous Lhx4 mutant mice die soon after birth with underdeveloped lungs that are unable to inflate; however heterozygotes have no obvious reported phenotypes (71).

De novo heterozygous mutations in *FOXA2* have recently been implicated in the etiology of CPHD and congenital hyperinsulinism (HI) with other features including craniofacial dysmorphic features, choroidal coloboma and endoderm-derived organ malformations in the liver, lung and gastrointestinal tract (72). A further case had CH with childhood-onset diabetes, cardiac malformation and anal atresia (73). These findings confirm those of a previous report describing a 277 kb heterozygous deletion on chromosome 20, incorporating *FOXA2*, in a family with CH, situs inversus, polysplenia, dysmorphic features, cardiovascular defects and biliary atresia (74). Expression profiling in human embryos by immunohistochemistry showed strong expression of *hFOXA2* in endoderm-derived organs including the pancreas, and transfection studies and western blot assays confirmed the causative role of FOXA2 in this syndrome (72, 75).

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Genes implicated in hypothalamic development

ARNT2 (aryl-hydrocarbon receptor nuclear translocator 2) is a member of the basic-helix-loop-helix-Per-Arnt-Sim (bHLH-PAS) superfamily of transcription factors. This protein forms heterodimers with sensor proteins from the same family that then bind regulatory DNA sequences. *Arnt2*(-/-) null murine embryos die perinatally and exhibit impaired hypothalamic development (76). Recent studies showed expression of *ARNT2* within the CNS, including the hypothalamus, as well as the renal tract during human embryonic development. A homozygous frameshift *ARNT2* mutation was previously described in six patients from a highly consanguineous pedigree with CPHD (GH, TSH and ACTH deficiencies associated with diabetes insipidus), post-natal microcephaly, fronto-temporal lobe hypoplasia and visual and renal abnormalities, proving lethal in several of the infants. This pedigree highlights the importance of ARNT2, in HP development and post-natal brain growth (77). A recent report described a second family with CPHD, congenital central hypotonia and hypoventilation, central diabetes insipidus, severe developmental delay, acquired microcephaly, cortical blindness with normal retinal examination, and seizures. Interestingly the 3 patients had a synonymous variant, c.378C>T; p.G126G, that is thought to create a cryptic donor splice site predicted to lead to a loss of function (78).

Signaling molecules

The Sonic hedgehog (SHH) signaling pathway

GLI2 encodes a transcription factor component of the SHH signaling pathway, and is implicated in the etiology of HPE and other midline neurodevelopmental anomalies (79, 80). Unlike mutated *SHH*, described to specifically cause HPE, mutated *GLI2* is also associated with CH in the absence of midline brain defects, giving rise to the Culler-Jones syndrome (81). These patients may have loss of function missense or truncation mutations that delete the entire C-terminus, with variable phenotypes ranging from IGHD to complex CPHD, in combination with variable polydactyly, cleft lip/palate, diabetes insipidus, dysmorphic features and an EPP on MRI (82-85). Phenotypic variability may

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be marked within pedigrees (86). Incomplete or variable penetrance may also be observed with *GLI2* mutations, where a heterozygous mutation with functional consequences in the child is also present in an unaffected parent or a parent with a mild form of the disease respectively (81). Although several variants have been identified in cohort studies, functional studies have been performed in a minority. Recently, Hayne et al. have proposed gene-environment interactions that may underlie the variable penetrance associated with *GLI2* mutations (87). On the C57BL/6J murine background, homozygous *GLI2* loss of function resulted in the characteristic brain and facial features seen in severe human HPE, including midfacial hypoplasia, hypotelorism and medial forebrain deficiency with loss of ventral neurospecification. Although normally indistinguishable from wild-type littermates, mice with single-allele *Gli2* mutations exhibited increased penetrance and severity of HPE in response to low-dose teratogen exposure. These data suggest that a genetic predisposition is associated with a Gli2 dosage-dependent attenuation of Hedgehog ligand responsiveness at the cellular level, and this may be determined by interactions with the environment.

Pituitary stalk interruption syndrome (PSIS) encompasses the presence of a thin or discontinuous pituitary stalk, a hypoplastic AP gland and an EPP on MRI. In rare cases, mutations in *HESX1, OTX2, SOX3, LHX4* and *PROKR2* have been described in patients with PSIS (88-90).

More recently, a mutation in *CDON*, a Shh co-receptor and a component of the SHH signaling pathway previously implicated in the etiology of HPE, has been identified in a patient with PSIS with GH, TSH, and ACTH deficiencies, and neonatal hypoglycemia and cholestasis (91). Interestingly, murine *Cdon* mutation was associated with optic nerve hypoplasia, and the effect was phenocopied by ethanol administration (92). A recessive variant in a further SHH component, *GPR161*, encoding the orphan G protein-coupled receptor 161, has also been reported in a consanguineous family with PSIS (93). These data suggest that the Shh signaling pathway is crItical for normal HP development.

Wnt signaling pathway

The WNT/β-catenin signaling pathway regulator, *TCF7L1,* has been implicated in the

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etiology of SOD. The conditional deletion of *Tcf7L1* in mice results in forebrain and eye defects with partially penetrant dwarfism (94). Heterozygous missense *TCF7L1* variants were subsequently identified in two unrelated SOD patients, one of whom had hypopituitarism (94), implicating this important signaling pathway and its components in HP development.

