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PHENOTYPIC SPECTRUM OF PATIENTS WITH CONGENITAL DISORDERS OF THE HYPOTHALAMO-PITUITARY AXIS

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If I have ever made any valuable discoveries, it has been owing more to patient attention than to any other talent.....

ISAAC NEWTON (1642 - 1727)

PHENOTYPIC SPECTRUM OF PATIENTS WITH CONGENITAL DISORDERS OF THE HYPOTHALAMO-PITUITARY AXIS

TABLE OF CONTENTS

ABSTRACT

Mutations within the cascade of pituitary transcription factors that play a crucial role in its development and differentiation have improved our understanding of variable hypopituitarism in children. The diagnosis of hypopituitarism is problematic as it is dependent on a series of endocrine tests varying in sensitivity and specificity, compounded by evolving hormonal deficiencies. In order to test the hypothesis that human phenotypes are determined by the neuroanatomy which is further influenced by the position of the abnormal gene within the pituitary developmental cascade, clinical, biochemical, magnetic resonance [MR] imaging and molecular data were retrospectively analysed in subgroups from a cohort of 825 patients with variable hypothalamo-pituitary [H-P] abnormalities. The major aims were to determine abnormalities on MR imaging that help predict the spectrum of hypopituitarism, to assess if genotype determined phenotype and to ascertain the optimum test for diagnosis of hormone deficiency. Results showed good structure-function relationships within the H-P axis. Anterior pituitary hypoplasia and an undescended posterior pituitary were 6.7 and 33.1 times more prevalent in patients with hypopituitarism as compared with those without. These abnormalities were also significantly associated with endocrinopathies in patients with optic nerve hypoplasia. Within patients with hypopituitarism, midline forebrain defects [MFD] and pituitary stalk abnormalities were found to be significantly associated with combined pituitary hormone deficiency as opposed to isolated GH deficiency. GH was critical for early postnatal growth, which was also influenced by other pituitary hormones and MFD. Regular evaluation of serum thyroxine concentration best revealed TSH deficiency, as the TRH test was normal in 23% of patients with central hypothyroidism limiting its role as a diagnostic test. A combination of the short Synacthen test and 0800-hour serum cortisol concentrations represented the optimal method of investigation for ACTH deficiency. The LHRH test in infancy needs careful interpretation, as responses were gender-specific with significantly exaggerated serum FSH concentrations in females. There was a poor genotype-phenotype correlation, particularly in patients with mutations in *HESX1, PROP1* and *SOX3,* both within and between pedigrees, indicating a role for other genetic or environmental factors on phenotypic expression.

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MANUSCRIPTS IN PREPARATION

Structure-function relationships within the hypothalamo-pituitary axis

Dominant splicing mutations are the commonest genetic cause of IGHD.

Post-natal basal and stimulated serum gonadotrophins in patients with hypothalamo-pituitary and midline brain disorders.

ABBREVIATIONS

ACTH	Corticotrophin
AVP	Arginine vasopressin
BMI	Body mass index
CPHD	Combined pituitary hormone deficiency
CRH	Corticotrophin releasing hormone
DI	Diabetes inspidus
FSH	Follicle stimulating hormone
FT4	Free thyroxine
GH	Growth hormone
GHR	Growth hormone receptor
GHRH	Growth hormone releasing hormone
GHRHR	Growth hormone releasing hormone receptor
Gn	Gonadotrophins
HH	Hypogonadotrophic hypogonadism
H-P	Hypothalamo-pituitary
HPE	Holoprosencephaly
IGF1	Insulin like growth factor 1
IGFBP3	Insulin like growth factor binding protein 3
IGHD	Isolated growth hormone deficiency
LCPE	London Centre for Paediatric Endocrinology

LH	Luteinising hormone
LHRH	Luteinising hormone releasing hormone
MFD	Midline forebrain defect
MFS	Midline forebrain structures
MR	Magnetic resonance
ONH	Optic nerve hypoplasia
POMC	Pro-opiomelanocortin
SD	Standard deviation
SDS	Standard deviation score
SOD	Septo-optic dysplasia
TRH	Thyrotrophin releasing hormone
TSH	Thyrotrophin
TT4	Total thyroxine
T3	Triiodothyronine

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 7

CHAPTER 8

LIST OF TABLES

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 8

CHAPTER 1

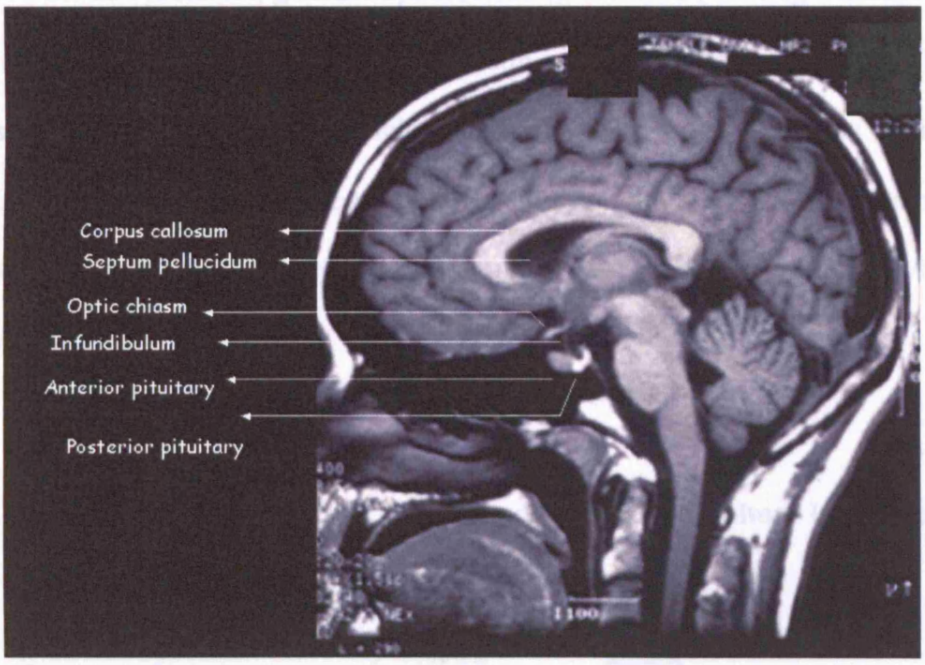
PITUITARY GLAND AND ITS FUNCTION

1.1 INTRODUCTION

The pituitary gland is the central regulator of growth, reproduction and homeostasis. The gland lies within a bony cavity called sella turcica at the base of the brain to which it is attached by the pituitary stalk or infundibulum [Figure 1.1]. A mature gland consists of the adenohypophysis [anterior and intermediate lobes] and neurohypophysis [posterior lobe]. The anterior pituitary secretes growth hormone [GH], thyrotrophin or thyroid stimulating hormone [TSH], corticotrophin or adrenocorticotrophic hormone [ACTH], follicle stimulating hormone [FSH], luteinising hormone [LH] and prolactin. The intermediate lobe produces pro-opiomelanocortin [POMC], which is a precursor to melanocyte stimulating hormone [MSH], and endorphins, and this intermediate lobe involutes in the adult. The posterior lobe secretes arginine vasopressin [AVP, also called antidiuretic hormone] and oxytocin. The hypothalamus lies in a superior position to the pituitary gland and the link between the two organs is critical for normal pituitary gland function.

The anterior pituitary develops from oral ectoderm while the posterior pituitary develops from neural ectoderm. Both lobes of the pituitary gland are histologically different as a result of embryological development and function almost as two separate glands.

Figure 1.1: Magnetic resonance scan of the brain illustrating neuroanatomical relations of the pituitary gland.

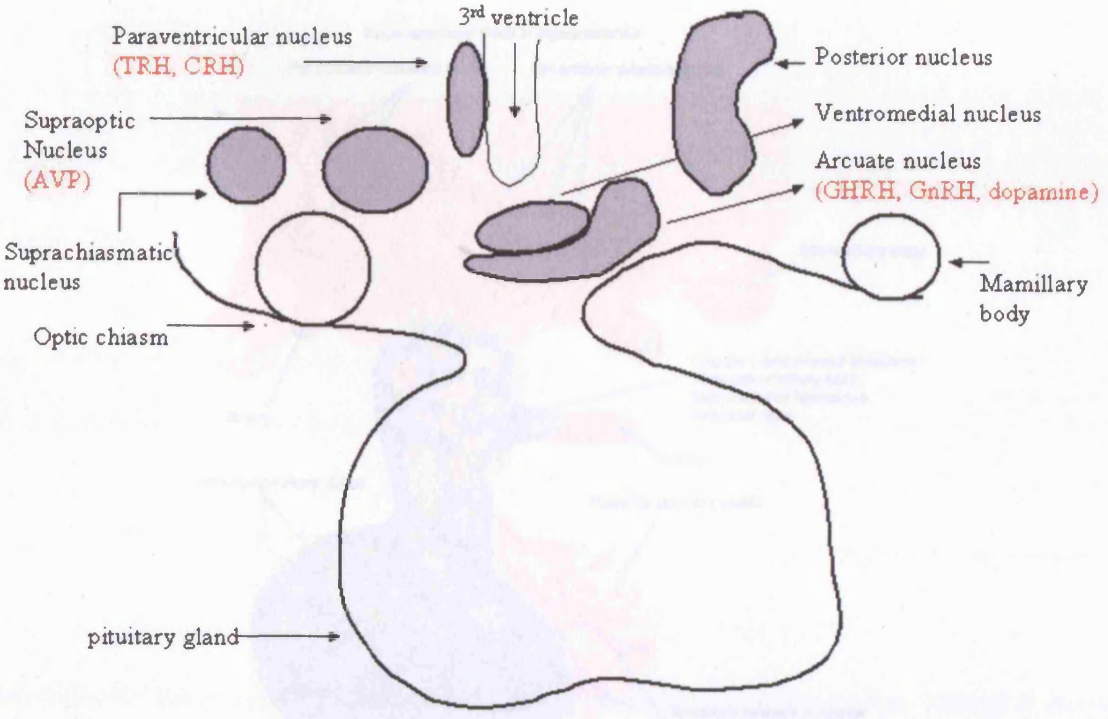


1.2 THE HYPOTHALAMO-PITUITARY AXIS

Complex functions of the pituitary gland are mediated via hormone-signalling pathways from the brain and hypothalamus that ultimately co-ordinate complex signals to various target organs. The hypothalamus virtually surrounds the third ventricle and has neural projections into the cerebral cortex and median eminence. Various stimulatory and inhibitory releasing hormones are secreted from hypothalamic nuclei that regulate the hypothalamo-pituitary [H-P] -target gland axis [Figure 1.2]. They include GH releasing hormone [GHRH], a 44-amino acid polypeptide which stimulates the release of GH;

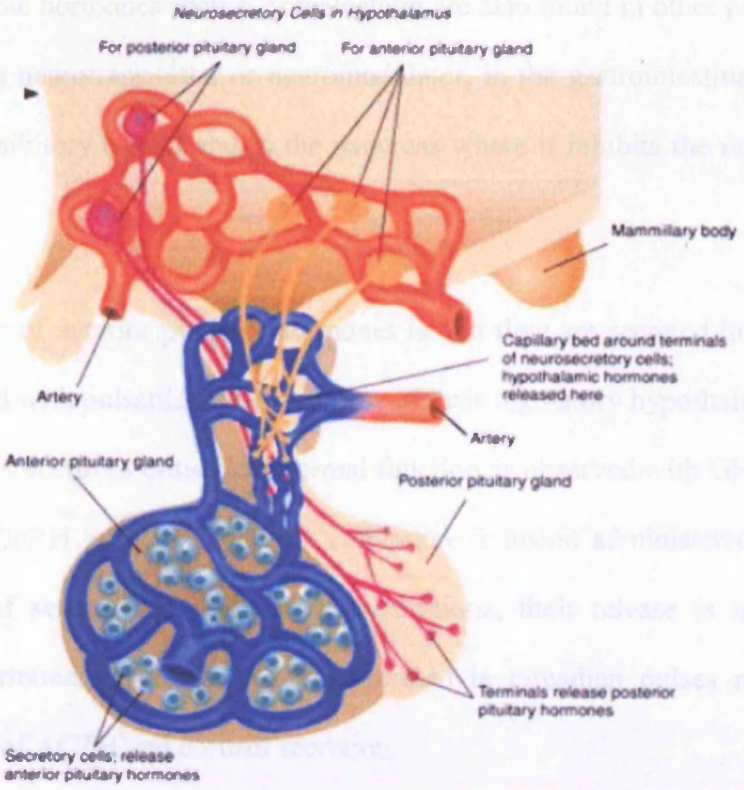
corticotrophin releasing hormone [CRH], a 41-amino acid polypeptide which stimulates the releases of ACTH; thyrotrophin releasing hormone [TRH], a tripeptide which stimulates the releases of TSH and prolactin; gonadotrophin releasing hormone [GnRH], a decapeptide that stimulates the release of FSH and LH; somatostatin, a 14-amino acid peptide which inhibits the release of GH; and dopamine, a single amino acid derivative that inhibits the release of prolactin. Hormones secreted by the posterior lobe of the pituitary gland are synthesised in magnocellular neurones of the paraventricular and supraoptic nuclei within the hypothalamus.

Figure 1.2: Hypothalamic nuclei and their stimulatory and inhibitory hormones



The hypothalamus is supplied by blood from the circle of Willis and most venous blood drains into the vein of Galen. Blood from the superior hypophyseal arteries, which arise from the internal carotid arteries, flows through a capillary plexus in the median eminence to enter a sinusoidal network in the pituitary stalk. Blood passes from these sinusoids into a second capillary network plexus in the anterior pituitary. This venous portal system linking these two capillary networks is called the H-P portal system [Figure 1.3]. The infundibulum or pituitary stalk carries both, the portal blood with delivery of hypothalamic hormones to the anterior pituitary, and neural tracts from magnocellular neurones of the paraventricular and supraoptic nuclei within the hypothalamus to the posterior pituitary. Any damage to the pituitary stalk can therefore result in anterior and posterior pituitary dysfunction.

Figure 1.3: Neurovascular connections within the infundibulum or pituitary stalk.



Several hypothalamic hormones are initially synthesised as prohormones according to specific gene sequences. Following transcription and translation on the endoplasmic reticulum, they are transferred to the Golgi complex where the final products of enzyme action are packaged into granules. These granules then migrate down their axons and their contents are released to the exterior by exocytosis following depolarisation of nerve terminals. Neurosecretory cells of the hypothalamic nuclei also have numerous connections with terminals of neurones originating in other parts of the central nervous system secreting known neurotransmitters such as noradrenaline, dopamine, acetylcholine, serotonin, opioids and γ amino-butyric acid [GABA]. This probably accounts for the important influence of external stimuli such as environmental changes, stress and exercise on anterior pituitary function.

Some hypothalamic hormones such as somatostatin are also found in other parts of the brain where it acts as a neurotransmitter or neuromodulator, in the gastrointestinal tract where it exerts various inhibitory effects and in the pancreas where it inhibits the release of insulin and glucagon.

One characteristic of anterior pituitary hormones is that they are secreted in discrete pulses that are associated with pulsatile release patterns of their regulatory hypothalamic hormones. The pulsatility is sometimes critical for normal function as observed with GH, FSH and LH. If pulsatility of GnRH is interrupted or a continuous infusion administered, then after an initial increase of serum FSH and LH concentrations, their release is inhibited. Other hypothalamic hormones such as CRH are secreted in circadian pulses resulting in the circadian rhythm of ACTH and cortisol secretion.

1.3 ANTERIOR PITUITARY HORMONES

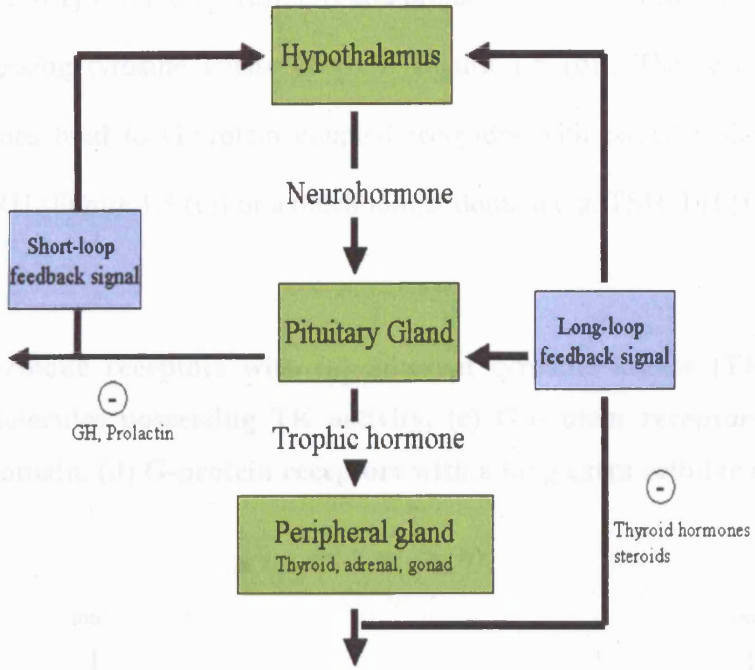
1.3.1 Introduction

The anterior pituitary or adenohypophysis consists of five different cell types secreting six hormones which can be classified arbitrarily as (1) those that have a primary effect on target tissues directly: two protein hormones with considerable homology, GH secreted by somatotrophs and prolactin secreted by lactotrophs and (2) those whose primary effect is to stimulate other endocrine glands to secrete their hormones including three glycoprotein and one polypeptide hormone. TSH secreted by thyrotrophs and gonadotrophins [FSH and LH] secreted by gonadotrophs are glycoproteins, while ACTH secreted by corticotrophs is a polypeptide [Table 1.2].

Table 1.1: Hormones secreted from the anterior lobe of the pituitary gland

Cell type	Hormone	% Cell population	Hypothalamic hormone	Hypothalamic nucleus of synthesis
Somatotroph	GH	40-50%	GHRH (+) Somatostatin (-)	Arcuate, Anterior periventricular
Thyrotroph	TSH	3-5%	TRH (+) Somatostatin (-)	Paraventricular, Anterior periventricular
Corticotroph	ACTH	15-20%	CRH (+) AVP -augments CRH	Paraventricular, Supraoptic
Gonadotroph	LH, FSH	10-15%	GnRH (+)	Arcuate
Lactotroph	Prolactin	10-25%	TRH (+) Dopamine (-)	Arcuate, Paraventricular

Figure 1.4: Feedback signals within the hypothalamo-pituitary-target gland axis.

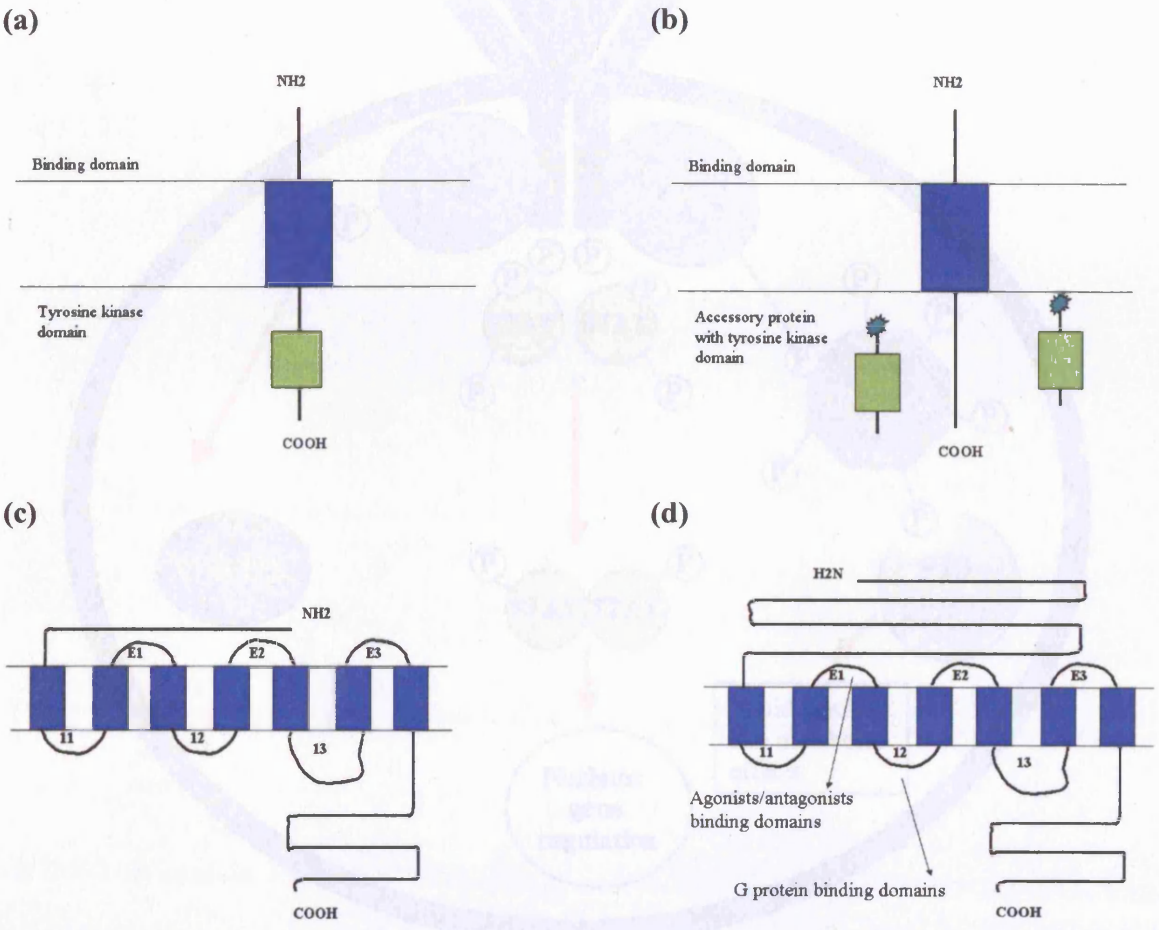


Hormones secreted from the anterior pituitary regulate growth, puberty, metabolism, response to stress, reproduction and lactation. To maintain function, it is essential that the pituitary gland and hypothalamic cells receive constant and rapid information about the state of systems regulated so that the release of hormones can be finely adjusted to the requirement of target tissues by these feedback mechanisms [Figure 1.4].

The hormones derived from the anterior pituitary and hypothalamus are peptide or protein hormones requiring transcription of a single gene apart from the α and β subunits of glycoprotein hormones [TSH, LH and FSH] which are derived from different genes. Hormone synthesis requires transcription followed by post-transcriptional modification by excision of introns, translation of messenger ribonucleic acid [mRNA] and post-translational modifications, in some cases involving cleavage of the prohormone into fragments.

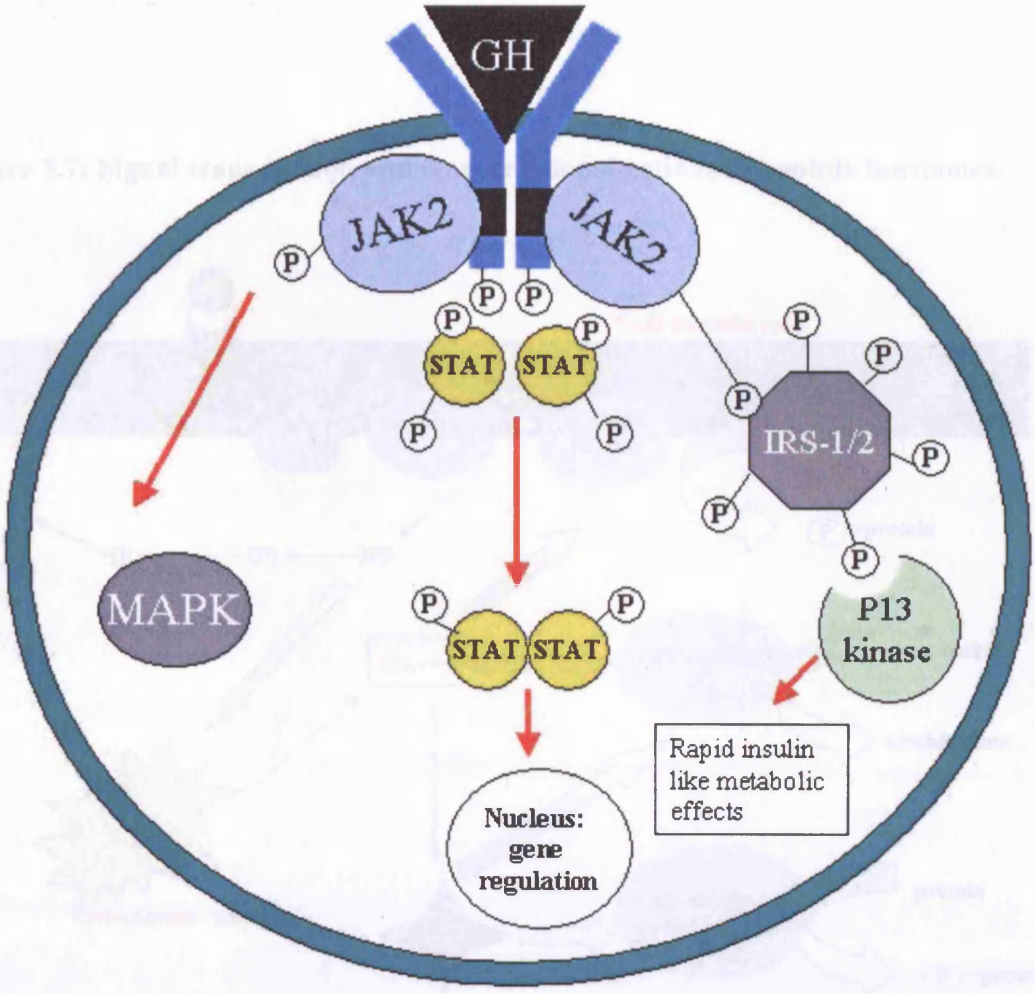
Unlike insulin and growth factors, which bind to transmembrane receptors with inherent tyrosine kinase activity [Figure 1.5 (a)], GH and prolactin bind to receptors with intracellular molecules possessing tyrosine kinase activity [Figure 1.5 (b)]. The rest of the anterior pituitary hormones bind to G-protein coupled receptors with either a short extracellular domain e.g. GnRH [Figure 1.5 (c)] or a much longer domain e.g. TSH, LH [Figure 1.5 (d)].

Figure 1.5: Hormone receptors with (a) inherent tyrosine kinase [TK] activity, (b) intracellular molecules possessing TK activity, (c) G-protein receptors with a short extra cellular domain, (d) G-protein receptors with a long extra cellular domain.



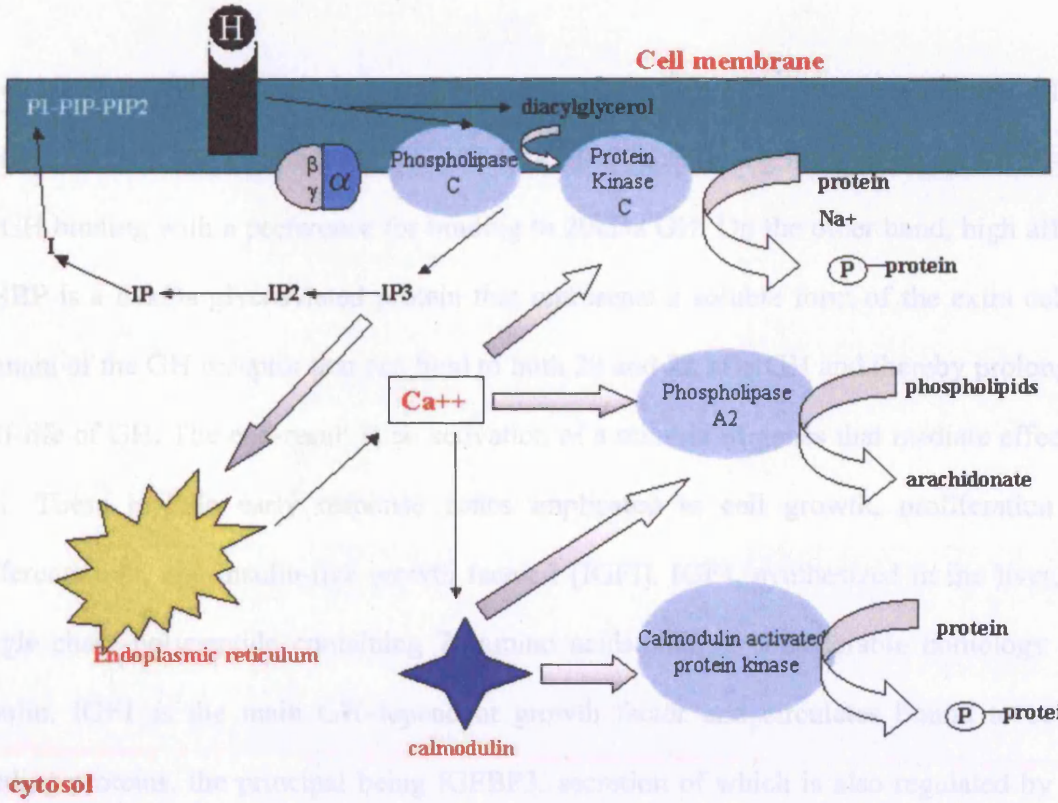
Binding of hormones, such as GH and prolactin, to the extra cellular domain of their receptor results in receptor dimerisation with phophorylation of Janus [JAK] kinases as well as of the receptor. This induces phosphorylation of signal transducers and activators of transcription [STAT] kinases, which translocate to the nucleus as dimers, and activate transcription factors [Figure 1.6].

Figure 1.6: The GH receptor, its dimerisation and activation of the JAK/STAT pathway of signal transduction.



Hormone-receptor interactions of other hormones induce dissociation of the intracellular trimeric G-protein that may either open ion channels in the membrane or activate a membrane bound enzyme that stimulates [or inhibits] production of a second messenger such as cyclic adenosine mono phosphate [cAMP] or diacylglycerol and inositol triphosphate. These products then activate serine/threonine kinases or phosphatases. Activation of these kinases leads to alterations in specific cytosolic enzyme activity, activation of nuclear transcription factors or initiation of a cascade of subsequent phosphorylations on serine and threonine residues of protein kinases that can regulate transcription [Figure 1.7].

Figure 1.7: Signal transduction and transcriptional actions of peptide hormones.



1.3.2 Growth hormone

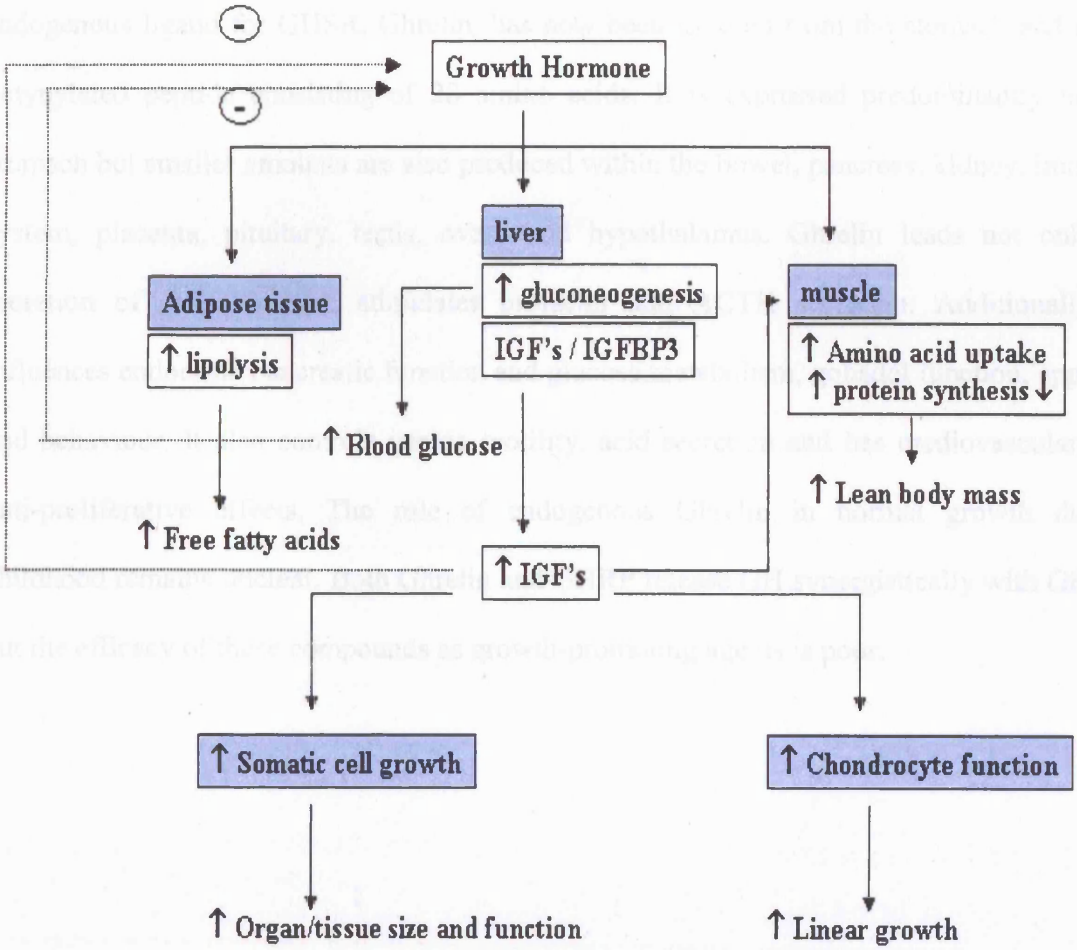
The human GH gene [*GH1*] forms part of a cluster of five homologous genes along with human chorionic somatomammotropic hormone pseudogene 1 (*CSHP1)*, human chorionic somatomammotropic hormone 1 [*CSH1*], *GH2* and human chorionic somatomammotropic hormone 2 [*CSH2*]. The gene is located on the long arm of chromosome 17 [17q22-24] spanning 66.5 kilo bases [Kb]. Its expression is regulated not only by a proximal promoter, but also by a locus control region 15-32 Kb upstream of the *GH1* gene. Full-length transcript from the *GH1* gene encodes a 191 amino acid, 22 kilo Dalton [kDa] protein that contains two disulphide bridges and accounts for 85-90% of circulating GH. Alternative splicing of the mRNA transcript generates a 20 kDa form of GH that accounts for the remaining 10-15%. Within both, the proximal promoter and the locus control region, are located binding sites for the pituitary-specific transcription factor *POU1F1*.

In circulation, GH binds to two binding proteins [BP], high affinity GHBP and low affinity GHBP. Little is known about the low affinity GHBP, accounting for approximately 10-15% of GH binding with a preference for binding to 20kDa GH. On the other hand, high affinity GHBP is a 61kDa glycosylated protein that represents a soluble form of the extra cellular domain of the GH receptor that can bind to both 20 and 22 kDa GH and thereby prolong the half-life of GH. The end-result is an activation of a number of genes that mediate effects of GH. These include early response genes implicated in cell growth, proliferation and differentiation, and insulin-like growth factor I [IGFI]. IGF1, synthesized in the liver, is a single chain polypeptide containing 70 amino acids sharing considerable homology with insulin. IGF1 is the main GH-dependent growth factor and circulates bound to several binding proteins, the principal being IGFBP3, secretion of which is also regulated by GH.

Measurement of IGF1 correlates well with spontaneous GH secretion and is widely used in the diagnosis of GH deficiency [GHD]. However, its concentration is altered in disease states such as hypothyroidism, malnutrition, poorly controlled diabetes and chronic diseases.

Apart from its indirect actions on linear growth through the synthesis of IGF1, GH is anabolic, lipolytic and diabetogenic [Figure 1.8]. It increases calcium absorption and is believed to improve bone density. Administration of GH results in a reduction in body fat and an increase in muscle mass.

Figure 1.8: Major actions of growth hormone.

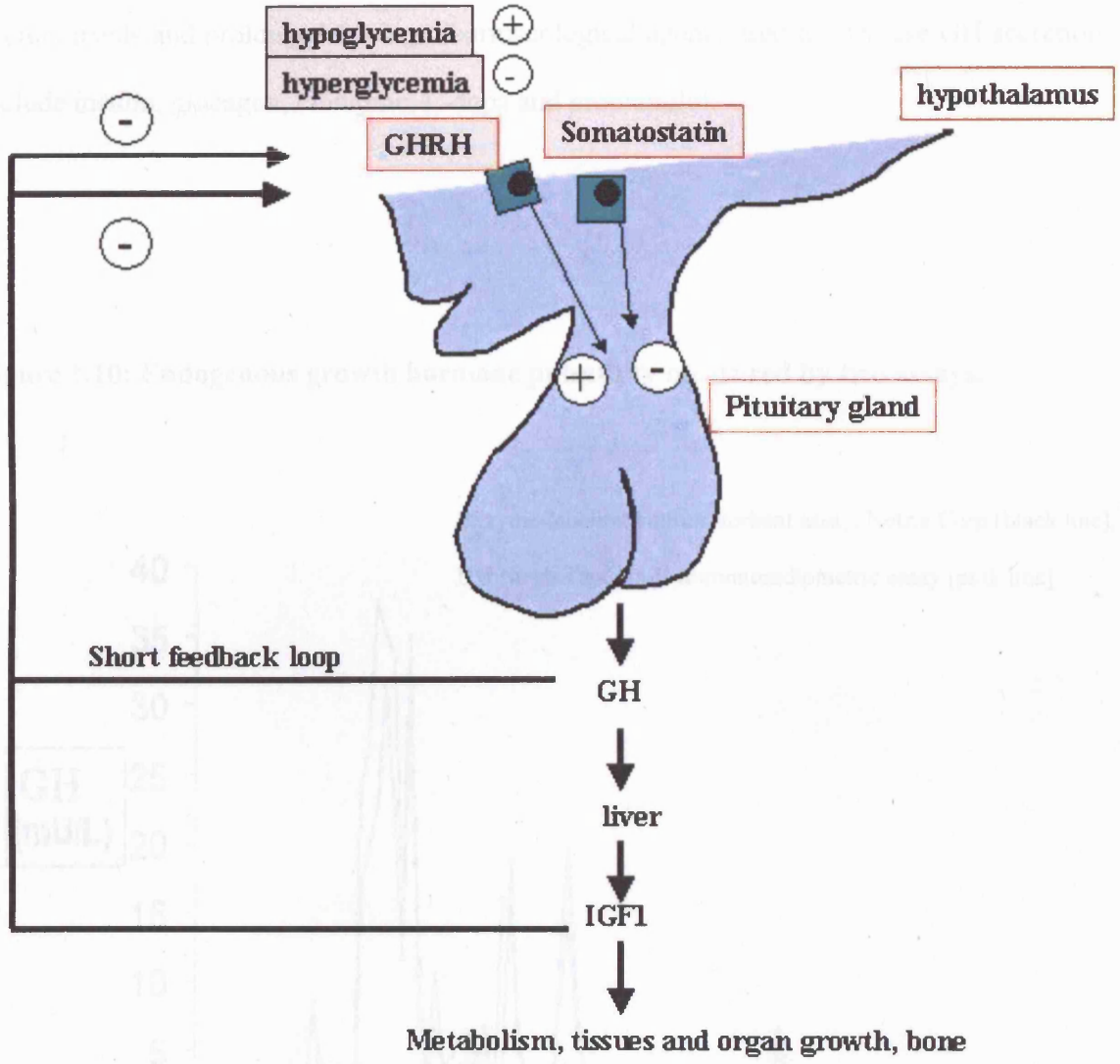


Two hypothalamic hormones regulate GH: GHRH, a 44 amino acid protein that stimulates GH secretion, and somatostatin, an inhibitory hormone containing 14 amino acids. Both GH and growth factors such as IGFI and IGFII exert negative feedback on hypothalamic regulators of GH secretion [Figure 1.9]. Sex steroids such as estrogen and testosterone also increase GH secretion, the latter having an indirect effect requiring prior aromatisation.

Recent use of synthetic GH-releasing peptides [GHRP] has led to the identification of a GH secretagogue receptor [GHSR type 1a]. The receptor is strongly expressed in the hypothalamus but specific binding sites for GHRP have also been identified in other regions of the central nervous system and peripheral endocrine and non-endocrine tissues. The endogenous ligand for GHSR, Ghrelin, has now been isolated from the stomach and is an octynylated peptide consisting of 28 amino acids. It is expressed predominantly in the stomach but smaller amounts are also produced within the bowel, pancreas, kidney, immune system, placenta, pituitary, testis, ovary and hypothalamus. Ghrelin leads not only to secretion of GH, but also stimulates prolactin and ACTH secretion. Additionally, it influences endocrine pancreatic function and glucose metabolism, gonadal function, appetite and behaviour. It also controls gastric motility, acid secretion and has cardiovascular and anti-proliferative effects. The role of endogenous Ghrelin in normal growth during childhood remains unclear. Both Ghrelin and GHRP release GH synergistically with GHRH but the efficacy of these compounds as growth-promoting agents is poor.

Figure 1.9: Feedback mechanism controlling growth hormone secretion.

+ positive feedback; - negative feedback



GH is secreted in a pulsatile fashion under the control of the hypothalamus [Figure 1.10]. Peak serum GH concentrations are achieved during sleep. Secretion of hypothalamic hormones is further influenced by neurotransmitters and neuropeptides [Table 1.2]. As a result, GH secretion is also increased during emotional stress, exercise, hypoglycaemia, protein meals and prolonged fasting. Pharmacological agents used to increase GH secretion include insulin, glucagon, clonidine, L-dopa and propranolol.

Figure 1.10: Endogenous growth hormone pulsatility measured by two assays.

Enzyme-labelled immunosorbent assay, Netria Corp [black line], Hybritech Tandem-R immunoradiometric assay [pink line].