Slit/Robo signaling

Variably penetrant mutations in the receptor ROBO1, regulating embryonic axon guidance and branching in the nervous system via Slit/Robo signaling during development (95), have been implicated in five PSIS patients. Four out of these five patients had ocular anomalies including hypermetropia with strabismus, and ptosis (96). Furthermore, a recent homozygous mutation was reported in a child with syndromic CPHD (97).

Isolated CPHD

Transcription factors implicated during later pituitary development

POU1F1 and PROP1 are the best functionally characterized intrinsic HP transcription factors in mice and humans (98). The pituitary-specific transcription factor POU1F1, formally known as PIT1, is expressed exclusively in the somatotroph, thyrotroph and lactotroph cell lineages during late AP differentiation (99). Functional studies are consistent with expression, showing that regulation of *GH, PRL, TSHβ* and *GHRHR* expression depends on POU1F1 (98). Homozygous loss-of-function mutations within a *Pou1f1* hotspot are known to give rise to the Snell dwarf mouse model phenotype (18, 100), which lacks the three Pou1f1 lineages. The importance of POU1F1 in these cell types was demonstrated *in vivo* by the identification of the first *POU1F1* mutation (homozygous p.R172*) in a patient with GH, TSH and PRL deficiencies, resulting in absent binding of mutant POU1F1 to GH and PRL promoters causing a loss of transcription (101). Both dominant and recessive *POU1F1* mutations occur in approximately 3% of CPHD cases that are highly variable, with GH and PRL deficiencies usually present, and TSH deficiency being more variable, ranging from early central

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congenital hypothyroidism through to maintained thyroid function in adulthood (55, 102) (103). Recently, an autosomal dominant heterozygous missense *POU1F1* mutation was identified in a large pedigree with IGHD, further expanding the phenotypic expression (104). MRI usually reveals a small AP with a normal PP and stalk.

The pituitary-specific transcription factor PROP1 is important for the production and secretion of GH, PRL, TSH and gonadotrophins (LH and FSH), and regulates *POU1F1* expression. Recent studies have suggested that all hormone-secreting cell types of both the anterior and intermediate lobes are descended from *Prop1*-expressing progenitors (105).

Mutant Prop1/PROP1 occurs in approximately 11% of all CPHD phenotypes, with an incidence of up to 50% of familial CPHD but rare in sporadic cases (~7%) (55, 106). Mutations appear to be more frequently identified in cohorts derived from Eastern Europe (107, 108). The phenotype is classically that of GH, PRL and TSH deficiencies. Other phenotypic features include congenital hypogonadism or arrested puberty (109), evolving ACTH deficiency (110), or an enlarged AP indicative of a tumor (111) that can wax and wane in size and thereafter regress with time, leading to complete pituitary involution and an empty sella syndrome. In murine studies, the p.S83P homozygous Prop1 mutation causes a lack of *Pou1f1* activation, preventing maturation of cells, giving rise to what is now known as the Ames dwarf mouse model (98, 112). This failure of cells to differentiate is specifically due to the retention of progenitor cells in the periluminal area (113). PROP1 stimulates stem cells to transform from epithelial to mesenchymal cells, and is essential for cell migration and differentiation (114), suggesting that PROP1 is essential for pituitary stem cell differentiation.

Isolated hormone deficiencies

Isolated growth hormone deficiency

Congenital isolated growth hormone deficiency (IGHD) has an incidence of 1/4000-

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10,000 live births with the majority being sporadic, with up to ~30% familial cases. Genetic causes are identified in approximately 34% of the familial cases compared to just 4% of sporadic cases (115). We have previously identified *GH1* mutations in 7.4% of our cohort, with a higher prevalence among familial cases (22.7%) compared to sporadic (2.7%) cases (116). Homozygous *GH1* deletions (~6.7kb in length) remain the most frequently identified *GH1* gene mutations in patients with autosomal recessive IGHD type IA (117), with other loss of function *GH1* mutations also being described. In this form of the disease, patients have severe growth failure with undetectable GH concentrations within 6 months of post-natal life. The MRI usually reveals a small AP with a eutopic PP. These patients variably develop growth-inhibiting anti-GH antibodies (118) on treatment with recombinant human (rh) GH; recombinant human insulin-like growth factor 1 (rhIGF1) is then the only therapeutic option to achieve growth. Patients with recessive GHD type IB may harbor *GH1* or *GHRHR* mutations, with the majority of reported cases originating from consanguineous pedigrees, often specifically from Brazil or the Indian subcontinent (4, 119). Type IB GHD caused by *GHRHR* mutations is usually termed Sindh dwarfism (119) and is phenotypically distinct from the classical GHD phenotype, with patients having minimal facial hypoplasia and no micropenis. The majority of *GHRHR* mutations (present in around 4% of IGHD cases) are associated with complete loss of function (120, 121) as evidenced by eg. cAMP production, such as the p.K329E substitution that fails to show any cAMP response following GHRH treatment in *in vitro* studies (122). The originally described and most common GHRHR mutation is the p.E72X truncation, which lacks the transmembrane and intracellular domains (123). Other frequent mutations include the recessive intronic c.57+1G>A mutation, originally described in 30 affected individuals from a large kindred with IGHD and dwarfism. (124). Additionally, a novel partial loss of function GHRHR homozygous mutation, p.P79L, has been described to give rise to an unusually mild form of IGHD in two unrelated families from Pakistan (125). The MRI findings in patients with Type1B IGHD reveal a small AP with a eutopic PP.