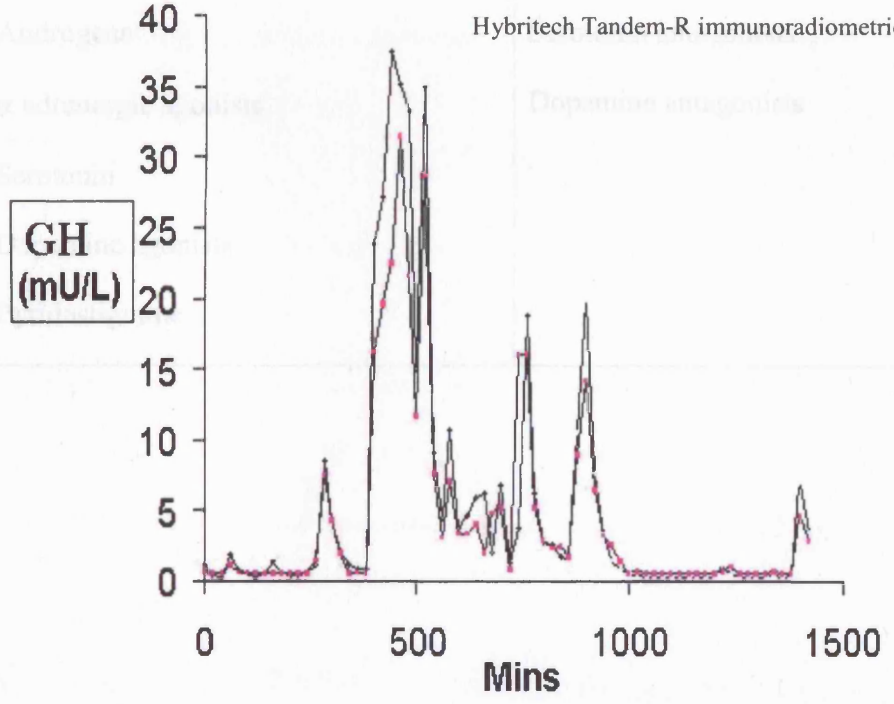


Table 1.2: Major factors controlling growth hormone secretion

Stimulation	Inhibition
Growth hormone releasing hormone	Somatostatin
Hypoglycaemia	Hyperglycaemia
Decreased free fatty acids	Increased free fatty acids
Starvation	Insulin-like growth factor 1
Sleep	Senescence
Exercise	Growth hormone
Stress	Progesterone
Puberty	Glucocorticoids
Estrogens	β adrenergic agonists
Androgens	Serotonin antagonists
α adrenergic agonists	Dopamine antagonists
Serotonin	
Dopamine agonists	
Pyridostigmine	

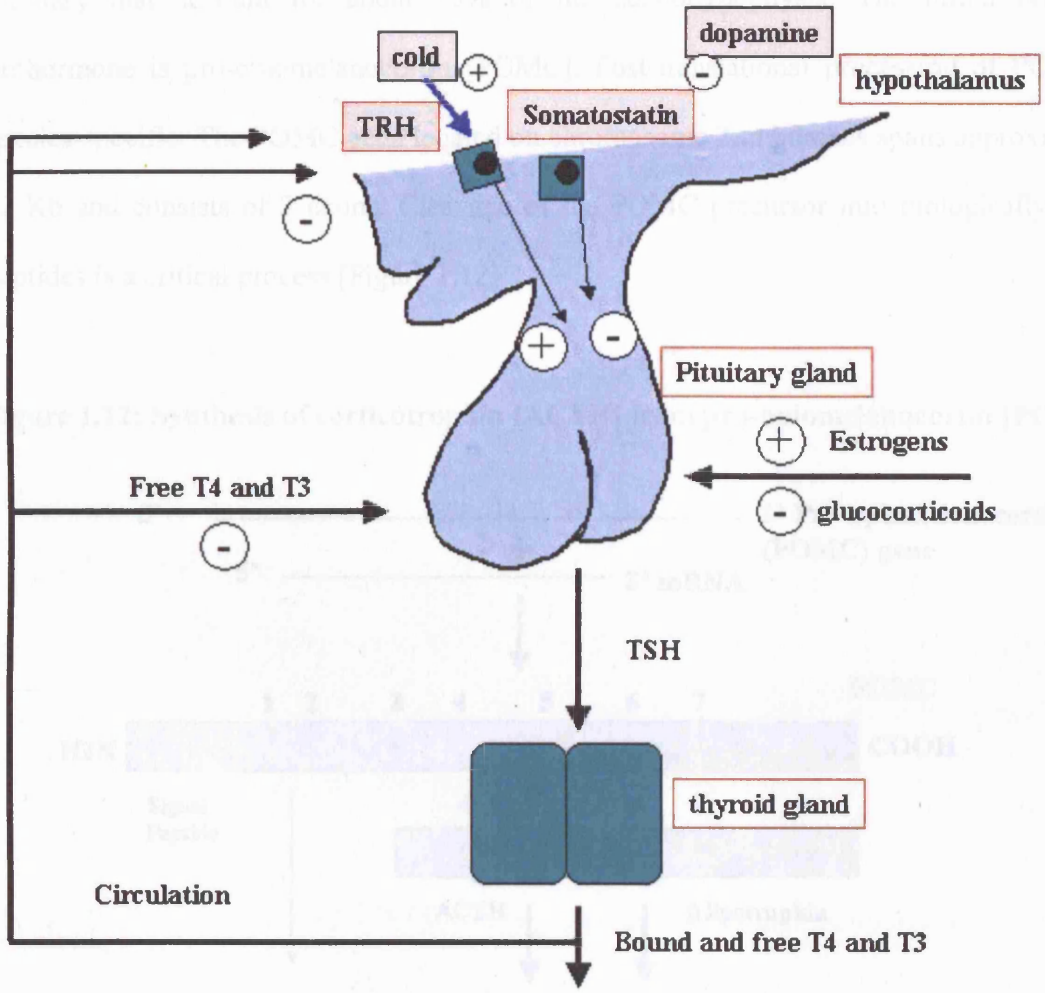
1.3.3 Thyrotrophin or Thyroid Stimulating Hormone

Thyrotrophin [TSH] is a glycoprotein consisting of two non-covalently bound chains of amino acids [α and β] and is synthesised and stored within the thyrotrophs of the anterior pituitary gland. The α chain consists of 92 amino acids and shares homology with other pituitary glycoproteins, FSH and LH. The gene encoding TSH α glycoprotein is located on chromosome 6q12-q21. The β chain contains 110 amino acids and is TSH-specific. The gene encoding the TSH β chain is located on chromosome 1p13.

The primary function of TSH is to stimulate the thyroid gland to secrete thyroid hormones; triiodothyronine [T3] and thyroxine [T4]. Its actions include stimulation of the iodide pump on the cell membrane transporting iodide into the cell, stimulation of synthesis of thyroidal storage protein thyroglobulin and stimulation, synthesis and release of T4 and T3 from their complexes with thyroglobulin.

TSH secretion is pulsatile with maximum concentrations attained at night. Its secretion is stimulated by hypothalamic TRH and inhibited by somatostatin and dopamine. Both thyroid hormones exert negative feedback at the pituitary level on TSH secretion and at the hypothalamic level on TRH [Figure 1.11]. Other factors impinging on TSH secretion include estrogen that increases the number of TRH receptors on the thyrotrophs and a decrease in the ambient temperature acting as a potent stimulator of TSH.

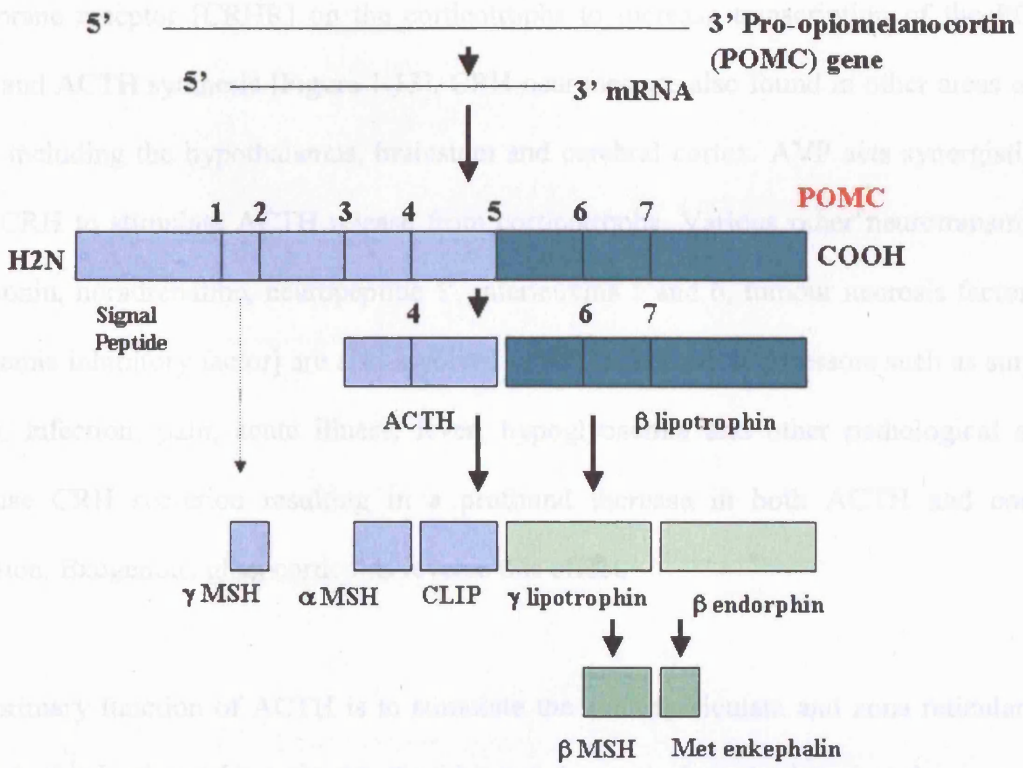
Figure 1.11: Feedback mechanisms within the hypothalamo-pituitary-thyroid gland axis. + positive feedback - negative feedback.



1.3.4 Corticotrophin or Adrenocorticotrophic hormone

Corticotrophin [ACTH] is a 39 amino acid polypeptide with a very short biological half-life of approximately 8 minutes. It is synthesised and stored within corticotrophs of the anterior pituitary that account for about 15% of the adenohypophysis. The initial precursor prohormone is pro-opiomelanocortin [POMC]. Post-translational processing of POMC is species-specific. The POMC gene located on chromosome 2 in humans spans approximately 12 Kb and consists of 3 exons. Cleavage of the POMC precursor into biologically active peptides is a critical process [Figure 1.12].

Figure 1.12: Synthesis of corticotrophin [ACTH] from pro-opiomelanocortin [POMC].



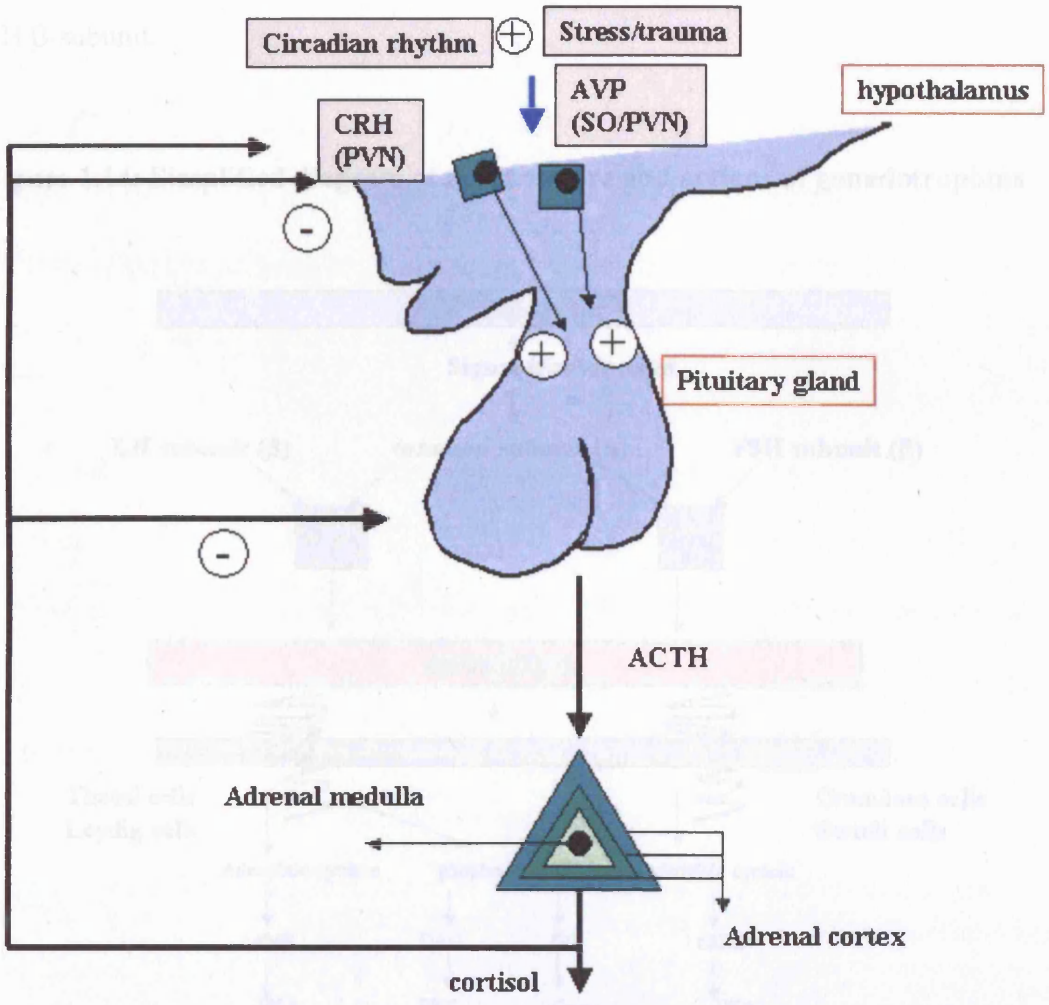
The main enzymes involved are prohormone convertases [PC], particularly PC1 within anterior pituitary corticotrophs. PC1 cleaves POMC to generate N-POC and β lipotrophin. N-POC is then cleaved to form pro-γ-melanocyte stimulating hormone [pro-γ-MSH], a joining peptide and ACTH. There is further evidence to suggest that another enzyme PC2 cleaves ACTH into αMSH and a corticotrophin-like intermediate lobe peptide [CLIP] within the intermediate lobe and β lipotrophin is cleaved into β endorphin and γ lipotrophin. αMSH plays an important role as an agonist for the melanocortin 1 [MC1] receptor in causing pigment deposition in the hair follicle and as an agonist for the MC4 receptor in the hypothalamus where it controls appetite.

Hypothalamic CRH, a 41 amino acid peptide, binds with high affinity to its specific cell membrane receptor [CRHR] on the corticotrophs to increase transcription of the POMC gene and ACTH synthesis [Figure 1.13]. CRH neurones are also found in other areas of the brain including the hypothalamus, brainstem and cerebral cortex. AVP acts synergistically with CRH to stimulate ACTH release from corticotrophs. Various other neurotransmitters [serotonin, noradrenaline, neuropeptide Y, interleukins 1 and 6, tumour necrosis factor and leukaemia inhibitory factor] are also involved in ACTH secretion. Stressors such as surgical stress, infection, pain, acute illness, fever, hypoglycaemia and other pathological states increase CRH secretion resulting in a profound increase in both ACTH and cortisol secretion. Exogenous glucocorticoids reverse this effect.

The primary function of ACTH is to stimulate the zona fasciculata and zona reticularis of adrenal glands to produce glucocorticoids, mainly cortisol, and adrenal androgens. Like other peptide hormones, ACTH binds to its specific membrane receptor on adrenocortical

cells to increase the formation of cyclic AMP and activation of various protein kinases. The secretion of ACTH follows a distinct circadian rhythm with peak concentrations in early hours of the morning and low concentrations in the late evening. As a result of this, cortisol secretion is also circadian with peak concentrations at around 0800 hours and a nadir at midnight. This rhythm can be disrupted by shifts in day-night patterns.

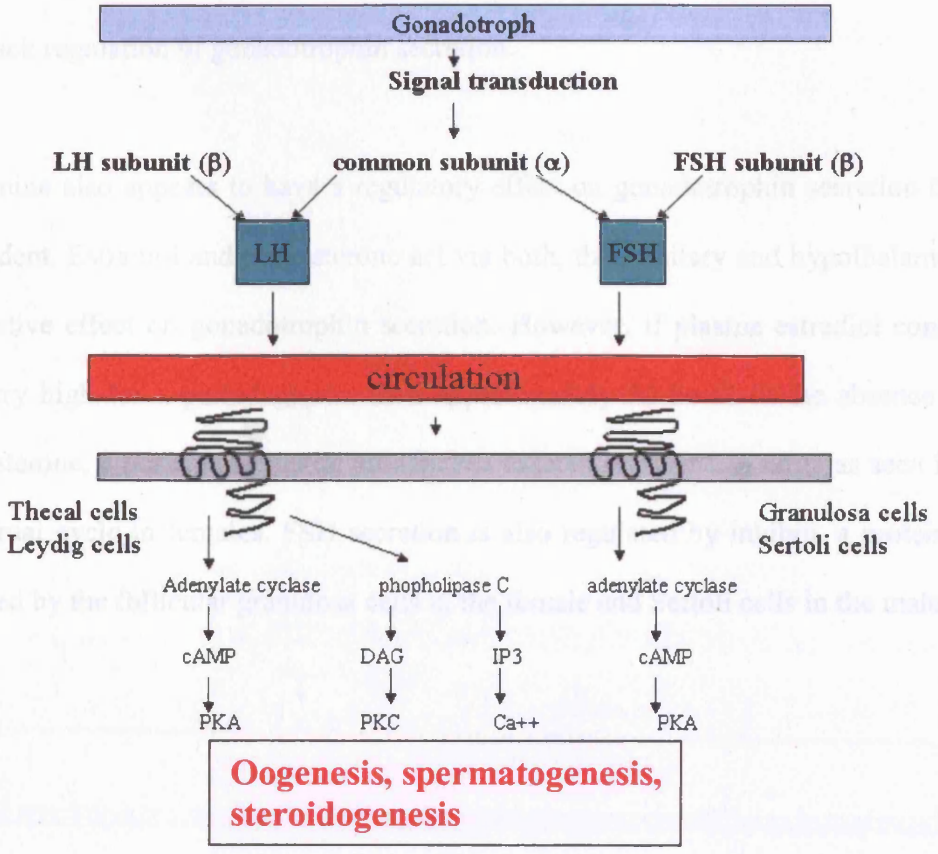
Figure 1.13: Hypothalamo-pituitary-adrenal axis and its feedback mechanisms;
+ positive feedback; - negative feedback



1.3.5 Gonadotrophins

The reproductive system is unique due to changes in the secretion of reproductive hormones taking place throughout life. Gonadotrophins, FSH and LH, are glycoproteins composed of 2 subunits, α and β [Figure 1.14]. The α-subunit is identical to the α-subunit of TSH [gene encoding the α-subunit is located on chromosome 6q12-q21] and the specific biological activity of both hormones resides in the β-subunit. The gene encoding the FSH β chain is found on chromosome 11p11.2 and that of the LH β chain is located on chromosome 19q13.2. The human chorionic gonadotrophin β-subunit has similar biological activity to the LH β-subunit.

Figure 1.14: Simplified diagram of the structure and actions of gonadotrophins



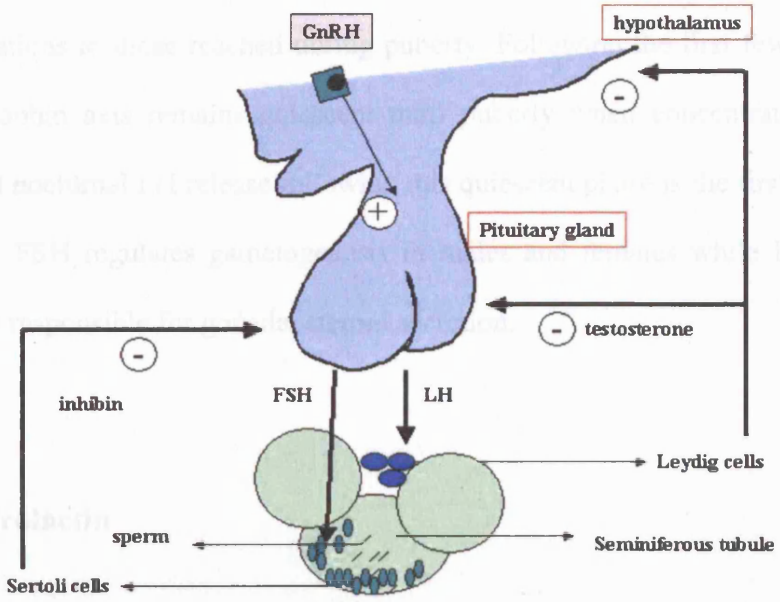
Pulsatile release of hypothalamic GnRH regulates the secretion of LH and FSH [Figure 1.15 a and b]. GnRH-synthesising neuronal migration, from their first appearance in the embryonic medial olfactory placode to their final position in the mediobasal hypothalamus, is complete by around the 19th week of gestation when pulsatile GnRH release is established. Several signalling factors such as anosmin-1 or KAL and FGFR1 are implicated in this migratory process. GnRH synthesis and release is also influenced by several neuroendocrine factors such as PC1 and leptin.

Kisspeptins are products of the *KiSS-1* gene, which bind to a G-protein coupled receptor known as GPR54. Although *KiSS-1* was initially discovered as a metastasis suppressor gene, recent evidence suggests the kisspeptin/GPR54 system is a key regulator of the reproductive system. GnRH neurons are direct targets for regulation by kisspeptins, and KiSS-1 mRNA is regulated by gonadal hormones, suggesting that KiSS-1 neurones play an important role in feedback regulation of gonadotrophin secretion.

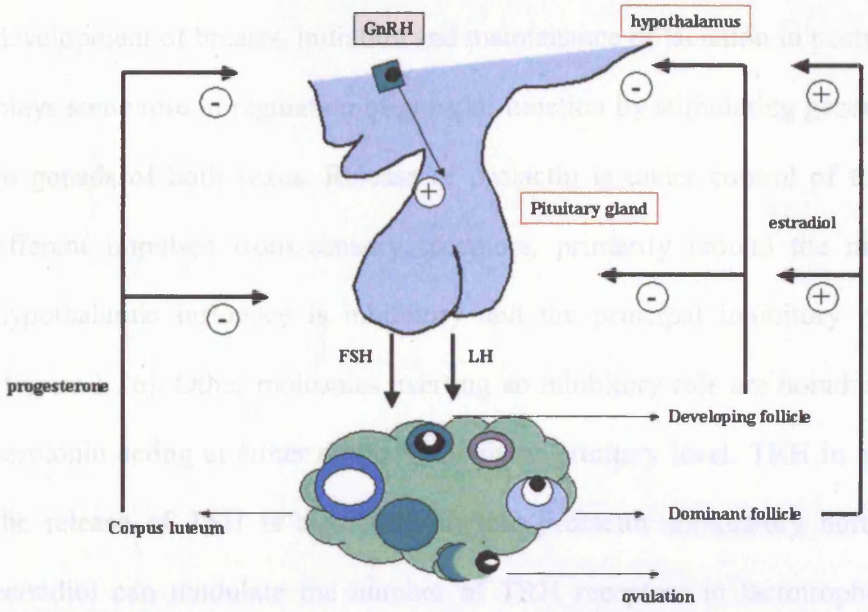
Dopamine also appears to have a regulatory effect on gonadotrophin secretion that is dose dependent. Estradiol and progesterone act via both, the pituitary and hypothalamus, to have a negative effect on gonadotrophin secretion. However, if plasma estradiol concentrations are very high for a period greater than approximately 36 hours in the absence of plasma progesterone, a positive feedback influence is exerted with an LH surge as seen in the mid-menstrual cycle in females. FSH secretion is also regulated by inhibin, a protein molecule secreted by the follicular granulosa cells in the female and Sertoli cells in the male.

Figure 1.15: Feedback mechanisms within the (a) hypothalamo-pituitary-testicular axis and (b) hypothalamo-pituitary-ovarian axis. +positive feedback, - negative feedback.

(a)



(b)



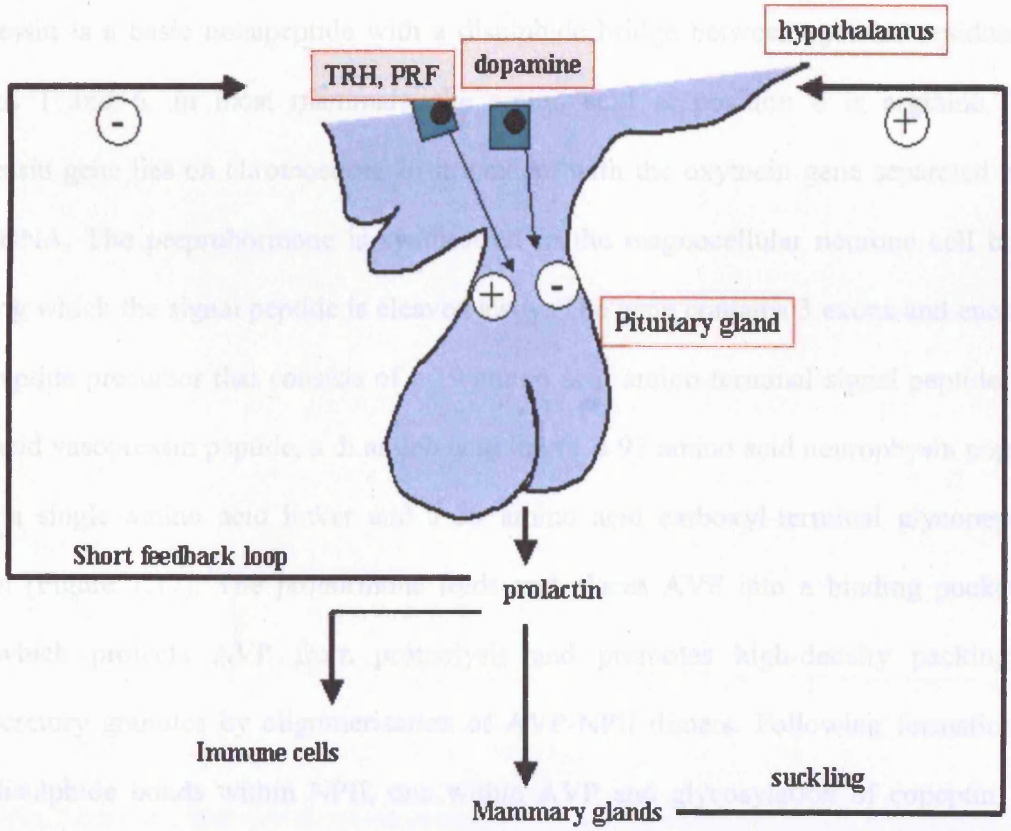
LH secretion is pulsatile in both sexes but sexual dimorphism in physiological secretory patterns becomes evident with maturity of the H-P-gonadal axis. There is a surge in gonadotrophin and gonadal steroid secretion in the neonatal period, with similar concentrations to those reached during puberty. Following the first few months of life, the gonadotrophin axis remains quiescent until puberty when concentrations rise again. An increased nocturnal LH release following this quiescent phase is the first sign of the onset of puberty. FSH regulates gametogenesis in males and females while LH is thought to be primarily responsible for gonadal steroid secretion.

1.3.6 **Prolactin**

The chemical structure of prolactin, a protein containing 199 amino acids, has similarity to that of GH. The prolactin gene is situated on chromosome 6 and the hormone is initially synthesized as a prohormone. The principal functions of prolactin are growth and development of breasts, initiation and maintenance of lactation in postpartum women. It also plays some role in regulation of gonadal function by stimulating generation of LH receptors in gonads of both sexes. Release of prolactin is under control of the hypothalamus with afferent impulses from sensory receptors, primarily around the nipples. The dominant hypothalamic influence is inhibitory and the principal inhibitory hormone is dopamine [Figure 1.16]. Other molecules exerting an inhibitory role are noradrenaline, histamine and serotonin acting at either a hypothalamic or pituitary level. TRH in addition to stimulating the release of TSH is also the principal prolactin stimulatory hormone. Thyroxine and estradiol can modulate the number of TRH receptors in lactotrophs thereby influencing

prolactin release. Thyroxine, by negative feedback, decreases the number of TRH receptors while estradiol increases their availability. Maternal pituitary gland is the main source of serum prolactin during pregnancy and the only known clinical effect of prolactin hyposecretion in adults is failure of lactation in puerperal women.

Figure 1.16: Factors controlling prolactin secretion. + positive feedback, - negative feedback



1.4 POSTERIOR PITUITARY HORMONES

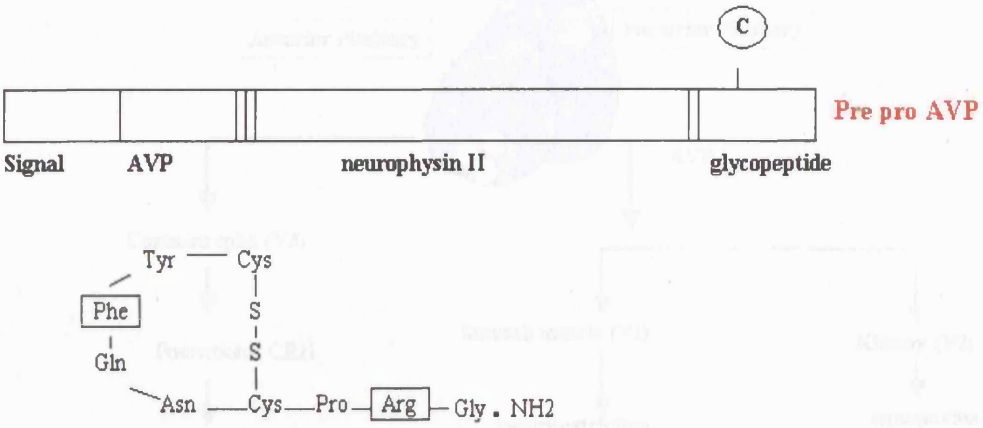
The posterior pituitary is composed of neural tissue that descends from the floor of the third ventricle. It does not synthesise hormones. It consists of axons of neurones with cell bodies located in the supraoptic and paraventricular nuclei of the hypothalamus. Posterior pituitary hormones, arginine vasopressin [AVP] and oxytocin, are synthesized in the hypothalamus, transported in the neurohypophyseal tract of the pituitary stalk, stored in the posterior pituitary and released in response to neurohypophyseal stimuli.

1.4.1 Arginine Vasopressin

Vasopressin is a basic nonapeptide with a disulphide bridge between cysteine residues at positions 1 and 6. In most mammals, the amino acid at position 8 is arginine. The vasopressin gene lies on chromosome 20 in tandem with the oxytocin gene separated by 8 Kb of DNA. The preprohormone is synthesized in the magnocellular neurone cell body, following which the signal peptide is cleaved away. The gene contains 3 exons and encodes a polypeptide precursor that consists of a 19 amino acid amino-terminal signal peptide, a 9 amino acid vasopressin peptide, a di amino acid linker, a 93 amino acid neurophysin peptide [NPII], a single amino acid linker and a 39 amino acid carboxyl-terminal glycopeptide copeptin [Figure 1.17]. The prohormone folds and places AVP into a binding pocket of NPII, which protects AVP from proteolysis and promotes high-density packing in neurosecretory granules by oligomerisation of AVP-NPII dimers. Following formation of seven disulphide bonds within NPII, one within AVP and glycosylation of copeptin, the prohormone is packaged into neurosecretory granules and cleaved into product peptides during axonal transport to the posterior pituitary. The mature hormone and NPII are stored

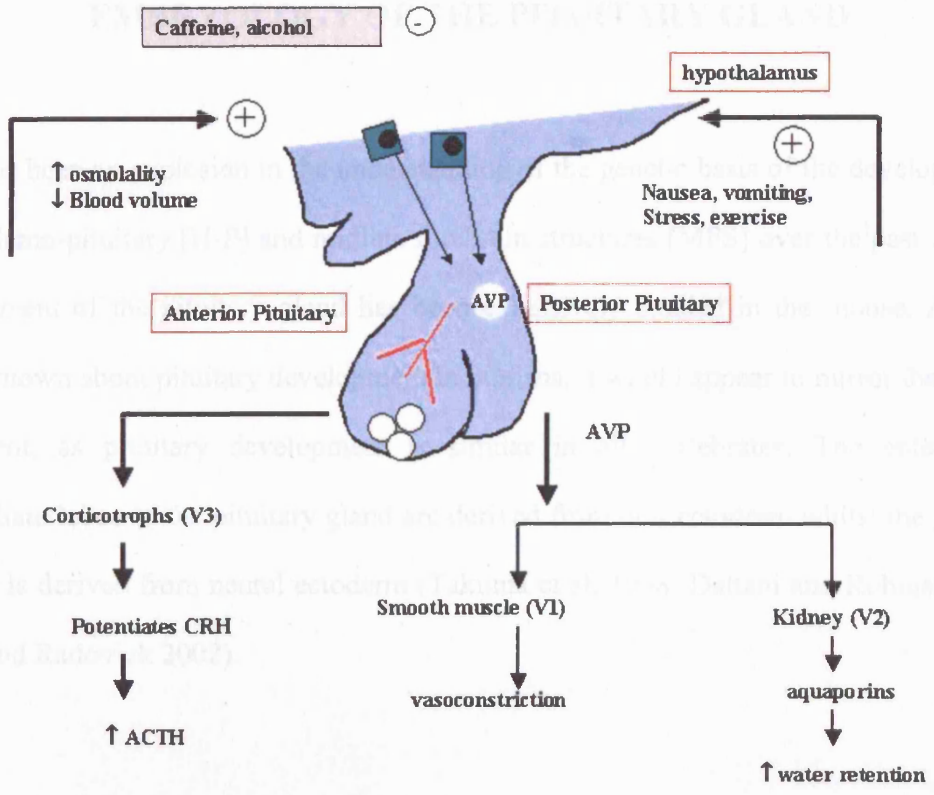
as a complex in secretory granules within nerve terminals of the posterior pituitary. Stimulation of vasopressinergic neurones releases their contents into the circulation.

Figure 1.17: Synthesis and structure of arginine vasopressin (AVP)



The half-life of AVP is short, approximately 5-15 minutes. AVP acts via three G-protein coupled receptors: it achieves its pressor effects via V1 receptors, its main renal effects via V2 receptors and it acts via V3 receptors on corticotrophs to secrete ACTH in synergy with CRH. Activation of V2 receptors leads to a biphasic increase in expression of the water channel protein Aquaporin 2. This allows re-absorption of water from the duct lumen along an osmotic gradient, with excretion of a concentrated urine [Figure 1.18]. The main regulatory factors in determining vasopressin secretion are osmotic status, blood pressure and circulating volume. Neurotransmitters, dopamine and norepinephrine and angiotensin II are also thought to play a role in vasopressin secretion.

Figure 1.18: Actions of arginine vasopressin and mechanisms controlling its secretion.



1.4.2 Oxytocin

The oxytocin gene found on chromosome 20 consists of 3 exons and like vasopressin, encodes a polypeptide precursor with an amino-terminal signal peptide, oxytocin peptide, neurophysin and a carboxy-terminal peptide. The human oxytocin promoter contains estrogen-response and interleukin-6 response elements although the significance of these is unclear. The half-life of oxytocin is short. It binds to a G-protein coupled receptor to mediate a variety of effects largely concerned with the regulation of lactation, parturition and reproductive behaviour. In humans, women lacking posterior pituitary function can breast-feed normally, indicating that oxytocin is not necessary for lactation in humans.

CHAPTER 2

EMBRYOLOGY OF THE PITUITARY GLAND

There has been an explosion in the understanding of the genetic basis of the development of hypothalamo-pituitary [H-P] and midline forebrain structures [MFS] over the past 25 years. Development of the pituitary gland has been extensively studied in the mouse. Although little is known about pituitary development in humans, it would appear to mirror that seen in the rodent, as pituitary development is similar in all vertebrates. The anterior and intermediate lobes of the pituitary gland are derived from oral ectoderm whilst the posterior pituitary is derived from neural ectoderm (Takuma et al. 1998; Dattani and Robinson 2000; Cohen and Radovick 2002).

2.1 STAGES OF PITUITARY DEVELOPMENT

Development of the pituitary gland occurs in 4 distinct stages [Figure 2.1].

(a) Formation of the pituitary placode from oral ectoderm: Cell types of the pituitary gland are derived from the most anterior midline portion of the embryo in a region contiguous with the anterior neural ridge. The anterior neural ridge is displayed ventrally to form the oral epithelium which gives rise to the roof of the oral cavity. Onset of pituitary organogenesis coincides with a thickening [the pituitary placode] in the roof of the oral ectoderm at embryonic day (E) 8.5, corresponding to 4-6 weeks gestation in humans.

(b) Formation of rudimentary Rathke's pouch: Invagination of the oral ectoderm forms a rudimentary pouch and evagination of the ventral diencephalon forms the posterior pituitary. The pituitary placode makes contact with the floor of the ventral diencephalon. Apposition between the rudimentary Rathke's pouch and neural ectoderm of the diencephalon is critical to normal development and is maintained throughout early pituitary organogenesis.

(c) Formation of definitive Rathke's pouch: The rudimentary Rathke's pouch deepens and folds on itself until it closes forming a definitive pouch. The infundibulum or pituitary stalk is formed by evagination of the posterior part of the presumptive diencephalon.

(d) Formation of the adult pituitary gland: The definitive pouch is completely detached from the oral cavity. Spatial and temporal differentiation of various cell types within the pituitary gland results in the development of individual hormone secreting cells in a sequential order.

Complex genetic interactions dictate normal pituitary development. A cascade of signalling molecules and transcription factors plays a crucial role in organ commitment, cell proliferation, cell patterning and terminal differentiation and the final product is a culmination of this coordinated process [Figure 2.2]. Initially, cells within the primordium of the pituitary gland are competent to differentiate into all cell types. Following expression of the earliest markers of pituitary gland development (e.g. *Hesx1*), further signalling pathways are established from within the gland and ventral diencephalon that direct these cells towards terminal differentiation into mature hormone secreting cell types.

Figure 2.1: Stages of rodent pituitary development:

a) Oral ectoderm

b) Rudimentary pouch

c) Definitive pouch

d) Adult pituitary gland

I, infundibulum; NP, neural plate; N, notochord; PP, pituitary placode; OM, oral membrane; H, heart; F, forebrain; MB, midbrain; HB, hindbrain; RP, Rathke's pouch; AN, anterior neural pore; O, oral cavity; PL, posterior lobe; OC, optic chiasm; P, pontine flexure; PO, pons; IL, intermediate lobe; AL, anterior lobe; DI, diencephalon; SC, sphenoid cartilage. [Taken from Sheng HZ, Westphal H 1999. Trends in Genetics, 15: 236-240].

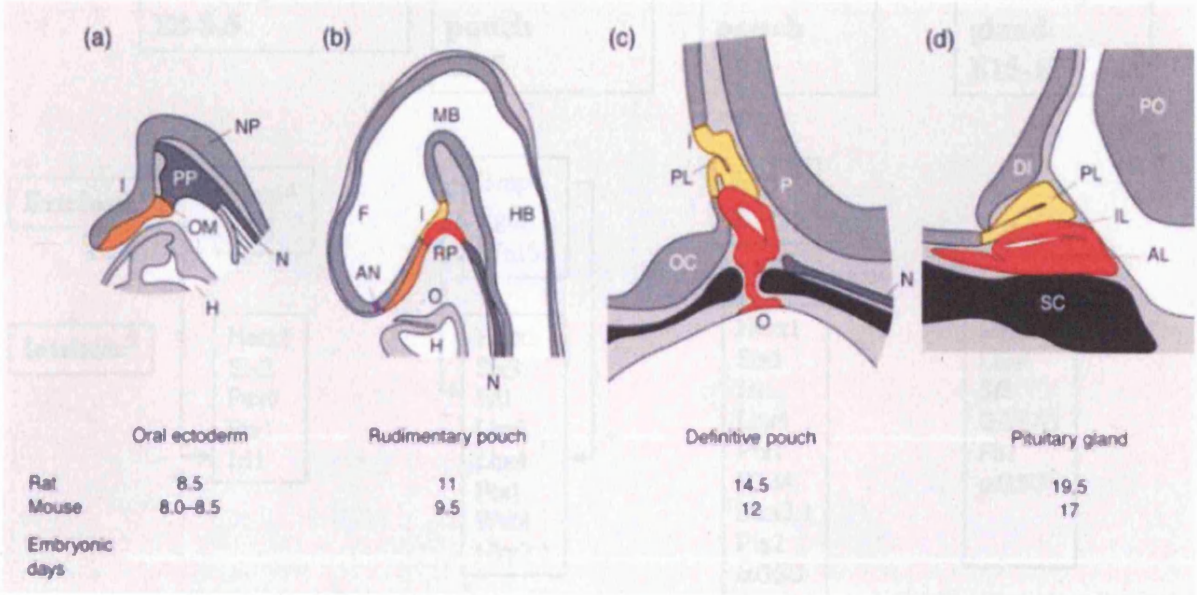
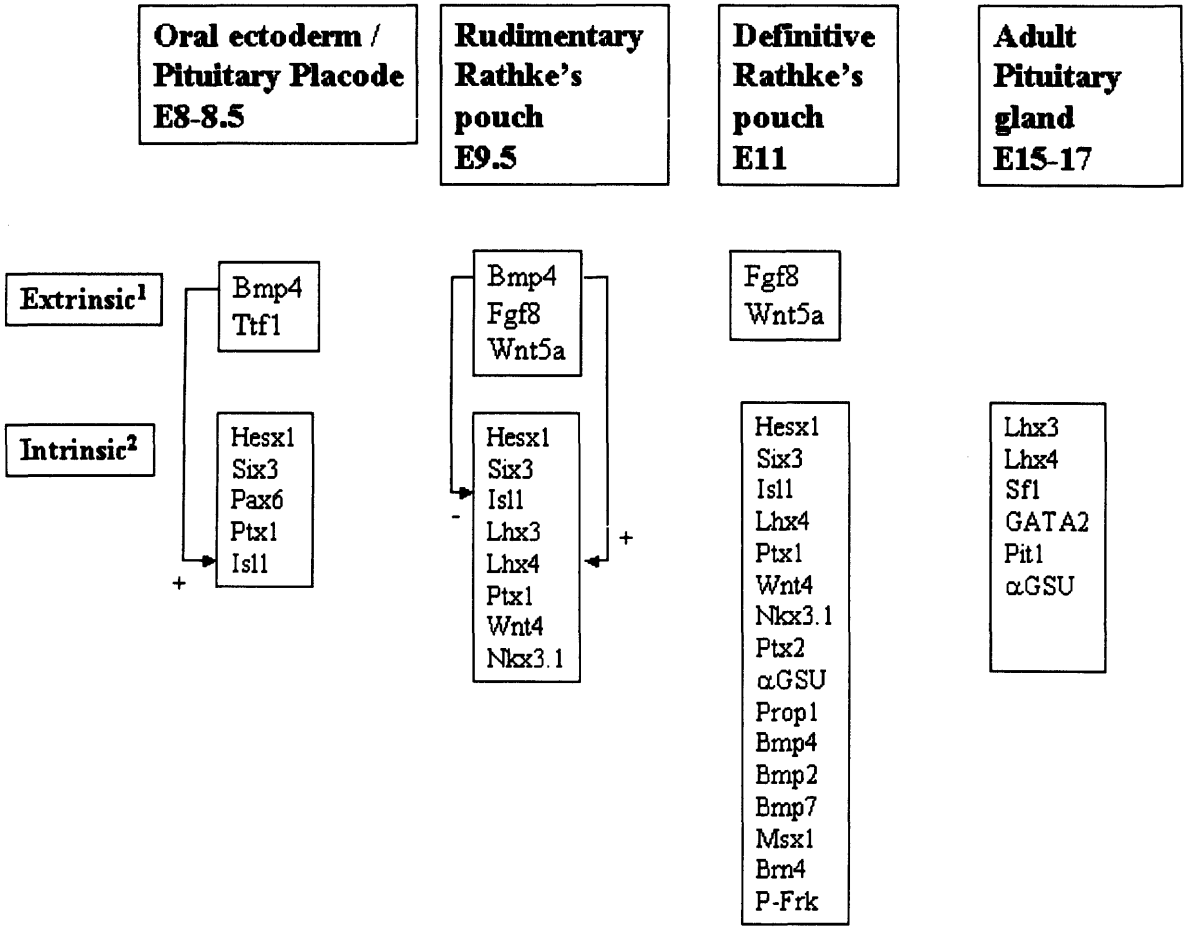


Figure 2.2: Transcription factors and signalling molecules involved in anterior pituitary development in the mouse.

E, embryonic day; [1] signalling molecules from the ventral diencephalon; [2] transcription factors and genes within the pituitary gland. Revised from Watkins-Chow DE, Camper SA 1998 Trends in Genetics, 14:284-290



Signalling molecules and transcription factors are expressed sequentially at critical periods of pituitary development and expression of many of these factors is attenuated subsequently [Figure 2.3]. Genes that are expressed early are implicated in organ commitment but are also implicated in repression and activation of downstream target genes that have specific roles in directing the cells towards a particular fate. Spontaneous or artificially induced mutations in the mouse have led to significant insights into human pituitary disease. Mutations involved specifically in human H-P disease are listed in Table 2.1.

Figure 2.3: Schematic representation of the pituitary developmental cascade with sequential expression of pituitary transcription factors and genes.