Alternative splicing causing exon skipping, caused by heterozygous *GH1* mutations, leads to autosomal dominant type II GHD (126), the most common genetic form of IGHD. The skipping of exon 3 yields the shorter GH 17.5kDa isoform, exerting a dominant negative effect on GH secretion, with expression levels directly related to disease severity

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(127) (128). Patients with Type II GHD may have variable height deficit with some mutation carriers even achieving a normal adult height without treatment, and with development of additional pituitary hormone deficiencies over time, including ACTH, TSH, and gonadotrophin deficiencies (129-132). In some patients, reversibility of GHD has also been observed (120, 132).

More recently, biallelic mutations in *RNPC3*, encoding a small protein component of the U12-type minor spliceosome (as opposed to the U2-type major spliceosome), have been reported in three sisters with severe IGHD and AP hypoplasia on MRI. Patient cells revealed anomalies in the formation of U11/U12 RNA-protein complexes (snRNPs) and U12-intron splicing (133). A zebrafish model with a lethal missense *rnpc3* mutation has provided a model of aberrant U12-type splicing *in vivo*, showing aberrant U11/U12 snRNPs with significantly impaired U12-type splicing, thus halting intestine, liver and pancreatic development (134).

Central congenital hypothyroidism

Mutations in genes such as *TSHB* and *TRHR* that regulate TSH biosynthesis and secretion have been identified in rare cases of isolated TSH deficiency (TSHD), also termed central congenital hypothyroidism (135, 136). TSH concentrations are highly variable, occasionally being undetectable (137, 138). The mutational 'hotspot' in *TSHB*, c.373delT (p.C105Vfs114X) (138), has been identified in several recessive forms of TSHD worldwide (139, 140). Haplotype analysis in six unrelated affected families with this deletion revealed a possible founder effect, believed to have a mutational age of 150 generations. Data suggested a monophyletic origin of the *TSHB* c.373delT mutation from a common ancestor with no significant population prevalence (140, 141). This hotspot has also been identified in compound heterozygosity with additional *TSHB* mutations such as p.Q49X, a 5.4kb deletion and p.M1P respectively (141, 142). Recessive biallelic inactivating *TRHR* mutations have been described in patients with TSHD with absent TSH and PRL responses to exogenous TRH (143, 144). In addition, deleterious *TRHR* mutations, p.P81R (145), and p.I131T (135), have been identified in TSHD patients, with the latter thought to decrease TRH affinity for its receptor. Interestingly, a *PROP1* homozygous frameshift has also been described in a patient with isolated TSHD (146).

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Two X-linked genes, *IGSF1* and *TBL1X,* have also been implicated in the pathogenesis of TSHD, occasionally in association with other pituitary hormone deficiencies (discussed in the X-linked disorders section).

Central congenital hypoadrenalism

The extremely rare and heterogeneous condition of isolated ACTH deficiency (IAD) is often lethal due to hypocortisolism, and can present with neonatal hypoglycemia, convulsions and hypercalcemia (147). *TBX19*, formally known as *TPIT* plays a critical role in corticotroph and melanotroph differentiation, the pituitary pro-opiomelanocortin (POMC) lineages. Mutations in *TBX19* have been identified in >60% of neonatal early-onset IAD (148), with *in vitro* assays depicting complete or severe loss of function (149). Mutations usually affect DNA binding or protein-protein interaction due to substitutions in the DNA binding Tbox domain; however, chromosomal deletions, truncations and mutations leading to alternative splicing have also been reported (149-151). Mutations in *POMC* have been reported in association with IAD patients, usually eliciting distinct phenotypic hallmarks such as early-onset obesity and red hair in addition to adrenal insufficiency with hypocortisolism and hypoglycemia. Initially, *POMC* mutations were described in a patient with compound heterozygosity for two mutations in exon 3 (G7013T, C7133Δ), and in an additional patient who was homozygous for the p.C3804A mutation (152). Recessive mutations have also been described in *PCSK1*, encoding PC1, a prohormone convertase that cleaves POMC to generate ACTH in corticotrophs, in ACTH-deficient patients (153). One such patient had ACTH and gonadotrophin deficiency, with severe obesity and glucose dysregulation (153), whilst another had predominant malabsorptive severe refractory neonatal diarrhea, with obesity, hypoadrenalism, reactive hypoglycemia, and elevated circulating prohormones (154). Interestingly, PC1-null mice have growth retardation instead of obesity; however they mirror PC1-deficient humans in having defective POMC and proinsulin processing (155). Exciting new studies have generated *PCSK1*/PC1-deficient human embryonic stem cells (hESC) that can differentiate into hypothalamic neurons. Neurons had increased levels of unprocessed POMC, and decreased levels of POMC-derived peptides in the knockout cell line, which mimics the abnormal POMC processing reported in both PC1-null mice

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and PC1-deficient patients (156). PC1/3-deficient patients may also manifest hypo- or hyper-thyroidism and -cortisolism respectively (157), or GHD, hypogonadotrophic hypogonadism and diabetes insipidus in rare cases (158), thereby expanding the range of endocrine abnomalities in patients with impaired *PCSK1*.