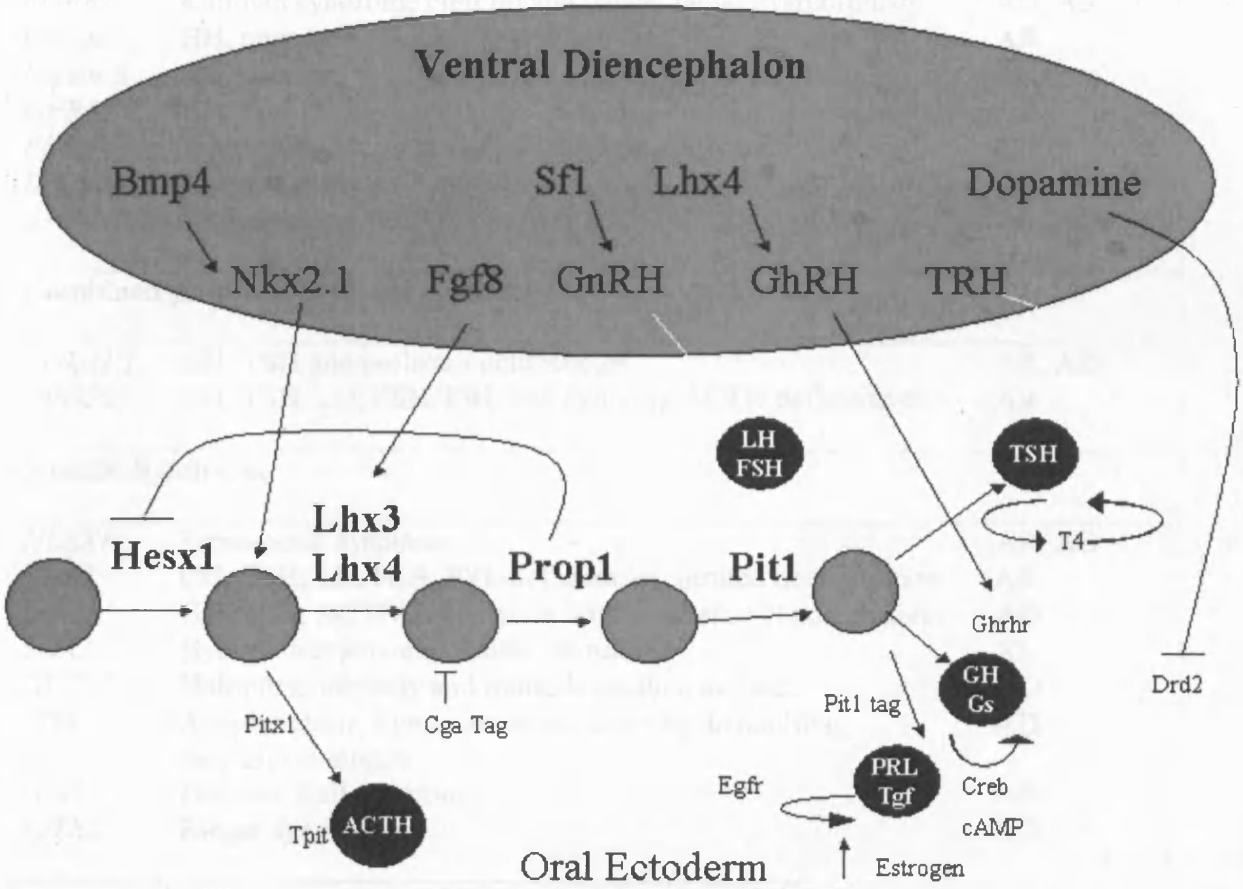


Table 2.1: Genetic disorders of hypothalamo-pituitary development in humans.

Gene	Phenotype	Inheritance
Isolated Hormone Abnormalities		
GH1	Isolated GH deficiency	AR, AD
GHRHR	Isolated GH deficiency	AR
TSH beta	Isolated TSH deficiency	AR
TRHR	Isolated TSH deficiency	AR
TPIT	Isolated ACTH deficiency	AR
GnRHR	Hypogonadotrophic hypogonadism (HH)	AR
PC 1	ACTH deficiency, hypoglycaemia, HH, obesity	AR
POMC	ACTH deficiency, obesity, red hair	AR
DAX1	Adrenal hypoplasia congenital and HH	XL
CRH	CRH deficiency	AR
KAL1	Kallman syndrome, renal agenesis, synkinesia	XL
FGFR1	Kallman syndrome cleft lip and palate, facial dysmorphism	AD, AR
Leptin	HH, obesity	AR
Leptin-R	HH, obesity	AR
GPR54	HH	AR
FSH beta	Primary amenorrhea, defective spermatogenesis	AR
LH beta	Delayed puberty	AR
AVP-NPII	Diabetes insipidus	AR, AD
Combined pituitary hormone deficiency		
POU1F1	GH, TSH and prolactin deficiencies	AR, AD
PROP1	GH, TSH, LH, FSH, PRL and evolving ACTH deficiencies	AR
Specific Syndrome		
HESX1	Septo-optic dysplasia	AR, AD
LHX3	GH, TSH, LH, FSH, PRL deficiencies, limited neck rotation	AR
LHX4	GH, TSH, ACTH deficiencies with cerebellar abnormalities	AD
SOX3	Hypopituitarism and mental retardation	XL
GLI2	Holoprosencephaly and multiple midline defects	AD
SOX2	Anophthalmia, hypopituitarism, learning difficulties, esophageal atresia	AD
GLI3	Pallister-Hall syndrome	AD
PITX2	Rieger syndrome	AD

R receptor; AR autosomal recessive; AD autosomal dominant; XL X-linked;
HH hypogonadotrophic hypogonadism

2.2 EARLY DEVELOPMENTAL GENES

2.2.1 Morphogenetic signals (B*mp, Fgf, Shh, Wnt)*

Extrinsic molecules within the ventral diencephalon and surrounding structures, such as bone morphogenetic proteins 2 and 4 [B*mp 2, 4*], fibroblast growth factor 8 [F*gf8*], sonic hedgehog [S*hh*], wingless [W*nt4*], thyroid transcription factor [T*tf1*; also called N*kx2.1*], and molecules involved in Notch signalling play critical roles in early organogenesis (Dasen and Rosenfeld 2001; Rizzoti and Lovell-Badge 2005). Recent studies in the mouse have shown that a close interaction between oral ectoderm and neural ectoderm is critical for initial development of the pituitary gland. Rathke's pouch develops in a two-step process that requires at least two sequential inductive signals from the diencephalon. First, induction and formation of the rudimentary pouch is dependent upon *Bmp4, and s*econdly, *Fgf8* activates key regulatory genes, *Lhx3* and *Lhx4*, that are essential for subsequent development of the rudimentary pouch into a definitive pouch. Both *Bmp4 and Fgf*8 are present only in the diencephalon and not in Rathke's pouch. Murine mutations within *Nkx2.1*, only expressed in the presumptive ventral diencephalon, can cause severe defects in the development of not only the diencephalon but also the anterior pituitary gland. Conditional deletion of *Rbp-J*, which encodes the major mediator of the Notch pathway, leads to conversion of the late [*Pit1*] lineage into the early [corticotroph] lineage. Notch signalling is required for maintaining expression of *Prop1,* which is required for generation of the *Pit1* lineage. Attenuation of Notch signalling is necessary for terminal differentiation in *Pit1* cells and maturation and proliferation of the GH-producing somatotroph (Zhu et al. 2006). There have been no reported mutations of these early morphogenetic signals in humans.

2.2.2 *Hesx1*

Hesx1 is one of the earliest markers of the pituitary primordium, suggesting that it has a critical role in early determination and differentiation of the pituitary gland. It is also called *Rpx* [Rathke's pouch homeobox] and is a member of the paired-like class of homeobox genes (Thomas et al. 1995; Hermesz et al. 1996; Dattani et al. 1998; Thomas et al. 2001). *Hesx1* is a transcriptional repressor, although its downstream targets are as yet unknown. A highly conserved region in the N-terminus of *Hesx1*, the engrailed homology domain, is crucial for its strong repressor function. The gene is first expressed during mouse embryogenesis in a small patch of cells in the anterior midline visceral endoderm as gastrulation commences. *Hesx1* continues to be expressed in the developing anterior pituitary until E12, when it disappears in a spatiotemporal sequence that corresponds to progressive pituitary cell differentiation. Extinction of *Hesx1* is important for activation of other downstream genes such as *Prop1*. It has been suggested that *Hesx1* and *Prop1* function as opposing transcription factors and that a careful temporal regulation of their expression is critical for normal pituitary development. Premature expression of *Prop1* can block pituitary organogenesis whereas prolonged expression of *Hesx1* can block *Prop1*-dependent activation. There is also evidence to suggest that *Prop1* activation is itself a prerequisite for extinction of *Hesx1*. *Lhx3* is also important for maintenance of *Hesx1* expression.

Targeted disruption of *Hesx1* in the mouse revealed a reduction in the prospective forebrain tissue, absence of developing optic vesicles, markedly decreased head size and severe microphthalmia reminiscent of the syndrome of septo-optic dysplasia [SOD] in humans. Other abnormalities included absence of the optic cups, the olfactory placodes and Rathke's pouch, reduced telencephalic vesicles, hypothalamic abnormalities and aberrant

morphogenesis of Rathke's pouch. In 5% of null mutants, the phenotype was characterized by complete lack of the pituitary gland. In the majority of mutant mice, they were characterized by formation of multiple oral ectodermal invaginations and hence multiple pituitary glands.

Mutations in *HESX1* in humans were first reported in two siblings with SOD and subsequently other mutations have been shown to present with varying phenotypes characterized by isolated GH deficiency [IGHD], combined pituitary hormone deficiency [CPHD] and SOD (Dattani et al. 1998; Brickman et al. 2001; Thomas et al. 2001; Carvalho et al. 2003; Tajima et al. 2003; Cohen et al. 2003; Sobrier et al. 2005; Sobrier et al. 2006).

2.2.3 *Pitx1* and *Pitx2*

Pitx1 and *Pitx2* are paired-like homeobox genes expressed in the fetal pituitary and in most cells of the adult pituitary gland. These genes play an important role in the development of Rathke's pouch and the anterior pituitary gland.

In the mouse, *Pitx1* is initially expressed in the first branchial arch mesenchyme at E9 and then throughout the oral epithelium lining the roof of the buccal cavity and in Rathke's pouch ectoderm. *Pitx1* expression continues throughout development in all regions of the anterior pituitary and overlaps with that of *Lhx3* and appears to be required for sustained expression of the latter. A T-box factor, *Tpit*, present only in POMC expressing cells within the pituitary is essential for initiating POMC cell differentiation and for activating POMC transcription synergistically with *Pitx1*. *Pitx1* also appears to modulate steroidogenic factor 1 [*sf1*] activity in gonadotrophs, activation of the GH promoter and synergistic activation of

the prolactin promoter with *Pit1*. Mice that are rendered deficient in *Pitx1* demonstrate abnormalities within the hindlimb and palate. Gonadotrophs and thyrotrophs are reduced with an increase in the concentration of ACTH transcripts and peptide in corticotrophs. In adults, *PITX1* is specifically expressed at higher levels in cells of the α-glycoprotein subunit lineage and a fraction of POMC-expressing cells. Most corticotrophs in humans however do not express *PITX1*.

Pitx2 is first expressed in mouse embryo in the oral epithelium and oral ectoderm. At E9.5 *Pitx2* is expressed in the developing Rathke's pouch in addition to mesenchyme near the optic eminence, basal plate of the central nervous system, forelimbs and domains of the abdominal cavity. It appears to be required for pituitary development shortly after formation of the committed pouch. It may be required for one or more anterior pituitary cell types or may act in concert with other transcription factors. It is also expressed in lungs, kidney, testes and tongue. Additionally, *Pitx2* is implicated in left-right asymmetry since it is expressed in the lateral plate mesoderm and then continues to be expressed asymmetrically in several organs that are asymmetric with respect to left-right axis of the embryo. There are at least 3 isoforms of *Pitx2*. *Pitx2a* and *Pitx2b* are expressed in the adult pituitary in thyrotrophs, gonadotrophs, somatotrophs and lactotrophs but not corticotrophs, where *Pitx1* is highly expressed. However, *Pitx2c* is expressed in all five cell lineages.

To date, no mutations have been described within *PITX1* in humans. Mutations in *PITX2* are associated with Rieger syndrome in humans (Semina et al. 1996).

2.2.4 *Lhx3* and *Lhx4*

Lhx3 and *Lhx4* belong to the LIM family of homeobox genes that are expressed early in Rathke's pouch. At least 3 different isoforms of *Lhx3* have been described in mammals, each with distinct expression patterns and transcriptional properties (Bach et al. 1995; Zhadanov et al. 1995; Schmitt et al. 2000).

Lhx3 is detected in the developing nervous system. *Lhx3a* isoform is first expressed at E8.5 in the mouse embryo whilst *Lhx3b* is first expressed at E9.5. Subsequently, *Lhx3* is expressed in the anterior and intermediate lobes of the pituitary gland, ventral hindbrain and spinal cord. Maintenance of *Lhx3* persists in the adult pituitary gland suggesting a maintenance function for one or more of the anterior pituitary cell types. *Lhx3* activates α glycoprotein subunit unit [αGSU] promoter and together with *Pit1* acts synergistically to activate TSH-β and prolactin promoters and the *Pit1* enhancer. *Lhx3* is one of the earliest markers for cells that are destined to form the anterior and intermediate lobes and continued expression is essential for formation of gonadotrophs, thyrotrophs, somatotrophs and lactotrophs. In *Lhx3* null mutant mice, Rathke's pouch is initially formed but then fails to grow and *Hesx1* expression is switched off early. There is failure of expression of αGSU, β subunit of TSH, GH and Pit1 transcripts. Specification of corticotroph cell lineage does occur, although there is failure of POMC cell proliferation.

Human mutations in *LHX3* are associated with GH, TSH, prolactin, and gonadotrophin deficiencies with a neck phenotype characterized by limited neck rotation (Netchine et al. 2000).

Lhx4 is a closely related gene that is expressed in specific areas of the brain and spinal cord. Like *Lhx3*, *Lhx4* is expressed throughout the invaginating pouch at E9.5. Subsequent expression at E12.5 is restricted to the future anterior lobe. Its expression is reduced by E15.5 (Sheng et al. 1997). Null mutants of *Lhx4* show formation of Rathke's pouch with expression of αGSU, β subunit of TSH, GH and Pit1 transcripts, demonstrating that various anterior pituitary cell lineages are specified although their numbers are reduced. *Lhx3*$^{-/-}$, *Lhx4*$^{-/-}$ double mutant mice show a more severe phenotype than either single mutant with an early arrest of pituitary development, thereby suggesting that these two genes may act in a redundant manner during early pituitary development (Sobrier et al. 2004).

LHX4 mutations in humans, reported in only one pedigree to date, resulted in GH, TSH and ACTH deficiencies with cerebellar hypoplasia (Machinis et al. 2001).

2.3 TERMINAL CELL DIFFERENTIATION GENES

Terminal pituitary cell differentiation is a culmination of a complex interaction between extrinsic signalling molecules and transcription factors such as *Lhx3*, *Lhx4*, *Sox* genes, *GATA2*, *Isl1*, *Prop1* and *Pit1*. *GATA2* encodes a transcription factor that is important in the differentiation of gonadotrophs and thyrotrophs. Other transcription factors involved in the maturation of gonadotroph lineage include S*f*1 and *Dax*1. *Pit1* and *Prop1* are best characterised in terms of function in both humans and mice.

2.3.1 *Prop1*

Prop1 [Prophet of *Pit1*] is a pituitary-specific paired-like homeodomain transcription factor first expressed in the dorsal portion of Rathke's pouch at E10-10.5 followed by maximal expression at E12 and subsequent extinction by E15.5 (Sornson et al. 1996). It is believed to be required for expression of *Pit1,* the critical lineage-determining transcription factor, since there is a failure of determination of *Pit1* lineages, lack of *Pit1* gene activation and absence of progression to mature cells in the Ames dwarf mice who harbour a homozygous missense mutation in the *Prop1* gene (Parks et al. 1999). *Prop1* is also important in regulating the expression of *Hesx1,* the lineage-inhibiting transcription factor. Beta-catenin acts as a binary switch by interacting with *Prop1* to simultaneously activate expression of *Pit1* and to repress *Hesx1*, acting via TLE/Reptin/HDAC1 co-repressor complexes (Olson et al. 2006).

Homozygous Ames dwarf mice exhibit severe proportional dwarfism, hypothyroidism and infertility and the emerging anterior pituitary gland is reduced in size by about 50% displaying an abnormal looping appearance. The adult Ames dwarf mouse exhibits GH, TSH and prolactin deficiency resulting from a severe reduction of somatotroph, lactotroph and caudomedial thyrotroph lineages. Additionally these mice have reduced gonadotrophin expression correlating with low plasma LH and FSH concentrations.

The size of the pituitary gland on magnetic resonance [MR] imaging is reduced considerably. Recent reports suggest that *Prop1*-deficient fetal mouse pituitary retains mutant cells in the perilumenal area of Rathke's pouch that fail to differentiate. The mutant pituitary then exhibits enhanced apoptosis and reduced proliferation (Ward et al. 2005). At postnatal day 11, apoptosis-independent caspase-3 activation occurs in thyrotrophs and

somatotrophs of normal but not *Prop1* and *Pit1* mutant pituitaries indicating a role for caspase-3 expression (Ward et al. 2006).

Humans with mutations in *PROP1* characteristically have GH, TSH, prolactin and gonadotrophin deficiencies suggesting a role for *PROP1* in gonadotroph differentiation in humans (Wu et al. 1998). The phenotype also includes evolving ACTH deficiency in some patients.

2.3.2 *Pit1*

Pit1 [called *POU1F1* in humans] is a pituitary specific transcription factor belonging to the POU homeodomain family. It has also been called GH factor 1 as it was first identified as a regulator of *GH1* transcription. Apart from *GH1*, *Pit1* binding sites have also been identified in promoters of the prolactin and TSH β-subunit genes. *Pit1* is expressed relatively late during pituitary development [E13.5 in the mouse] and its expression persists throughout life. *Pit1* is also essential for the development of somatotrophs, lactotrophs and thyrotrophs in the anterior pituitary. Transcripts first appear in cells within the caudomedial region of the anterior pituitary at E14.5, followed by detection of the protein within somatotrophs and lactotrophs and subsequent expression of *GH1* and prolactin genes on E16 and E17 respectively. *Pit1* dependent thyrotrophs arise on E15.5.

In the Snell dwarf mouse, a recessive point mutation results in absence of somatotrophs, lactotrophs and thyrotrophs. A similar phenotype results in the Jackson dwarf mouse, which harbours a recessive null mutation of *Pit1*. Apart from its role in proliferation and

maintenance of somatotrophs, lactotrophs and thyrotrophs, Pit1 binding sites have also been found in promoter regions of the *GHRHR* and the *Pit1* gene itself. Data suggest that auto regulation of Pit1 is required to sustain *Pit1* gene expression once the Pit1 protein has reached a critical threshold (Li et al. 1990; Andersen and Rosenfeld 2001).

Humans with mutations in *POU1F1* characteristically present with a pituitary phenotype characterized by GH, TSH and prolactin deficiencies (Tatsumi et al. 1992).

2.3.3. Sox3

Sox3 is a single-exon gene located on the X-chromosome in all mammals. It contains a high mobility group [HMG] box and is believed to be the gene from which the testis-determining gene *SRY* evolved. It is closely related to *Sox1* and *Sox2* and all 3 genes belong to the *Soxb1* subgroup and are expressed throughout the central nervous system. *Sox* genes are expressed in the neuroepithelial progenitor and stem cells from the earliest stages with considerable overlap in their expression patterns. Over-expression in the fish leads to hypoplasia in other tissues.

Duplications of Xq26-27 and mutations in *SOX3* have been implicated in variable hypopituitarism and mental retardation in humans (Hamel et al. 1996; Hol et al. 2000; Solomon et al. 2002; Laumonnier et al. 2002).

CHAPTER 3

CONGENITAL HYPOPITUITARISM

3.1 INTRODUCTION

Hypopituitarism encompasses a group of disorders with variable aetiologies. Hormone dysfunction may occur in isolation, combined with other pituitary hormone deficiencies or associated with extra-pituitary features. The reported incidence of GH deficiency [GHD], isolated or combined with other pituitary hormone deficiencies is between 1 in 4,000 to 1 in 10,000 live births, the majority of cases being idiopathic in origin (Lacey and Parkin 1974; Vimpani et al. 1977; Rona and Tanner 1977). The exact incidence of combined pituitary hormone deficiency [CPHD] is unknown. Familial CPHD may account for 5-30% of cases of hypopituitarism (Phillips and Cogan 1994). Idiopathic hypopituitarism is generally associated with a male to female preponderance. Clinically, the phenotype is highly variable, both in severity and in the number of hormone deficiencies. The onset of clinical features may be early in the neonatal period often with a stormy perinatal course or later with growth failure. Isolated GHD [IGHD], by far the commonest endocrinopathy, presents with growth failure and short stature. The root of the problem appears to lie within the hypothalamus rather than the pituitary gland in many patients with hypopituitarism. Additionally, the evolution of hormone deficiencies with time is now a well-recognised feature of hypopituitarism. Evolving endocrinopathies are observed more commonly in patients with septo-optic dysplasia (SOD), autosomal dominant familial GHD (type II) and in patients with mutations in *PROP1* and *HESX1*.

3.2 ISOLATED HORMONE DEFICIENCY

3.2.1 Growth hormone [GH] deficiency

IGHD occurs in four well-described familial forms, as shown in Table 3.1, and described in detail below.

Table 3.1: Types of isolated growth hormone deficiency [IGHD].