Hypogonadotrophic hypogonadism

Congenital hypogonadotrophic hypogonadism (CHH) is a rare disorder characterized by absent production, secretion, or action of gonadotrophin releasing hormone (GnRH), the master hormone of the reproductive axis. It is clinically characterized by absent puberty, which may be complete or partial, and impaired or absent fertility. In approximately 50% of CHH patients, the patients complain of a defective sense of smell (anosmia or hyposmia); this association is termed Kallmann syndrome and results from incomplete embryonic migration of GnRH neurons, which originate outside the CNS in the olfactory placode and migrate into the brain during embryonic development. CHH can be difficult to diagnose in the absence of anosmia or hyposmia, particularly when attempting to differentiate it from constitutional delay of puberty. Timely diagnosis and treatment to induce puberty are critical for sexual, bone, and metabolic health and may help minimize some of the psychological impact of CHH. Fertility may need to be induced using specialized treatment regimens. CHH is clinically and genetically heterogeneous with >30 different causal genes identified to date, but accounting for only 50% of all cases identified to date. A number of developmental anomalies including cleft lip or palate, dental agenesis, ear anomalies, congenital hearing impairment, renal agenesis, bimanual synkinesis, and skeletal anomalies may occur in a variable proportion of CHH patients, depending on the genetic etiology. The causative genes include a number of ligands and their receptors, as well as signaling molecules such as FGF8. Oligogenicity is estimated to occur in up to 20% of cases, with reversibility of the condition reported to occur in up to 10% of cases. A full discussion of this hormone deficiency is beyond the scope of this review, and the reader is referred to several existing reviews (159-161).

X-linked hypopituitarism

Aside from the previously discussed transcription factor *SOX3*, a number of other X-linked

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causes of CH have recently come to light. Mutations in Immunoglobulin Superfamily Member 1 (*IGSF1*) have been associated with an X-linked form of central hypothyroidism, often associated with macroorchidism, GH deficiency, and variable prolactin deficiency (162-164). Murine *Igsf1* is expressed in thyrotrophs, lactotrophs, and somatotrophs (162), and in Leydig and, albeit at low levels, in Sertoli cells in murine/human testes (165). *Igsf1*-deficient male mice have lower pituitary and serum TSH concentrations, pituitary TRH receptor expression and triiodothyronine concentrations, with an increase in body mass (162). Mutant mice with loss of function in the C-terminus have decreased TSH subunit gene expression, and TSH and TRH protein expression (166). IGSF1 has recently been reported to stimulate TRHR transcription, thus increasing TSH synthesis and bioactivity, via involvement with the TGFβ1-Smad signaling pathway. Garcia et al. recently reported a large hemizygous ~208 Kb deletion on Chr. Xq26.2 associated with hypothyroidism and macroorchidism from 3 years of age, with reduced TSH biopotency and increased FSH secretion in neonatal minipuberty (165). Interestingly, female carriers of *IGSF1* mutations occasionally manifest mild hypothyroidism (167). Furthermore, a component of the thyroid hormone receptor-corepressor complex, *TBL1X,* has been associated with an X-linked form of TSHD, with six mutations being identified in unrelated pedigrees with isolated TSHD (168), whereas previous studies had implicated the gene in sensorineural hearing loss (169).

The eukaryotic translation initiation factor (eIF) 2 subunit 3 (*EIF2S3*) encodes the eIF2γ subunit. Protein synthesis is initiated by eIF2 forming a ternary complex with initiator methionyl-tRNA and GTP, to bind to mRNA and scan for the AUG start codon. Hemizygous missense and frameshift mutations in *EIF2S3* have been described in patients with MEHMO syndrome, an X-linked disorder characterized by mental retardation, epileptic seizures, hypogonadism with hypogenitalism, microcephaly and obesity (170-172). These patients usually have a severe intellectual disability, GHD and microcephaly, with a few reports of hypoglycemia. We recently reported a novel *EIF2S3* variant, p.P432S, in a pedigree with endocrine deficits including hypopituitarism and a unique form of glucose dysregulation that fluctuates between hyperinsulinemic hypoglycaemia and post-prandial hyperglycemia, with only mild learning difficulties (173). The phenotype observed in this family contrasts with all previously reported patients with

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an *EIF2S3* mutation, in that the patients do not have severe intellectual disability, microcephaly, epilepsy or obesity, but instead have a much milder phenotype. This milder phenotype was reflected through functional assays in corresponding yeast residues, which showed a milder loss of function of *EIF2S3*/eIF2γ p.P432S in start codon selection stringency compared to all previously described mutations (173). It has been proposed that the neurological phenotype in the majority of previous cases may have been exacerbated by their untreated hypoglycemia, resulting in their more severe intellectual impairment and seizures. Furthermore, *EIF2S3* is expressed in the human brain specifically at high levels in the hypothalamus and Rathke's pouch during embryonic development, and functional studies showed an increase in caspase activity and thus cell death, when *EIF2S3* was knocked down in human pancreatic 1.1 B4 cells (173).

Recently described molecular causes of CH

Channelopathy genes implicated in hypopituitarism

KCNQ1, encoding a voltage-gated ion channel Kv7.1 subunit, is known to be associated with cardiac arrhythmia syndromes (174). However, patients with variably penetrant phenotypes including GH and gonadotrophin deficiencies, maternally inherited gingival fibromatosis, and accompanying mild craniofacial dysmorphic features have recently been reported to harbor missense mutations in this paternally imprinted gene (175). Studies have revealed transcripts in somatotrophs and gonadotrophs in mice and humans, in embryonic murine hypothalamic GHRH neurons and in the human hypothalamus (175). Previous reports of currents through voltage-gated potassium channels in pituitary cells, together with these data, suggest that ion channels may be imperative regulators of pituitary function in humans (176-178).

Genes implicated in cell membrane integrity

Neuropathy target esterase (NTE), encoded by *PNPLA6*, known to be involved in rare neurodegenerative conditions (179, 180), has also been implicated in disorders of HP dysfunction. NTE is an enzyme that catalyzes the de-esterification of the membrane

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phosphatidylcholine into fatty acids and glycerophosphocholine. The gene is thus important for membrane integrity.

Phenotypes include Oliver–McFarlane and Laurence–Moon syndromes, and are characterised by chorioretinopathy, spinocerebellar ataxia, spastic paraplegia, learning difficulties and trichomegaly. The HP phenotype includes variable GHD and HH associated with a small AP on MRI. Human embryonic expression studies reveal *PNPLA6* transcripts in the developing eye, pituitary and brain. These data suggest that recessive *PNPLA6* mutations may give rise to pituitary-related neurodegenerative disorders.

Genes implicated in ciliary function

Compound heterozygosity for mutations in *IFT172* was identified in a patient with early growth retardation, AP hypoplasia and an EPP, in addition to retinopathy, and hypertension with renal failure. The phenotype was consistent with a ciliopathy (181), and was the first report of a mutation in this gene associated with a pituitary defect. *IFT172* encodes an intraflagellar transport subcomplex (IFT-B) subunit, essential for ciliary assembly and maintenance. Previously, mutations have been described in skeletal ciliopathies with variable polydactyly as well as retinal, cerebellar, or hepatorenal malformations (182-184). Consistent with these data, Alström syndrome, a rare autosomal recessive disease, consists of multiorgan dysfunction with GHD, and is caused by mutated *ALMS1* that encodes a protein localizing to centrosomes and basal bodies of ciliated cells (185). These studies imply a critical emerging role for cilia in HP development.

Summary

Several reports of mutations in novel genes associated with CH have been recently published, further increasing the list of CH candidate genes (Table 2). Many of the reports are those of single cases or a single patient, and these genes have not been routinely screened previously in CH cohorts. Hence, their actual mutation frequency is at present unknown. Additionally, few patients have been screened for mutations in all the genes by using targeted sequencing panels. It is important to note that between 80-90% of CH

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cases remain unsolved (186). The ever-increasing list of candidate genes associated with HP development and the phenotypic heterogeneity among patients has emphasized the growing need for a fast and high throughput screening approach in genotyping CH patients to uncover the genetic etiology. The laborious and outdated Sanger sequencing methods have been exchanged for targeted gene panels, which can screen hundreds of known causative genes in multiple patients simultaneously. However, next generation sequencing (NGS) techniques including whole exome and genome sequencing are potentially the most efficient methods for identifying pathogenic variants. These methods have identified combinations of rare variants in multiple genes, which is reflective of the incomplete penetrance often seen in CPHD for example (5). The cost and speed of NGS is decreasing rapidly, enabling a higher volume of patients to benefit from its resources, however at present the number is limited to only a select few unique cases where no clear causative genes are suspected, or to familial cases or those born to consanguineous unions. It is important to note that the identification of variants by NGS is only a first step; ultimately, functional studies of novel variants in both known and novel CH genes are critical for the interpretation of genetic findings to establish their pathogenicity and impact on the patient phenotype. These could take the form of *in vivo* assays in animal models such as the mouse and zebrafish, or *in vitro* assays designed to exploit the properties of the molecule concerned.

In the years to come, especially with the use of NGS methods as the first screening approach, there is no doubt that we will discover more genes that are critical for normal HP development. We will define more representative frequencies of mutations in known and novel genes associated with CH, and unravel the phenotypic spectrum associated with such mutations. Importantly, we will discover novel pathways implicated in CH, and understand pituitary organogenesis and disease pathogenesis better, thereby leading to the development of novel therapeutic modalities.

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Figure 1: Adapted from *Gregory LC et al., Contemporary Endocrinology: Pituitary Disorders of Childhood. Springer Nature, first edition. 2018. 1, pp 3-27.* A flowchart illustrating human embryonic hypothalamo-pituitary development. A complex spatio-temporal genetic cascade of transcription factors and signaling molecules, intrinsic or extrinsic to the developing Rathke's pouch. A series of tightly regulated steps result in cell proliferation and differentiation to give rise to the five different specialized AP cell types that secrete six hormones. Specific peptides derived from the hypothalamus regulate the synthesis of these hormones by binding to their respective receptors on each AP cell type.