Inheritance	Type	Phenotype	Gene	Nature of mutations
AR	IA	Short stature, anti GH antibodies	*GH1*	Deletions, amino acid substitutions
	IB	Short stature. No anti GH antibodies	*GH1* / *GHRHR*	Splice site mutations, amino acid substitutions
AD	II	Short stature. No antibodies	*GH1*	Splice site mutations, missense mutation [R183H]
XLR	III	Short stature with ? learning difficulties ? agammaglobulinemia	Unknown *? SOX3*	

AR, autosomal recessive; AD, autosomal dominant; XLR, X-linked recessive

3.2.1.1 IGHD Type IA

Patients with IGHD type IA have complete absence of GH and lack tolerance to exogenous GH treatment with production of human GH [hGH] antibodies. They present with early and profound growth failure, a characteristic facial appearance of a large vaulted forehead and a small nose with a depressed nasal bridge, and a markedly reduced adult height. Serum GH concentrations are undetectable or extremely low on provocation testing.

The exact prevalence of this disorder is unclear as sporadic cases may go unrecognised. All families reported to date with IGHD type IA are consanguineous with an autosomal recessive mode of inheritance. There is marked heterogeneity in the phenotype of these patients in addition to a considerable variability in antibody production and response to hGH treatment even within families with the same mutation.

The majority of patients with IGHD type IA have large deletions within the *GH1* gene (Phillips, III et al. 1981). However, micro deletions and point mutations have also been described. Patients with larger deletions (>7.6 Kb) respond better to GH treatment as compared with those with smaller deletions. Recombinant human IGF1 [rhIGF1] treatment has also been used, particularly in patients with a poor initial response to hGH treatment and production of high antibody titres. With improvements in recombinant technology, purer forms of rhGH can now be produced which may alleviate the problem of antibody production to some extent. Table 3.2 lists deletions and mutations within *GH1* reported to date.

Table 3.2: Mutations identified to date within the *GH1* gene.

Mutation	Anti GH antibodies	Type
Gross deletions		
6.7 Kb gross deletion	√	1A
7.0 Kb gross deletion	√	1A
7.6 Kb gross deletion	√ / X	1A
45.0 Kb gross deletion	√	1A
Double (GH1; CSH1, GH2, CSH2)	√	1A
Micro deletions		
C10del micro deletion	√	1A
IVS3 18 base pair del micro deletion		II
2 base pair deletion S54del [Compound heterozygote with 6.7Kb del]	X	1A
Point mutations		
W7X nonsense mutation	√	1A
E4X nonsense mutation	X	1A
W20X nonsense mutation	X	1A
P89L missense mutation	X	II
R183H missense mutation	X	II
V110F missense mutation	X	II
R77C missense mutation	X	II
Splice site mutations		
E3+1 G>T	X	II
E3+5 A>G	X	II
IVS2 - 2 A>T	X	II
IVS3+1 G>A	X	II
IVS3+1 G>C	X	II
IVS3+2 T>C	X	II
IVS3+5 G>A	X	II
IVS3+5 G>C	X	II
IVS3+6 T>C	X	II
IVS3+6 T>G	X	II
IVS3+28 G>A	X	II
IVS4+1 G>C	X	IB
IVS4+1 G>T	X	IB
IVS4+5 G>C	X	II

✓, production of anti GH antibodies; X, no production of anti GH antibodies.

3.2.1.2 IGHD Type IB

This disorder is also associated with a prenatal onset of IGHD, but is milder than IGHD type IA, with detectable concentrations of GH after provocation testing. The condition is inherited as an autosomal recessive trait. Children present with short stature and a poor growth velocity with a good response to exogenous hGH treatment with no formation of GH antibodies. IGHD type IB is manifest due to either homozygous splice site mutations within the *GH1* gene or mutations within the GHRH receptor gene (*GHRHR*).

The human *GHRHR* gene consists of 13 exons spanning approximately 15Kb, and has been mapped to chromosome 7p15. It encodes a protein containing 423 amino acids. The receptor is a G-protein coupled receptor with a high binding affinity for GHRH. The expression of *GHRHR* is up regulated by POU1F1. *GHRHR* is also required for proliferation of somatotrophs and therefore plays an important role in anterior pituitary development.

The first reported cases of *GHRHR* mutations were found in two first cousins, from the Indian subcontinent, who were found to have a G>T substitution leading to a stop codon and a severely truncated protein lacking membrane spanning domains and an inability to bind to GHRH (Wajnrajch et al. 1996). Since then, several patients with *GHRHR* mutations have been reported, including splice site mutations (Carakushansky et al. 2003) [Table 3.3].

Table 3.3: *GHRHR* mutations identified to date.

Mutation
Point mutations
E72X nonsense mutation L144H missense mutation F242C missense mutation (compound heterozygote with L144H) A222E missense mutation H137L missense mutation (compound heterozygote with del 1140-1144) K329E missense mutation (compound heterozygote with promoter region mutation) Q43X missense mutation (compound heteozygote with splice site mutation) A176V missense mutation R357C missense mutation
Splice site mutations
IVS1+1G→A IVS7 + 1G→C IVS12 + 2T→A
Small deletions
Del 1121-1124 Del 1140-1144
Promoter region
- 124 A> C

3.2.1.3 IGHD Type II

This condition is inherited in an autosomal dominant manner. The patients present with short stature and respond well to exogenous hGH treatment with no formation of antibodies. IGHD type II is most commonly a result of splice site mutations in intron III (IVSIII) within the *GH1* gene [See Table 3.2]. In addition, four missense mutations (R77C, R183H, P89L and V110F) have also been implicated in IGHD type II. More recently, mutations in an exon splice enhancer within exon 3 of the *GH1* gene have been associated with autosomal dominant GHD (Moseley et al. 2002). Splice site mutations lead to the production of two alternatively spliced GH molecules, 20kDa and 17.5kDa hGH. The 17.5kDa form of GH generated as a result of the skipping of exon 3 and subsequent loss of amino acids 32-71 has

a dominant negative effect preventing secretion of normal wild-type 22kDa GH with a consequent deleterious effect on pituitary somatotrophs. In a murine model of this dominant negative mutation, there is evolution of the phenotype with later failure of prolactin, TSH and gonadotrophin secretion (McGuinness et al. 2003). Patients presenting with a splice site mutation within the first 2 base pairs of intervening sequence 3 (5'IVS +1/+2 bp) leading to a skipping of exon 3 were found to be more likely to present at follow-up with other pituitary hormone deficiencies (Mullis et al. 2005). The development of multiple hormonal deficiencies is not age dependent and there is a clear evidence of variability in the onset, severity and progression, even within the same family. A detailed analyses of different mutations identified in IGHD type II showed different mechanisms of secretory pathophysiology at a cellular level resulting in a different extent of co-localization and a different effect on GH secretion. This might be caused by different folding or aggregation problems necessary for sorting, packaging or secretion through the regulated secretory pathway (Salemi et al. 2005).

3.2.1.4 IGHD Type III

The disorder is inherited in an X-linked recessive manner. In addition to GHD, patients may also manifest agammaglobulinaemia. No abnormalities have been documented within the *GH1* gene and the exact mechanism for the phenotype is as yet unknown. Mutations in *SOX3* associated with X-linked mental retardation and GHD (Laumonnier et al. 2002) may account for some patients although the association with agammaglobulinaemia suggests the involvement of other genes.

3.2.2 Thyrotrophin [TSH] deficiency

Isolated central hypothyroidism, characterized by deficient TSH secretion resulting in reduced concentrations of thyroid hormones, is a very rare disorder. It is most often sporadic although familial cases have been reported. The reported prevalence is 1 in 50,000 live births. Neonates may present with non-specific symptoms such as lethargy, poor feeding, prolonged hyperbilirubinaemia and cold intolerance. Central hypothyroidism is generally milder than primary hypothyroidism, where a more severe phenotype characterised by coarse facies and severe mental retardation may be characteristic.

Dacou-Voutetakis et al first reported a homozygous nonsense mutation in exon 2 of the TSH β-subunit gene in three children affected by congenital TSH-deficient hypothyroidism within two related Greek families (Dacou-Voutetakis et al. 1990). Affected individuals showed symptoms of severe mental and growth retardation. This mutation gave rise to a truncated peptide including only the first 11 of 118 amino acids of the mature TSH beta-subunit peptide.

Collu et al were the first to report an inactivating mutation of the TRH receptor gene as a cause for isolated central hypothyroidism in a patient with short stature and delayed bone maturation. Although he had a subnormal intelligence quotient, this was possibly related to the low socio-economic status as the intelligence quotients of unaffected sibs was similar (Collu et al. 1997). The mutation resulted in a failure of TRH binding to its receptor and a consequent failure of TSH and prolactin secretion.

3.2.3 Corticotrophin [ACTH] deficiency

Isolated congenital ACTH deficiency is rare. Patients usually present in the neonatal period with non-specific symptoms such as poor feeding, failure to thrive and hypoglycaemia. More acute signs of adrenal deficiency include vascular collapse, shock and bradycardia. Serum aldosterone secretion is controlled by the renin-angiotensin system and hence abnormalities in salt excretion are unusual. Females rely on adrenal androgens for the development of pubic and axillary hair and women lack both if they are ACTH deficient.

Krude et al first described two patients with mutations in the POMC gene (Krude et al. 1998). Both patients presented with early-onset ACTH deficiency, obesity and red hair due to lack of αMSH. Symptoms of hypoglycaemia and cholestasis resolved with hydrocortisone treatment. A compound heterozygous mutation in *PC1* in a female patient with extreme early-onset obesity and ACTH deficiency with defective processing of other prohormones, abnormal glucose homeostasis and hypogonadotrophic hypogonadism has been described (O'Rahilly et al. 1995; Jackson et al. 1997). More recently, a child with ACTH deficiency, red hair and a severe enteropathy was found to harbour mutations in *PC1* (Jackson et al. 2003). Twelve independent mutations have been identified in *TPIT,* with a recessive mode of inheritance, resulting in severe ACTH deficiency, profound hypoglycemia associated with seizures in some cases and prolonged cholestatic jaundice in the neonatal period. Neonatal deaths have been reported in 25% of families with *TPIT* mutations, in a large series, suggesting that isolated ACTH deficiency may be an underestimated cause of neonatal death (Lamolet et al. 2001; Vallette-Kasic et al. 2005).

3.2.4 Gonadotrophin deficiency

Hypogonadism may be due to abnormalities within the H-P axis [hypogonadotrophic hypogonadism (HH)] or within the gonad itself [hypergonadotrophic hypogonadism]. It is rare condition with an incidence variably reported from 1 in 10,000 to 1 in 86,000 and is four times more common in males (Fromantin et al. 1973; Filippi 1986). HH is particularly heterogeneous with a phenotype, in males, ranging from undescended testes at birth, absent pubertal development alone, to normal puberty but infertility at the other extreme. Overall, there is a low prevalence of genital abnormalities at birth in these patients suggesting that maternal human chorionic gonadotrophin may also play a significant role in testosterone secretion by the fetus.

An association between isolated HH and anosmia, Kallman syndrome [KS], was first reported by Maestre de San Juan and Kallman described the genetic basis to this disorder. Mutations in the *KAL1* gene result in an X-linked inheritance of KS (Bick et al. 1992; Hardelin et al. 1993). Anosmia is a result of agenesis of olfactory bulbs, development of which is closely linked to that of GnRH secreting neurones. Although these patients are capable of synthesising and secreting a normal GnRH protein, the abnormal location of GnRH neurones results in an inability of GnRH to reach the pituitary gland to stimulate gonadotrophs. Other features such as mirror movements, renal aplasia, high-arched palate, deafness and pes cavus are associated with this disorder (Hardelin et al. 1993). Loss of function mutations in *FGFR1* are thought to be responsible for autosomal dominant [and rarely recessive] forms of KS. These patients have a milder phenotype and may have adult-onset HH. They may also have abnormalities such as a cleft lip or palate.

Disrupted GPR54 signalling causes HH in rodents and man. Recently, a homozygous missense mutation was identified within the gene in a highly consanguineous pedigree of patients with HH (de Roux et al. 2003). A second patient was found to be a compound heterozygote and, since, several pedigrees have been identified that harbour deletions within this gene. Mutations or targeted disruptions in its ligand kisspeptin-1 also cause autosomal recessive HH in humans and mice. Central or peripheral administration of kisspeptin potently stimulates the H-P-gonadal axis, increasing circulating serum gonadotrophin concentrations in a number of animal models underlying the importance of kisspeptin and its receptor in the secretion of gonadotrophins.

DAX-1 [dosage sensitive sex reversal, adrenal hypoplasia congenita critical region on the X chromosome] mutations in humans cause HH and adrenal hypoplasia congenita that can result in a severe neonatal adrenal crisis. More often, HH presents itself at puberty (Tabarin et al. 2000). The condition is inherited as an X-linked disorder. *DAX1* is a transcription factor that is expressed in several tissues including the hypothalamus and pituitary gland and closely interacts with *SF1*, another transcription factor critical for adrenal and gonadal development, acting as a repressor of *SFI*. Duplications of *DAX1* result in persistent Mullerian structures and XY sex reversal suggesting that the gene acts in a dosage sensitive manner similar to the *SOX3* gene. Females with duplications of *DAX1* are phenotypically normal.

Inactivating mutations of the GnRH receptor gene (*GnRHR*) were first reported in 1997. Since then seven different pedigrees with *GnRHR* mutations with a varying phenotypes, ranging from complete hypogonadism with undescended testes at birth to those who present with mild pubertal delay, have been described (de Roux et al. 1997). Mutations in *GnRHR*

are rare but patients with milder phenotypes harbouring mutations may not, as yet, have been identified.

Leptin is a secretory product of the adipocyte, which acts as a satiety factor and also plays an important role in several neuroendocrine functions at a hypothalamic level. Mutations in leptin and its receptor are associated with obesity, marked hyperphagia, metabolic abnormalities and HH (Montague et al. 1997; Farooqi et al. 1999). Mutations in *PC1* associated with defective processing of prohormones also result in HH (Jackson et al. 1997).

3.2.5 Prolactin deficiency

Isolated prolactin deficiency is extremely rare (Kauppila et al. 1987). More commonly it occurs in combination with other anterior pituitary hormone deficiencies, sporadically or in patients with *POU1F1* and *PROP1* mutations.

3.2.6 Central Diabetes Insipidus

Central diabetes insipidus [DI] is most often acquired and congenital causes are rare. Familial central DI is an autosomal dominant disorder of AVP secretion. Affected patients present with polyuria and polydipsia, usually within the first 10 years of life. Neonatal manifestations are uncommon suggesting that the pathophysiology of familial central DI involves progressive postnatal degeneration of AVP-producing magnocellular neurones. During infancy, common clinical features include hyperthermia, vomiting, failure to thrive

and constipation. Overt hypertonic dehydration occurs only if the patient is unable to obtain water. Food consumption is decreased leading to loss of weight and poor growth.

A number of mutations have been described in children with central DI in the *AVP-NPII* gene. These include signal peptide mutations with a decreased ability of the signal peptidase to initiate removal of the signal peptide from the preprohormone (Ito et al. 1993). A second group of mutations occurs within the *AVP* or amino-terminal domain of NPII-coding sequence interfering with binding of AVP to NPII or in folding of NPII (Bahnsen et al. 1992). A third group of mutations results in the synthesis of a truncated neurophysin molecule (Nagasaki et al. 1995). All mutations may lead to abnormal folding and processing of the preprohormone. The mutant protein may then accumulate within the endoplasmic reticulum killing cells by interfering with the orderly processing of other essential proteins. Hence, heterozygous mutations within the *AVP-NPII* gene may result in production of an abnormal preprohormone that cannot be processed properly destroying AVP-processing neurones. A pedigree with autosomal recessive DI has also been described.

Other causes include Wolfram syndrome, an autosomal recessive condition with DI, diabetes mellitus, optic atrophy and sensorineural deafness. The *WFS1* gene, on chromosome 4, encodes an 890 amino acid glycoprotein, wolframin, and is predominantly localised in the endoplasmic reticulum. Mutations in *WFS1* underlie Wolfram syndrome. Mutations have been reported to date over the entire coding region and are typically inactivating, suggesting that a loss of function causes the disease phenotype (Cryns et al. 2003). DI may also be a feature of midline disorders such as SOD and holoprosencephaly [HPE].

3.3 COMBINED PITUITARY HORMONE DEFICIENCY [CPHD]

3.3.1 Idiopathic CPHD

Most patients with CPHD do not have a known underlying etiology and are classified as having idiopathic disease. The condition is highly variable with respect to clinical presentation. Even if hypopituitarism is not recognized in the immediate postnatal period, a careful history at the time of diagnosis may indicate the presence of perinatal symptoms.

Craft et al have suggested a causal relationship between gestational-perinatal complications [65%] and hypopituitarism with risk factors such as prematurity, gestational bleeding, complications of delivery, fetal distress or asphyxia (Craft et al. 1980). It is difficult to differentiate whether hypothalamo-pituitary defects are actually responsible for these perinatal complications or whether perinatal problems themselves lead to hypopituitarism. The discovery of pituitary transcription factors, mutations of which can lead to abnormalities of hypothalamo-pituitary [H-P] morphology, suggests that the former may be the case at least in some patients. The fact that many males with hypopituitarism are born with genital abnormalities such as undescended testes and a micropenis also suggests that, in some cases at least, hypopituitarism is of prenatal onset.

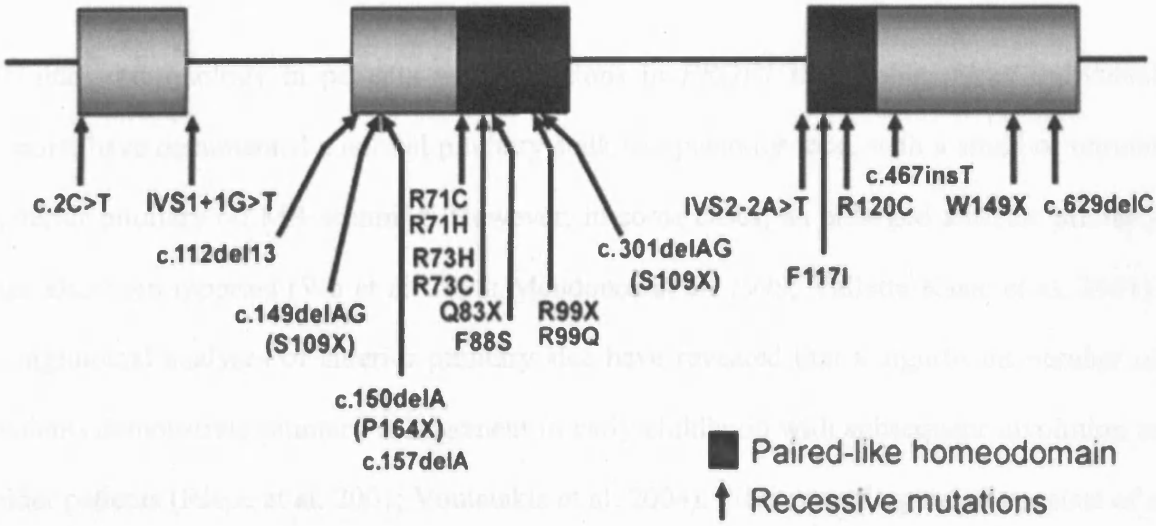
3.3.2 Non-Syndromic Genetic CPHD

3.3.2.1 Mutations in PROP1

Human *PROP1* gene has been mapped to chromosome 5q and is a member of the paired-like homeobox gene family. The gene spans 3Kb and consists of 3 exons encoding a protein product of 226 amino acids. The DNA binding homeodomain consists of 3 alpha helical regions and most mutations reported to date affect this region.

Wu et al first reported mutations in *PROP1* in four unrelated pedigrees with an endocrine phenotype consistent with GH, TSH, prolactin, LH and FSH deficiencies (Wu et al. 1998). To date, 22 distinct mutations have been identified in over 170 patients [Figure 3.1], suggesting that mutations in *PROP1* are the most common cause of CPHD, in approximately 50% of familial cases (Cogan et al. 1998; Deladoey et al. 1999). The incidence in sporadic cases is, however, much lower (Cogan et al. 1998]. Affected individuals exhibit recessive inheritance. The most common *PROP1* mutation (50%-72%) detected in multiple unrelated families is a 2 base pair [bp] deletion within exon 2 resulting in a frame shift at codon 101 and has been referred to as 301-302delAG and 296delGA in different reports (Wu et al. 1998; Fofanova et al. 1998; Mendonca et al. 1999; Deladoey et al. 1999). This probably represents a mutational hot spot (Cogan et al. 1998) and along with the 150delA mutation accounts for approximately 97% of all mutations in *PROP1*.

Figure 3.1: Human *PROP1* mutations.



The timing of initiation and severity of hormonal deficiencies in patients with mutations in *PROP1* is highly variable. The spectrum of gonadotrophin deficiency can range from hypogonadism and lack of puberty, to spontaneous pubertal development with subsequent arrest, and infertility (Parks et al. 1999). Individuals with mutations in *PROP1* exhibit normal ACTH and hence cortisol concentrations in early life but often demonstrate an evolving cortisol deficiency associated with increasing age (Mendonca et al. 1999; Agarwal et al. 2000; Asteria et al. 2000; Pernasetti et al. 2000; Riepe et al. 2001), although it has also been described in a 7 year old patient (Agarwal et al. 2000). The underlying mechanism for cortisol deficiency is unknown, especially as *PROP1* is not expressed in corticotrophs, although it appears to be required for maintenance of the corticotroph population. Various

hypotheses have been postulated such as a gradual attrition of corticotrophs or an expanding pituitary and its subsequent involution (Asteria et al. 2000), although there appears to be no correlation between involution of the pituitary gland and development of ACTH deficiency.

Pituitary morphology in patients with mutations in *PROP1* is variable. Most individual reports have documented a normal pituitary stalk and posterior lobe, with a small or normal anterior pituitary on MR scanning. However, in some cases, an enlarged anterior pituitary has also been reported (Wu et al. 1998; Mendonca et al. 1999; Vallette-Kasic et al. 2001). Longitudinal analyses of anterior pituitary size have revealed that a significant number of patients demonstrate pituitary enlargement in early childhood with subsequent involution in older patients (Riepe et al. 2001; Voutetakis et al. 2004). Pituitary enlargement consists of a mass lesion interposed between the anterior and posterior lobes, possibly originating from the intermediate lobe (Voutetakis et al. 2004).