Figure 2: (taken from Endocr Rev. 2009 Dec; 30(7): 790–829)

A, Midsagittal MRI scan of the head of a normal child. Note the well-formed corpus callosum (CC), the optic chiasm (OC), and the posterior pituitary (PP), which appears as a bright spot within the sella turcica. **B**, Sagittal MRI scan of two siblings with a homozygous p.R160C mutation in *HESX1*. In the first sibling (i) the splenium of the corpus callosum is more hypoplastic than the rest of the structure and the PP is partially descended as compared with the other sibling (ii) who has a severely hypoplastic corpus callosum, ectopic posterior pituitary (EPP), and lack of visible pituitary stalk (PS). **C**, Coronal and sagittal MRI scans from one patient [panels (i) and (ii)] and sagittal scan from a second patient (iii) with *SOX3* duplication showing anterior pituitary (AP) hypoplasia, partial hypoplasia of the infundibulum (I) in the first patient, which is completely absent in the second, and an EPP which is more severe in patient 2. **D**, MRI scan from patients with *SOX2* mutations. Sagittal section from patient with c.60insG mutation showing AP hypoplasia with normal PP and infundibulum (i) and a hypothalamic hamartoma (h). **E**, Sagittal MRI scan in patient with compound heterozygosity for p.E230K and p.R172Q mutations in *POU1F1*, showing hypoplasia of the AP gland with a normal PP and infundibulum. **F**, Sequential MRI scanning of a patient with a 13-bp deletion (c.112_124del13) in *PROP1* reveals waxing and waning of a pituitary mass (*arrow*); (i) on initial presentation, (ii) after 4 months, (iii) after 12 months, and (iv) 21 months after initial MRI.

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Tables:

Table 1: Phenotypes associated with Congenital Hypopituitarism and genes implicated to date

Table 2: List of genes with reported pathogenic variants known to cause hypothalamo-pituitary disease.

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Table 1: Summary of CH phenotypes associated with genetic mutations

Congenital hypopituitarism phenotype	**Incidence**	**Description**	**Candidate genes**
Multiple pituitary hormone deficiency (MPHD) without midline defects	1 in 4000	Deficiencies in one or more of the 6 anterior pituitary hormones: GH, TSH, LH, FSH, PRL, ACTH ± Diabetes insipidus; APH or structural abnormalities of HP but with no other brain abnormalities	*HESX1, SOX3, GLI2, LHX3, LHX4, PROP1, POU1F1, IGSF1, KAL1, PROKR2, GPR161, CDON, ROBO1*
Septo-optic dysplasia	1 in 10000	Optic nerve hypoplasia (ONH), Midline forebrain neuroradiological abnormalities Pituitary hypoplasia - consequent endocrine deficits - GH, TSH, LH, FSH, PRL, ACTH ± Diabetes insipidus	*HESX1,* *SOX2, OTX2* *PROKR2, FGF8,* *KAL1, TCF7L1* *RAX*
Holoprosencephaly	1 in 10000 - 1 in 20000	Incomplete cleavage of the prosencephalon, affecting both the forebrain and the face: Alobar (no forebrain division) Semilobar (some separation) Lobar (complete separation) Microcephaly, hypotelorism, a single central maxillary incisor, cleft lip and/or palate. Endocrine deficits including ACTH, TSH and gonadotrophin	*SHH* *GLI2* *ZIC2* *SIX3* *TGIF1* *PTCH1* *FGF8 etc.* Sub-microscopic deletions at a number of loci

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		deficiencies with DI; GH deficiency rare	
Other syndromic forms of CH	Unknown	Several syndromes associated with APH or structural abnormalities of the pituitary	*ARNT2, EIF2S3, FOXA2, IFT172, KCNQ1, PC1, PNPLA6, ROBO1 (recessive)*
Hypogonadotropic hypogonadism (HH)/ Kallmann syndrome (KS	Males: 1/10,000 Females:1/50,000	Failure to activate pulsatile secretion of GnRH, causing deficiencies in LH, FSH. Delay in onset/complete/partial failure of puberty Anosmia	*GnRHR* *KAL1* *PROK2* *PROKR2* *FGF8* *FGFR1 etc* Please see Ref 159 for more detailed list
Isolated growth hormone deficiency (IGHD)	1/4000 - 1/10,000	The most common isolated deficiency - short stature, delayed growth velocity and skeletal maturation	*GH1, GHRHR, RNPC3* *HESX1, OTX2* *SOX3* *POU1F1*
Isolated TSH deficiency	1/20,000 - 1/80,000	Usually normal brain MRI. Variable presentation – may be neonatal or presentation in childhood/adolescence or in asymptomatic adults	*TSHβ, TRHR, TBL1X, IGSF1*
Isolated ACTH deficiency	Rare – true incidence unknown	Neonatal hypoglycaemia	*TBX19 (TPIT), POMC*

Abbreviations: APH – Anterior pituitary hypoplasia; DI – Diabetes Insipidus

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Table 2: List of genes with reported pathogenic variants known to cause hypothalamo-pituitary disease

Gene with reported variants	Phenotype	Mode of inheritance
ARNT2	CPHD, congenital abnormalities of the kidneys and urinary tract	Recessive
CDON	PSIS	Dominant
EIF2S3	GHD, TSHD, Glucose dysregulation, MEHMO syndrome	X-linked
FGF8	HH/KS; HPE	Dominant
FGFR1	HH/KS, SOD	Dominant
FOXA2	CPHD, HI, childhood-onset diabetes, choroidal coloboma, biliary atresia (cardiac/endoderm-derived organ abnormalities)	Dominant
GH1	IGHD Type IA	Recessive
	IGHD Type IB	Recessive
	IGHD Type II	Dominant
GHRHR	IGHD Type IB	Recessive or Dominant (rare)
GLI2	HPE, IGHD/CPHD, polydactyly, single central incisor	Dominant: haploinsufficiency
GPR161	PSIS	Recessive
HESX1	IGHD, CPHD, SOD	Dominant or Recessive
IFT172	GHD, retinopathy, metaphyseal dysplasia, renal failure (ciliopathies)	Compound heterozygous
IGSF1	TSHD, hypoprolactinemia, transient GHD; usually with macroorchidism	X-linked
KAL1	HH/KS	X-linked
KCNQ1	GHD, maternally inherited gingival fibromatosis	Dominant
LHX3	CPHD, short neck with limited rotation	Recessive
LHX4	CPHD, Chiari malformation, cerebellar abnormalities, respiratory distress	Dominant or Recessive
OTX2	IGHD, CPHD, SOD, anophthalmia/microphthalmia, retinal dystrophy	Dominant: haploinsufficiency or dominant negative
PCSK1	IAD, GHD, TSHD, DI, malabsorption	Dominant, Compound heterozygous
PNPLA6	Oliver–McFarlane and Laurence–Moon syndrome; GH and gonadotrophin deficiencies	Recessive
POMC	IAD; early-onset obesity and red hair pigmentation	Recessive
POU1F1	GH, TSH and ACTH deficiencies	Dominant or Recessive
PROKR2	HH/KS	Recessive
PROP1	CPHD, pituitary tumors	Recessive
RAX	Anophthalmia/microphthalmia, CPHD, DI, and Cleft Palate	Recessive or Compound heterozygous

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RNPC3	IGHD	Recessive
ROBO1	PSIS	Dominant
SOX2	HH, anophthalmia/microphthalmia, learning difficulties, Hypothalamo-Pituitary tumors,	Dominant
SOX3	GHD, CPHD, absent infundibulum, persistent craniopharyngeal canal	X-linked
TBL1X	TSHD, ASD	X-linked
TBX19	IAD	Recessive
TCF7L1	SOD	Dominant
TRHR	TSHD	Recessive
TSHB	TSHD	Recessive

CPHD, combined pituitary hormone deficiency; PSIS, pituitary stalk interruption syndrome; GHD, growth hormone deficiency; TSHD, thyroid-stimulating hormone deficiency; MEHMO, mental retardation, epileptic seizures, hypogonadism with hypogenitalism, microcephaly and obesity; HH, hypogonadotropic hypogonadism; KS, Kallmann syndrome; HPE, holoprosencephaly; SOD, septo-optic dysplasia; HI, congenital hyperinsulinism; IGHD, isolated growth hormone deficiency; IAD, isolated adrenocortical deficiency; DI, diabetes insipidus; ASD, autism spectrum disorder.