To date, the underlying mechanism leading to pituitary gland enlargement remains unknown. The only biopsy report of the "tumour" was non-specific with presence of amorphous material, no signs of apoptosis and no recognisable cells. Prolonged expression of *HESX1* or *LHX3* and hence of undifferentiated precursor cells that remain viable for a longer time have been implicated (Sornson et al. 1996). A recent report suggesting that *Prop1*-deficient fetal mouse pituitary retains mutant cells in the perilumenal area of Rathke's pouch that fail to differentiate and exhibit enhanced apoptosis and reduced proliferation may be a possible explanation (Ward et al. 2005).

3.3.2.2 Mutations in POU1F1

The human *POUIFI* gene has been localised to chromosome 3p11 and consists of 6 exons spanning 17Kb. It encodes a 291 amino acid protein with a molecular mass of 33kD. The protein has three functional domains: a transactivation domain, a POU specific domain and a POU homeodomain. The POU specific and POU homeodomains are both critical for high affinity DNA-binding on GH and prolactin promoters.

The first mutation within *POUIFI* was identified by Tatsumi et al in a child with GH, prolactin and profound TSH deficiency (Tatsumi et al. 1992). The patient was homozygous for a nonsense mutation in *POU1F1* resulting in a truncated protein of 171 amino acids. Since then, a total of 27 mutations within *POUIFI* have been described [22 recessive, five dominant] in over 60 patients, all with a broadly similar phenotype of GH, TSH and prolactin deficiency [Figure 3.2] (Cohen and Radovick 2002). Although the majority of mutations within *POU1F1* are recessive, the heterozygous point mutation R271W appears to be a "hotspot" for mutations within *POUIFI* (Cohen et al. 1995), and has been identified in several unrelated patients of different ethnic backgrounds (Ohta et al. 1992; Radovick et al. 1992; Okamoto et al. 1994; Holl et al. 1997; Aarskog et al. 1997; Ward et al. 1998; Rodrigues Martineli et al. 1998).

The spectrum of hormone deficiency varies considerably in patients with mutations within *POUIFI.* MR imaging demonstrates a small or normal anterior pituitary with no other extra-pituitary abnormalities. Deficiency of GH, prolactin and TSH is more profound in patients harbouring mutations in *POU1F1* than in patients with *PROP1* mutations [Table 3.4].

Figure 3.2: Human *POU1F1* mutations

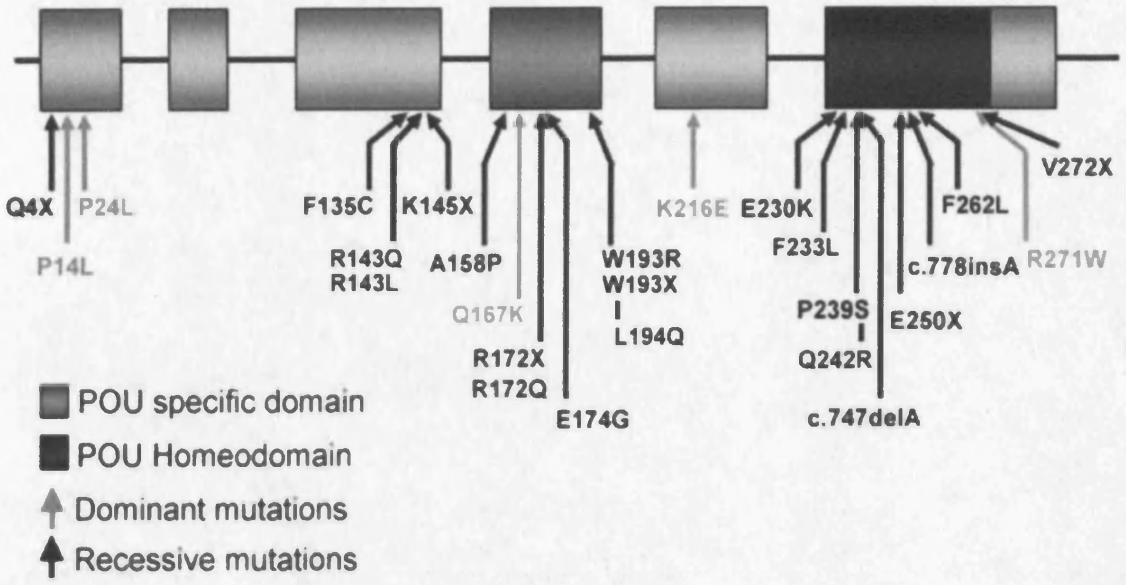


Table 3.4: Differences in phenotype due to mutations in *POU1F1*, *PROP1* and *LHX3*.

Phenotype	***PROP1***	***POU1F1***	***LHX3***
Presentation	Delayed	Congenital	Congenital
GH	Deficient	Deficient	Deficient
TSH	Deficient	Deficient	Deficient
Prolactin	Deficient	Deficient	Deficient
LH, FSH	Deficient	Normal	Deficient
ACTH	May evolve	Normal	Normal
Pituitary size	H, N, E	H, N	H, N, E
Extra-pituitary phenotype	Nil	Nil	Short cervical spine

H hypoplastic; N normal; E enlarged

3.3.3 Syndromic CPHD

3.3.3.1 Septo-optic dysplasia

Septo-optic dysplasia [SOD] is a rare congenital heterogeneous anomaly with a prevalence ranging from 6.3-10.9 per 100,000 (Tornqvist et al. 2002; Patel et al. 2006). The condition is defined by presence of any two of three features: midline forebrain defects [MFD], optic nerve hypoplasia [ONH] and hypopituitarism. Approximately 30% of patients with SOD manifest the complete clinical triad, 62% of patients have some degree of hypopituitarism, and 60% have an absent septum pellucidum (Arslanian et al. 1984; Morishima and Aranoff 1986). The condition is equally prevalent in males and females. ONH may be unilateral or bilateral; the latter is more common.

Pituitary dysfunction may manifest as endocrine deficits varying from IGHD to CPHD. Decrease in growth rate as a result of GHD is often the commonest feature, with hypoglycaemia, polyuria and polydipsia being less common. Either sexual precocity or failure to develop in puberty may occur. Abnormal hypothalamic function with diabetes insipidus [DI] may be a feature. The endocrinopathy may evolve with a progressive loss of endocrine function over time. Gonadotrophin secretion may be retained in the face of other pituitary hormone deficiencies (Nanduri and Stanhope 1999). Commencement of GH treatment in patients with SOD may be associated with accelerated pubertal maturation.

Neurological deficits range from global retardation to focal deficits such as epilepsy or hemiparesis. Other neuroanatomical abnormalities can include a cavum septum pellucidum, cerebellar hypoplasia, schizencephaly and aplasia of the fornix. An association between

SOD and other congenital anomalies such as digital abnormalities has been reported (Pagon and Stephan 1984; Orrico et al. 2002; Harrison et al. 2004).

Both genetic and environmental factors have been implicated in the etiology of the condition (Wales and Quarrell 1996; Rainbow et al. 2005). As forebrain and pituitary development occurs as early as 3-6 weeks gestation in the human embryo, any insult at this critical stage of development could account for the features of SOD. There has been an increased understanding of the genetic basis of this condition led by the discovery of *Hesx1*. *HESX1* maps to chromosome 3p21.1 – 3p21.2, and its coding region spans 1.7Kb with a highly conserved genomic organization consisting of four coding exons. The 185-amino acid frame is highly conserved compared with the mouse. The first homozygous missense mutation [Arg160Cys] was found in the homeobox of *HESX1* in two siblings with SOD (Dattani et al. 1998). The overall frequency of *HESX1* mutations in SOD has, however, been low suggesting that mutations in other known or unknown genes may contribute to this complex disorder.

Environmental agents such as viral infections, vascular or degenerative changes and exposure to alcohol or drugs have also been implicated in the etiology of SOD. The condition presents more commonly in children born to younger mothers and clusters in geographical areas with a high frequency of teenage pregnancies (Murray et al. 2005; Patel et al. 2006).

3.3.3.2 Holoprosencephaly

Abnormal cleavage of the forebrain leads to holoprosencephaly [HPE]. Three types have been identified: alobar, semilobar and lobar. The condition is also associated with other anomalies such as nasal and ocular defects, abnormalities of the olfactory nerves and bulbs, corpus callosum, hypothalamus and pituitary gland. Phenotypes can be highly variable and the pituitary abnormality most commonly associated with HPE is DI, although anterior pituitary hormone deficiencies may be associated with the condition.

Major advances have recently been made in understanding the aetiology of this condition. At least twelve chromosomal regions on eleven chromosomes contain genes implicated in HPE. Autosomal recessive and dominant forms of the condition have been described. Mutations in various genes such as *SHH*, *ZIC2*, *TG1F* and *SIX3* have now been implicated in this condition. Three *GLI* genes have been implicated in the mediation of SHH signals. Heterozygous mutations within *GLI2* were identified in seven of 390 patients with HPE (Roessler et al. 2003). The phenotype and penetrance was variable, with the parent carrying a mutation but showing no obvious phenotype in some cases. In all affected patients, pituitary gland function was abnormal, accompanied by variable craniofacial abnormalities.

Other features included post-axial polydactyly, single nares, single central incisor and partial agenesis of the corpus callosum.

3.3.3.3 Neck abnormalities

Homozygous mutations in *LHX3* have currently been identified in five patients from three unrelated consanguineous families (Netchine et al. 2000; Sobrier et al. 2004; Bhangoo et al. 2006). Patients presented with an endocrine phenotype similar to that observed in individuals with mutations in *PROP1* with a deficit in all anterior pituitary hormones except ACTH. Hypopituitarism was additionally associated in all patients with a short rigid cervical spine with limited head rotation and trunk movement. Pituitary morphology on MR scanning was variable in these patients, as with mutations in *PROP1*, ranging from a small to a markedly enlarged anterior pituitary not evident in a previous MR scan, to a recent report of a hypointense lesion with a "microadenoma" (Bhangoo et al. 2006).

3.3.3.4 Cerebellar Abnormalities

Probands with a mutation within *LHX4,* only reported in one three generation family, presented with GH, TSH and ACTH deficiency (Machinis et al. 2001). MR imaging revealed anterior pituitary hypoplasia, an undescended posterior pituitary, an absent pituitary stalk, a poorly formed sella and pointed cerebellar tonsils, suggesting that *LHX4* tightly coordinates brain development and skull shape. Haploinsufficiency of *LHX4* results in defective regulation of *POU1F1* and downstream activation of *GH1* expression, providing a mechanism at the molecular level in patients with mutations in *LHX4* (Machinis and Amselem 2005). However, ACTH deficiency observed in these patients cannot be explained by this mechanism suggesting that other *LHX4*-dependent pathways may exist independent of *POU1F1* in the developing pituitary gland.

3.3.3.5 Hypopituitarism and mental retardation

A number of pedigrees have been described with X-linked hypopituitarism involving duplications of Xq26-q27 (Hamel et al. 1996; Hol et al. 2000; Solomon et al. 2002). The phenotype is that of variable mental retardation and hypopituitarism. All duplications encompass the *SOX3* gene. Further implication of *SOX3* in hypopituitarism comes from identification of patients with hypopituitarism and an expansion of a polyalanine tract within the gene (Laumonnier et al. 2002).

3.3.3.6 Rieger Syndrome

Mutations within *PITX2* or *RIEG* are associated with Rieger syndrome, an autosomal dominant condition with anomalies of the anterior chamber of the eye, dental hypoplasia, protuberant umbilicus, mental retardation and variable pituitary abnormalities in humans. All mutations identified within *PITX2* to date are heterozygous, affecting the homeodomain of the gene, although none of the patients with these mutations presented with pituitary hormone deficiencies (Cohen and Radovick 2002).

3.3.3.7 Other Midline Abnormalities

Other conditions associated with hypopituitarism include Pallister-Hall syndrome, Fanconi anaemia, solitary single central incisor, cleft lip/palate and ectrodactyly-ectodermal dysplasia-clefting syndrome.

3.4 DIAGNOSIS OF HYPOPITUITARISM

Normal secretion of a hormone is dependent upon the presence of an intact H-P-target gland axis. The axis can be stimulated to test for hormone deficiency and can be suppressed to test for hormonal excess and this forms the basis for several pituitary stimulation tests. Various pharmacological and physiological agents have been used to stimulate the axis and have been extensively studied. Laboratory evaluation of the H-P-target gland axis is performed for the diagnostic evaluation of the patient presenting with symptoms suggestive of pituitary insufficiency or to evaluate patients at risk of developing hypopituitarism such as those with ONH and midline forebrain defects [MFD].

Children with congenital abnormalities of the H-P axis are at risk of serious morbidity. It is important to establish the diagnosis quickly in order to avoid the risk of hypoglycaemia, adrenal crisis, delayed mentation and even mortality. In spite of the serious consequences of this disorder and a multitude of tests available, diagnosis of hypopituitarism in infants and children is still problematic. To add to this, patients have markedly variable phenotypes ranging from IGHD to CPHD, with evolution of hormone deficiency, that makes a precise definition of the underlying abnormality difficult.

Once the diagnosis of hypopituitarism has been made, appropriate mutational screening is an important adjunct to assessment and management of the patient. Not only does genetic screening provide a better understanding of the pathophysiological process but it can also help in management of the patient as observed in those with IGHD type II and those with mutations in *PROP1*.

3.5 OBJECTIVES OF THE PRESENT STUDY

The hypothesis of the present study was that the phenotype of a patient with hypopituitarism is determined by the morphology of hypothalamo-pituitary and midline forebrain structures, which is further influenced by position of the abnormal gene within the developmental cascade.

The major aims of the study were as follows:

1) to define possible structure – function relationships within the hypothalamo-pituitary axis in order to determine risk factors associated with hypopituitarism,

2) to determine if birth size and growth in infancy are influenced by the endocrine and neuroanatomical phenotype,

3) to establish if phenotype correlated with genotype in patients with a molecular genetic cause of hypopituitarism and

4) to ascertain the optimal test for the diagnosis of specific hormone deficiency.

CHAPTER 4

PATIENTS AND METHODOLOGY

4.1 PATIENT RECRUITMENT AND STUDY COHORT

Eligibility criteria for inclusion into the study were: (1) patients with congenital hypopituitarism or (2) patients "at-risk" of hypopituitarism including those with developmental defects of the eye [e.g. optic nerve hypoplasia] or midline facial dysmorphism. Patients with a later confirmation of an alternative diagnosis not related to a pituitary pathology or those with acquired hypopituitarism were excluded from the study.

825 patients with a male: female ratio of 1.8:1 satisfied the eligibility criteria. The majority of patients had sporadic disease with 109 familial cases belonging to 73 unrelated pedigrees. Clinical data and blood [or DNA] of patients recruited in the study, from June 1998 to December 2004, were obtained in two ways depending on the referral centre:

(1) directly from 268 patients (32.5% of patients) referred to the London Centre for Paediatric Endocrinology (LCPE), based at Great Ormond Street Hospital for Children and University College London Hospitals. LCPE is a tertiary referral centre for the diagnosis and management of complicated forms of hypopituitarism. Patients are generally referred by their local Paediatrician or General Practitioner and occasionally, have had a brief initial assessment prior to referral to the endocrinology department,

(2) by the referring doctor of a patient evaluated at another national (232 patients, 28.1%) or international (325 patients, 39.4%) hospital. Clinical data were obtained through a proforma that was supplied to each caring physician, paediatrician or endocrinologist [Appendix I]. The proforma was completed and forwarded along with the patient's blood or DNA sample for mutational screening of pituitary transcription factors.

The study cohort consisted of the following broad endocrine phenotypes:

(1) patients with isolated hormone deficiencies

   a. isolated growth hormone deficiency (IGHD, n=325)
   b. isolated thyrotrophin [TSH] deficiency (n=2)
   c. isolated gonadotrophin deficiency (n=7)
   d. isolated diabetes insipidus (n=7)

(2) patients with combined pituitary hormone deficiency [CPHD, n=399] and

(3) patients "at-risk" of hypopituitarism but normal endocrinology to date

   a. optic nerve hypoplasia (n=68)
   b. microphthalmia / anophthalmia (n=2)
   c. midline facial dysmorphism (n=15)

4.2 STUDY GROUPS

Various subgroups from the above cohort were analysed in order to achieve the objectives.

(1) Determine structural abnormalities on neuroimaging associated with hypopituitarism:

The endocrinology and findings on magnetic resonance [MR] imaging of 170 of 268 patients referred to a single endocrine centre [LCPE] were retrospectively analysed in order to identify structural abnormalities associated with hypopituitarism. Patients were selected if they had undergone baseline pituitary function testing and MR imaging at a single centre to exclude inter-observer variation. As MR imaging and / or endocrine tests were not available in 98 patients, they were excluded from analysis.

(2) Determine factors influencing birth size and early growth:

Length, weight and body mass index of patients from birth to 2 years of age or onset of GH treatment (whichever was earlier), with congenital GHD, isolated or as CPHD, were analysed retrospectively in order to ascertain if birth size and postnatal growth were influenced by the severity of the endocrine and neuroanatomical phenotype. Patients were selected if sufficient growth data (at least three data points) from birth to 2 years of age, from a single endocrine centre [LCPE] were available. 44 of 268 patients from the study cohort met the selection criteria.

(3) Determine genotype-phenotype correlation:

Based on studies in mice and reported mutations in humans, patients were selected for appropriate mutational screening [Table 2.1]. All patients were screened for mutations in *HESX1* given the phenotypic variability associated with these mutations. Patients with IGHD [without developmental defects of the eye or midline structures] were screened for mutations in *GH1, GHRHR* and selected patients for *PROP1, LHX3, LHX4* and *POU1F1*. Patients with CPHD [without developmental defects of the eye and midline structures] were screened for mutations in *POU1F1*, *PROP1*, *LHX3* and *LHX4* depending on the hormonal phenotype. Patients with a X-linked inheritance of hypopituitarism were screened for *SOX3* mutations.

Table 4.1: Mutational screening of appropriate candidate genes within study cohort.

Gene	Number of patients screened
HESX1	825
PROP1	233
LHX3	233
LHX4	233
POU1F1	129
GH1	103
GHRHR	103
SOX3	76

(4) Determine the optimal test for specific hormone deficiency:

As tests performed for the diagnosis of GHD have been extensively studied and some of the controversies surrounding its diagnosis resolved by guidelines set by the Growth Hormone Research society and National Institute for Clinical Excellence, only tests performed for the diagnosis of other anterior pituitary hormone deficiencies were analysed.

a. **Thyrotrophin [TSH] deficiency:** Unstimulated serum thyroxine and TSH concentrations in patients investigated at a single endocrine centre [LCPE] with central hypothyroidism [CH, n=54] were analysed retrospectively. In order to determine whether TSH releasing hormone [TRH] tests have a role to play in the diagnosis and in differentiating between "pituitary" and "hypothalamic" CH, TRH-stimulated TSH responses were analysed in 30 of 54 patients.

b. **Corticotrophin [ACTH] deficiency:** To assess the usefulness of the short Synacthen test [SST] as a marker of endogenous cortisol secretion, serum cortisol concentrations obtained after the SST were compared prospectively with those obtained on spontaneous secretion in 28 consecutive patients investigated for ACTH deficiency at a single endocrine centre [LCPE] from March 2002 to June 2004.

c. **Gonadotrophin deficiency:** Peak serum follicle stimulating hormone [FSH] and luteinising hormone [LH] concentrations in 30 consecutive patients, at a single endocrine centre [LCPE], who underwent a LH releasing hormone [LHRH] stimulation test within 18 months of birth, were prospectively analysed to ascertain usefulness of the test in infancy and assess gender-specific responses.

4.3 ASSESSMENT OF PATIENTS REFERRED DIRECTLY TO THE LONDON CENTRE FOR PAEDIATRIC ENDOCRINOLOGY [LCPE]

4.3.1 Clinical Evaluation

268 patients were referred to LCPE directly. Parents or patients were given the information sheet [Appendix II] prior to obtaining an informed written consent for participation in the study and obtaining a blood sample for genomic analysis [Appendix III]. Ethical committee approval was obtained from the Institute of Child Health Research and Ethics Committee. Clinical information obtained from the parent and from admission notes included birth details [antenatal history, mode of delivery, gestation, history of perinatal complications], family details [parental ages and heights, relevant family history], age at puberty and final adult height if relevant. Birth size [height and weight] measurements were obtained from individual patient held records. Data were recorded on to the proforma [Appendix I].

4.3.2 Endocrine Evaluation

4.3.2.1 Pituitary function testing

Dynamic tests to assess pituitary function were performed in day care planned investigation units by nurses based on standardized protocols shown in Appendix IV. Children between 0-12 years of age were admitted to the Programmed Investigation Unit at Great Ormond Street Hospital for Children and those over 12 years of age were admitted to the Adolescent Unit at University College London Hospitals. Tests performed and "cut-off" levels used for diagnosis of individual pituitary hormone deficiency at LCPE are shown in Table 2.2. Blood samples from patients were also collected at the same time for genetic analysis.

Table 2.2: Investigations performed to assess pituitary function at the London Centre for Paediatric Endocrinology (LCPE)

Hormone	Primary Test	Diagnostic "cut-off" value	Alternative test
Growth Hormone	- Glucagon provocation (< 12 years) - Insulin induced hypoglycaemia (> 12 years) - Serum IGF1, IGFBP3	- Peak GH <20 mU/L - Peak GH <20 mU/L - < 2SD below mean for age and sex	- Overnight GH profile
Thyrotrophin	- Free thyroxine (FT4) or total thyroxine (TT4) - Serum TSH	- FT4 <12.0 pmol/L or TT4 <65 nmol/L with serum TSH concentration <5 mU/L.	- TRH stimulation
Corticotrophin	- Standard Synacthen test - Glucagon provocation (< 12 years) - Insulin induced hypoglycaemia (> 12 years)	Peak serum cortisol < 540 nmol/L	- 24 hour cortisol profiles - Low dose Synacthen test - Serum ACTH
Gonadotrophins	- LHRH test (if age appropriate)	Absent LH and FSH response	
Prolactin	- Serum prolactin	- < 100 mU/L (elevated serum prolactin > 500 mU/l)	- TRH test
Arginine Vasopressin	- Early morning plasma and urine osmolalities - Water deprivation and DDAVP tests	- inappropriate osmolalities that respond to DDAVP treatment	

IGF1, insulin like growth factor 1; IGFBP3, insulin like growth factor binding protein 3

4.3.2.2 Measurement of plasma hormone concentration

Hormones and metabolites were measured in the biochemistry departments at LCPE as a service provision. Unless otherwise stated, hormone concentrations are expressed in SI units.