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Figure 1

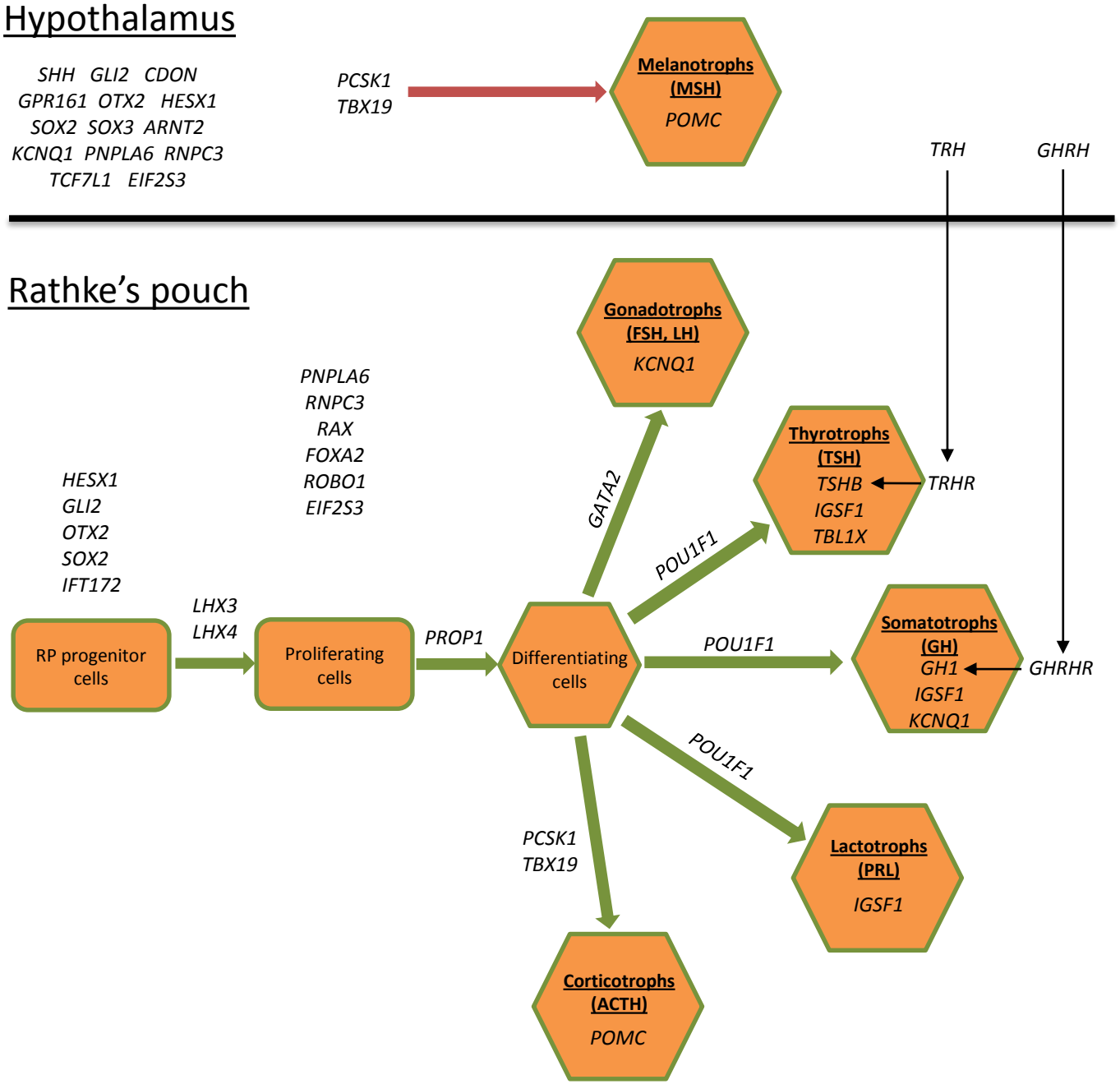

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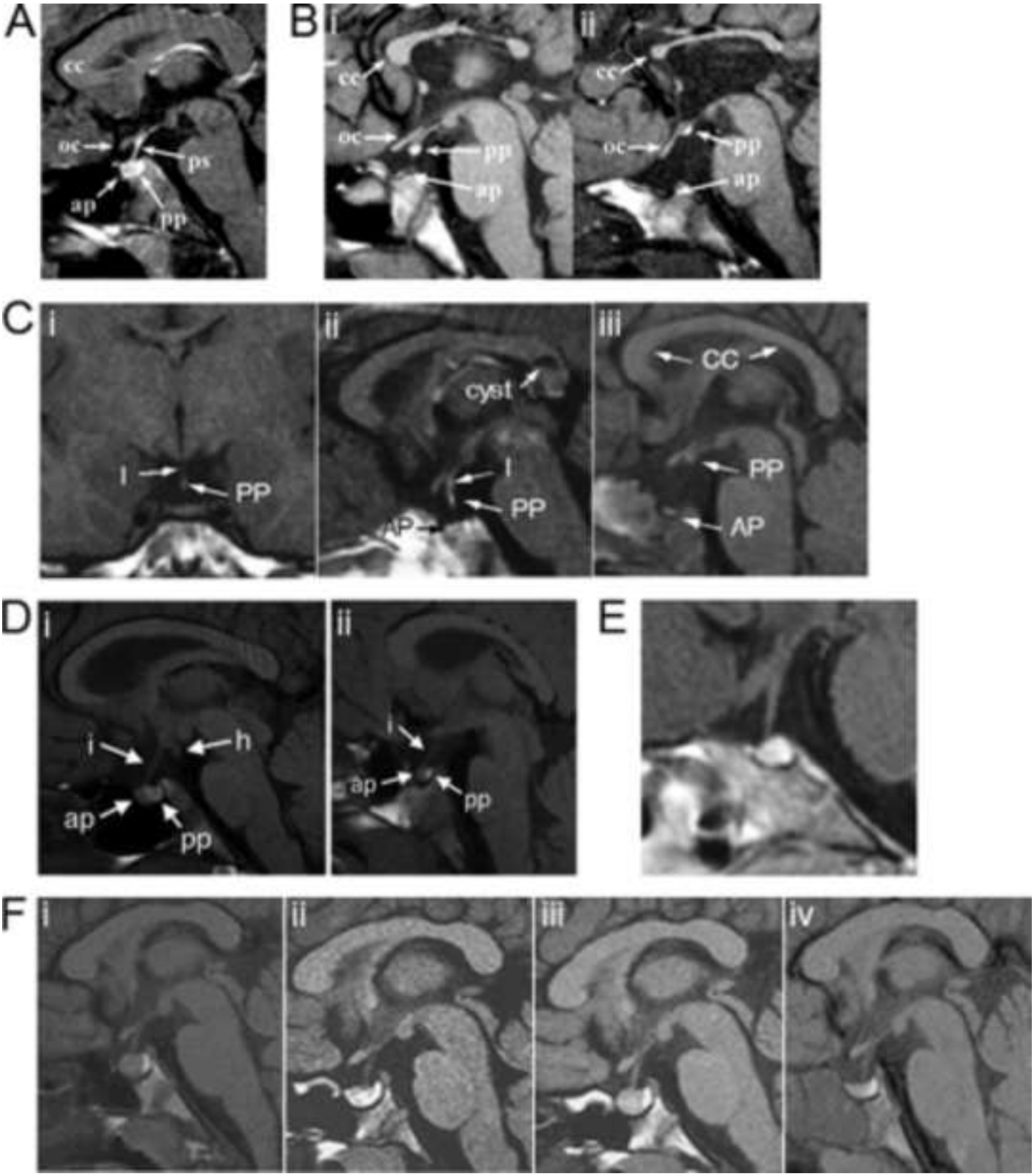
Figure 2



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