Samples for GH, FT4 or TT4, TSH, cortisol, LH, FSH and prolactin were analysed on the IMMULITE 2000 analyser as a chemiluminescent immunometric analysis (Diagnostic Products, Gwynedd, UK). IMMULITE 2000 is a continuous random-access analyser with a processing throughput of 200 tests per hour and has been designed specifically for optimum efficiency and consolidation in medium - and high-volume laboratories. The IMMULITE 2000 software package offers information management from remote test ordering to sophisticated analysis of results. Workflow enhancing features such as primary tube sampling, automatic reflex testing and on-board dilution have been incorporated for optimum efficiency. The system's open architecture also accommodates interfacing to laboratory automation systems. The IMMULITE system uses assay-specific antibody or antigen coated plastic beads as the solid phase, alkaline phosphatase-labelled reagent and a chemiluminescent enzyme substrate. The bead is housed in a proprietary test unit. This unit serves as the reaction vessel for the immune reaction, incubation, washing process and signal development. The IMMULITE system automates the entire assay process. After incubating the patient sample with the alkaline phosphatase reagent, the liquid reaction mixture in the test unit is rapidly separated from the bead, the bead is washed and the test unit is spun at a high speed in its vertical axis.

The fluid contents (sample, reagent and wash solution) are transferred to a coaxial waste chamber in the Test Unit. The bead is left with no residual unbound label. The bound label is then quantified with a dioxetane substrate, which produces light. A photo multiplier tube detects light emission and the systems computer generates printed reports for each sample. Performance data for various hormones on the IMMULITE 2000 is shown in Table 4.2.

IGF1 and IGFBP3 were analysed on the Diagnostics Systems Laboratories 5600 ACTIVE and 6600 ACTIVE [Oxon, UK] respectively. Principles of analysis were same for both metabolites. Analyte to be measured was sandwiched between two antibodies. The first antibody is immobilized to the inside wall of the tube and the other is radiolabeled for detection. Both assays are immunoradiometric assays. There are 3 reagents used for IGF1 analysis: IGF1 in buffer, I^{125} labeled anti IGF1 and anti IGF1 immunoglobulin. The assay has a sensitivity value for the lowest limit of detection at 0.80 ng/ml (27 pg/ml). The intra-assay coefficients of variation were 3.4% and 1.5% at concentrations of 9.4 ng/ml and 263.6 ng/ml respectively. The inter-assay coefficients of variation were 8.2% and 3.7% at concentrations of 10.4 ng/ml and 155.9 ng/ml respectively. The reagents used for analysis of IGFBP3 were IGFBP3 in a protein-based buffer and I^{125} labeled goat anti-IGFBP3 polyclonal antibody. Lowest limit of detection was 0.5 ng/ml and intra-assay coefficients of variation were 1.8% and 3.9% at concentrations of 82.73 ng/ml and 7.35 ng/ml respectively. Inter-assay coefficients of variation were 1.9% and 0.6% at concentrations of 76.9 ng/ml and 8.03 ng/ml respectively.

Table 4.3: Performance data for various hormones on the IMMULITE 2000 analyser

Hormone	Concentration	CV		Sensitivity	Antibody	Reagent	Volume of blood	Incubation (cycles)	Calibration range
		Intra-assay	Inter-assay						
GH (mU/L)	7.8 5.1	3.5% 4.2%	6.5% 6.6%	0.03	Murine MC	Rabbit PC	25 µL	1 X 30	Upto 120
TSH (mU/L)	0.021 68	19% 5%	23.8% 5.7%	0.01	Murine MC	Goat PC	75 µL	1 X 30	Upto 75
FSH (U/L)	6.8 103	2.9% 3/1%	4.1% 7.9%	0.1	Murine MC	Murine MC	50 µL	1 X 30	Up to 170
LH (U/L)	0.15 170	13.1% 3.5%	23.9% 7.1%	0.05	Murine MC	Goat PC	75 µL	1 X 30	Up to 200
Prolactin (mU/L)	98 3280	2.2% 2.3%	6.9% 7.9%	3.2	Murine MC	Goat PC	25 µL	1 X 30	Up to 3000
Cortisol (nmol/L)	91.4 859	6.1% 7.4%	8.2% 9.4%	5.5	Rabbit PC	Cortisol	10 µL	1 X 30	28-1380
FT4 (pmol/L)	8.6 65.9	7.5% 5.2%	9% 7.7%	3.9	Murine MC	3 reagents[1]	10 µL	2 X 30	3.9-77.2

GH, growth hormone; TSH, thyrotrophin; FSH, follicle stimulating hormone; LH, luteinising hormone; FT4, free thyroxine; CV, coefficient of variation; MC, monoclonal; PC, polyclonal; [1], 2 reagents - ligand labeled T4 labeled, one reagent- anti-ligand in buffer.

4.3.3 Ophthalmologic Evaluation

Detailed ophthalmologic evaluation for the presence of ONH was carried out by the paediatric ophthalmologists in patients with visual disturbances. Examination by ophthalmologists included an examination of the optic disc by direct and indirect ophthalmoscopy, presence of pigmentary halo, comparison of the optic disc diameter with blood vessels and with the macula. Visual acuity was tested in older children. Where indicated, patients also underwent an electroretinogram and visual evoked responses following fundoscopy to confirm ONH.

4.3.4 Magnetic resonance imaging

Data on neuroanatomy were obtained following MR imaging using a 1.5 Tesla Siemens Magnetom Symphony scanner. T1 and T2 weighted high-resolution slices through the H-P axis (T1 sagital 3mm slices, T1 and T2 coronal 3mm slices) were obtained before and following gadolinium contrast. The Siemens Magnetom Symphony scanner is an advanced high speed, short bore imaging system. It has a short [1.6 meter] length and a 60 cm inside diameter with a flared 120 cm opening. Details noted included anterior pituitary morphology, position of the posterior pituitary signal, presence and morphology of the optic nerves, optic chiasm, pituitary stalk and midline structures such as the septum pellucidum and corpus callosum and were reported by a single paediatric neuroradiologist.

4.4 MOLECULAR ANALYSIS

Genomic DNA was extracted from peripheral lymphocytes by salt extraction using standard methods. Coding and splice donor/acceptor regions of the relevant candidate genes were amplified by polymerase chain reaction using flanking primer pairs.

HESX1, *LHX4*, *GHRHR* and *GH1* were analysed using a heteroduplex protocol on the MegaBACE 1000 (Amersham Biosciences, Buckinghamshire, UK), according to manufacturer's recommendations. *POU1F1* and *PROP1* were analysed using single stranded conformational polymorphism analysis [CleanGel SSCP kit, Amersham Biosciences, Buckinghamshire, UK]. *LHX3* was analysed using dHPLC WAVE [Transgenomic Ltd., Cheshire, UK], optimized and run according to manufacturers' instructions. Duplications of the *SOX3* locus were analysed by interphase Flourescent In Situ Hybridization using a human genomic BAC clone, bA51C14, containing the *SOX3* gene. Candidates that screened positive were directly sequenced using BigDye v3.1 [Applied Biosystems, Warrington, UK) and analysed on the MegaBACE1000 using Sequencher (Ann Arbor, Michigan, USA]. The mutations were confirmed by repeat polymerase chain reaction and direct sequencing in both orientations.

Mutational screening for genes causing isolated hormone deficiencies other than IGHD or for *GLI2, GLI3, PITX1* and *SOX2* was not included in the present study.

4.5 STATISTICAL ANALYSIS

Data manipulation was performed using the Microsoft ® Excel 2000 spreadsheet [Microsoft Corp., USA] and statistical analysis performed using the SPSS 13 for Windows statistical package [SPSS Inc., Illinois, USA]. A probability [p] value of <0.05 was regarded as significant.

Data are presented in this study as mean ± standard deviation (SD). In situations where the distribution was highly skewed, the median was used to describe the overall measure of the center, with the range quoted.

The height and weight of patients were measured at the time of initial presentation and subsequently by a single auxologist with an intra-observer co-efficient of variation of 0.15% and 0.1% at lengths of 75 cms and 100 cms respectively. Height, weight and body mass index [BMI, weight in kilograms ÷ (length in meters)^2] were expressed as standard deviation scores [SDS] to allow comparisons to be made between sexes and at different ages using current reference data (Freeman et al. 1995).

A statistical significance test measures the strength of evidence, which the data sample supplies for, or against, some proposition of interest. This proposition is known as a "null hypothesis" since it usually relates to there being no difference between groups or "a no effect" of treatment. The unpaired-samples student t-test was applied to ascertain statistically significant differences between two groups of parametric data. It computes differences between values of two variables for each case and tests the hypothesis whether the samples are compatible with a population difference in means of zero.

For 3 or more groups of parametric data, analysis was performed using the One-way Analysis of Variance [ANOVA] to test the null hypothesis that there was no significant difference between groups. The One-Way ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable by a single factor [independent] variable. This technique is an extension of the two-sample t-test. The ANOVA test determines whether the means of groups differ by more than could be expected by chance. A probability value <0.05 shows that at least one of the group samples has a mean value that was unlikely to have risen by chance if the population that the groups were randomly sampled from had identical means.

The ANNOVA was followed by Least Significant Difference [LSD] Post Hoc test to determine that differences exist among means. Examination of group confidence intervals indicated which of the groups was different.

The one-way ANOVA Contrast was used to partition the between-groups sums of squares into trend components and to specify a priori contrasts. The test partitions the between-groups sums of squares into trend components in order to test for a trend of the dependent variable across the ordered levels of the factor variable. Employing user-specified a priori contrasts were then tested by the t-statistic. The order of the coefficients corresponded to the ascending order of the category values of the factor variable. The first coefficient on the list corresponded to the lowest group value of the factor variable and the last coefficient corresponded to the highest value. This test was used particularly to compare a variable across different phenotypic states to assess the level of severity.

When the data were categoric, it was summarized as the proportions [or percentages] of the total falling into each category. When comparing the proportions having a feature in two different groups, a 2X2 frequency table followed by the Pearson's Chi-square test was used to analyse the data. Odds ratio were used as an estimate of relative risk when the occurrence of the factor was rare.

In order to quantify the relationship between two continuous variables, a scatter plot of the data was first produced. The raw data were examined and variables, which had skewed distributions, underwent logarithmic transformation before analysis to normalize the distributions and reduce skewness on correlations. Correlation analysis was performed using Pearson's correlation coefficient with a null hypothesis value of zero or no linear association.

Test performance was assessed using principles outlined by Sox (Sox, Jr. 1986). Performance of a test when compared to another or a "gold standard" test can be considered under two circumstances assuming that both tests are performed on different days and are independent:

(1) the "rule-in" scenario, when both tests need to be positive for a diagnosis maximising specificity but possibly missing many treatable individuals,

(2) the "rule-out" scenario, when both tests need to be negative to exclude abnormality possibly resulting in misdiagnoses by falsely labelling normal children.

Given the potentially fatal consequences of missing a diagnosis, the "rule-out" approach was considered safer although clinically this would place an onus on a clinician to reassess all individuals at a later stage.

CHAPTER 5

STRUCTURAL BRAIN ABNORMALITIES ASSOCIATED WITH HYPOPITUITARISM

5.1. INTRODUCTION

The spectrum of hypopituitarism [growth hormone deficiency (GHD) in isolation or as combined pituitary hormone deficiency (CPHD)] is highly variable, both in its clinical phenotype and abnormalities within the hypothalamo-pituitary [H-P] axis on magnetic resonance [MR] imaging. With the advent of MR imaging technology, it is now possible to relate neuroanatomy in patients with hormone deficiencies to the likely underlying pathophysiological process. The causal relationship between gestational-perinatal complications and hypopituitarism due to traumatic injury to the pituitary stalk has been suggested previously (Craft et al. 1980; Fujisawa et al. 1987; Kikuchi et al. 1988; Triulzi et al. 1994). However, it is difficult to differentiate whether perinatal insults such as breech delivery lead to hypopituitarism by traumatic disruption of the pituitary stalk or whether hypopituitarism with structural H-P defects result in an increased prevalence of perinatal complications. Identification of structural abnormalities on imaging in patients with mutations in pituitary transcription factors suggests that the latter is more likely. MR imaging abnormalities in patients with mutations in *PROP1* and *HESX1* are more variable as compared with mutations in *GH1*, *GHRHR*, *PIT1*, *LHX3 and LHX4,* although the number of patients reported to date with mutations in *LHX3* and *LHX4* are small. Overall, incidence of mutations within genes known to be implicated in hypopituitary states has

been low indicating that mutations in other unknown transcription factors may be responsible (Sloop et al. 2000; Osorio et al. 2002).

GHD with midline forebrain defects [MFD] such as those observed within the phenotypic spectrum of septo-optic dysplasia [SOD] and holoprosencephaly [HPE] are more likely to present with hypothalamic abnormalities leading to diabetes insipidus, precocious puberty and a propensity for weight gain in later life (Hoyt et al. 1970; Huseman et al. 1978; Arslanian et al. 1984; Izenberg et al. 1984; Roessmann et al. 1987; Hanna et al. 1989; Yukizane et al. 1990; Fitz 1994). Abnormalities on imaging such as an undescended posterior pituitary, absent septum pellucidum and cerebral hemispheric abnormalities in patients with optic nerve hypoplasia [ONH] have been suggested to increase the prevalence of endocrine abnormalities and neurodevelopmental deficits (Miyamoto et al. 2001; Lange et al. 2003; Birkebaek et al. 2004). This can be particularly useful as identifying these structural abnormalities in patients diagnosed with ONH may suggest the possibility of hypopituitarism and a lower threshold for investigation. Additionally, hypopituitarism in ONH may evolve several years after the initial diagnosis (Stanhope et al. 1984). Neuroimaging has also helped to identify those patients with isolated GHD [IGHD] who continue to be GHD as adults as the finding of an undescended posterior pituitary has been suggested as a specific marker of permanent GHD (Maghnie et al. 2004).

The aim of this part of the study was to assess if there were good structure-function relationships within the H-P axis and to identify abnormalities on MR imaging following gadolinium injection that predict endocrine dysfunction in a large cohort of patients.

5.2 PATIENTS AND METHODS

Patients were recruited into the study if they had undergone full clinical assessment, pituitary function evaluation and MR imaging, at a single endocrine centre [LCPE]. Genomic analysis for appropriate candidate genes [*GH1, HESX1, POU1F1, PROP1, LHX3, LHX4, SOX3]* depending on the phenotype was performed in all patients. Where indicated, the paediatric ophthalmologist evaluated patients with visual problems such as blindness, decreased visual acuity, strabismus or nystagmus. MR imaging was reviewed by a single paediatric neuroradiologist. 137 children with hypopituitarism [Appendix V-1] and 33 children [Appendix V-2] with normal endocrinology, serving as a "control" group, met the selection criteria. The children within the "control" group with normal endocrinology continued in clinical follow up as they had evidence of ONH on clinical examination and might be considered "at-risk" of developing endocrine dysfunction in the future. All individuals within this "control" group had abnormal MR imaging. A number of patients had extra-pituitary abnormalities as shown in Table 5.1.

The study was divided into 3 sections for further analysis:

1. Identify abnormalities on MR imaging predictive of endocrine dysfunction:

Group A: 124 of 137 patients with hypopituitarism who demonstrated abnormal MR imaging [patients 14-137, Appendix V-1]

Group B: "Control" group, 33 children with abnormal MR imaging but normal endocrinology [patients 138-170, Appendix V-2].

2. Identify abnormalities on MR imaging predictive of CPHD over IGHD:

Patients with hypopituitarism [n=137] were selected for this part of the study and divided based on their endocrinology into:

Group C: 44 patients with IGHD [patients 1-5 and 14-52, Appendix V-2]

Group D: 93 patients with CPHD [patients 6-13 and 53-137, Appendix V-2]

3. **Identify abnormalities on MR imaging predictive of endocrine dysfunction in cohort of patients with ONH**

Group E: 51 of 137 hypopituitary patients with ONH [Appendix V-2, patients 46-96]

Group B: "Control" group [n=33] with ONH but normal endocrinology [patients 138-170, Appendix V-3]

Table 5.1: Extra-pituitary abnormalities in study cohort.

Abnormality	**Patient Number**
Eye abnormalities	
Entropion	149
Pigmentary retinal dystrophy	13, 53, 75
Coloboma	52, 168, 170
Unilateral microphthalmia	52, 168
Closed funnel retinal detachments	96
Optic nerve hypoplasia	46-96, 138-170
Systemic abnormalities	
Congenital heart disease	6, 83, 85, 122, 147, 152
Persistent hyperinsulinaemia hypoglycaemia of infancy	55
Cleft lip and/or palate	47, 168
Situs inversus	106
Deafness	76, 77, 93, 152
Anosmia	76
Digital abnormalities	51
Other neuroradiological abnormalities	
Polymicrogyria	122, 139, 142
Cerebral cysts	74, 76, 117, 155
Lipoma rostrum	169
Macrocephaly	60, 104, 110
Craniosynostosis	162

5.3 RESULTS

5.3.1 Identify abnormalities on MR imaging predictive of endocrine dysfunction

The results of MR imaging of 124 patients with hypopituitarism [group A] were compared with the MR imaging of 33 patients with normal endocrinology ["control" group B]. There were no significant differences between the male: female ratio, gestational age or mode of delivery between patients in both groups [Table 5.2, page 121]. 51 of 124 patients in group A had evidence of ONH.

All patients within group A demonstrated at least one structural abnormality of the H-P axis as compared with 76% of group B patients and this difference was statistically highly significant [Table 5.3, page 122]. The risk of an undescended posterior pituitary was significantly greater in patients in group A as compared with group B [$p<0.0001$, odds ratio (OR) 33.05, 95% confidence interval (CI) 4.38, 249.45], as only one patient in group B demonstrated this abnormality. Anterior pituitary hypoplasia was also observed in a significantly greater number of patients in group A as compared with group B [$p=0.0001$, OR 6.78, 95% CI 2.63, 16.98]. Prevalence of an abnormal pituitary stalk was however not significantly different between patients in group A [54%] and group B [48.5%]. The presence of an undescended posterior pituitary [n=46] was significantly [$p=0.0001$, OR 3.69] greater in patients with an abnormal stalk [n=83] as compared to those with a normal stalk [18 of 73]. MFD were, however, observed in a significantly greater number of patients in group B as compared with group A. These differences were statistically significant for abnormalities of the corpus callosum [$p=0.001$, OR 0.27, 95% CI 0.12, 0.62] but not the septum pellucidum.

To summarise, the odds ratios suggest a 33 times greater risk of an undescended posterior pituitary and a 6.7 times greater risk of anterior pituitary hypoplasia in patients with hypopituitarism as compared with those without endocrine abnormalities in a cohort of patients with abnormal neuroimaging.

5.3.2 Identify abnormalities on MR imaging predictive of CPHD over IGHD

The cohort of 137 patients with hypopituitarism was further analysed to identify risk factors on neuroimaging that predict the endocrine spectrum with respect to development of IGHD [n=44, group C] or CPHD [n=93, group D]. There was a 2.1:1 male to female preponderance within the cohort. Although males were more commonly affected within both groups as well, there were no significant gender differences between the 2 groups. The male to female ratio was significantly different between patients with ONH [n=51, 1.1:1] and those without [n=86, 3.3:1].

Patients with CPHD presented significantly earlier than those with IGHD [Table 5.4, page 123]. Within patients with CPHD, GH, TSH, ACTH and gonadotrophin deficiencies were found in 99%, 82%, 78.5% and 69% of patients tested. 18 patients had diabetes insipidus [DI], 14 of whom had ONH. Prevalence of both DI and ACTH deficiency were significantly [$p=0.0001$, $p<0.00001$ respectively] greater in patients with ONH [relative risk 2.5, 4.1 respectively] as compared with those without ONH [relative risk 0.3, 0.5 respectively]. The posterior pituitary was undescended in only 39% of patients [n=7] with DI; it was enlarged in one patient [patient 92] and was normally sited in the remaining 10 patients.

Five patients were found to harbour mutations in pituitary transcription factors and genes. Patients 36 and 40 with IGHD were found to have mutations in *GH1*. The MR scan of patient 36 revealed an empty pituitary sella with extension of chiasmatic cistern into the sella, while that of patient 40 revealed anterior pituitary hypoplasia. Patient 43, with IGHD and MR scan revealing anterior pituitary hypoplasia, an undescended posterior pituitary and a hypoplastic stalk and had a mutation in *HESX1*. Patient 117 with CPHD had a mutation in *PROP1* and an MR scan characterized by a massively enlarged anterior pituitary. Patient 112 with CPHD and anterior pituitary hypoplasia had a mutation in *POU1F1*. These patients are further discussed in Chapter 7 in sections 7.6, 7.3, 7.4 and 7.5 respectively along with a larger cohort of patients with a molecular genetic cause of hypopituitarism.

The MR scan was normal in 11% (n=5) of patients with IGHD and 9% (n=8) of patients with CPHD [Table 5.5, page 124]. All 5 patients with IGHD [patients 1-5] have reached their end of growth, been re-tested with an insulin induced hypoglycaemia test and been found to have IGHD as adults. The presence of anterior pituitary hypoplasia, an undescended posterior pituitary or abnormalities with the septum pellucidum were not significantly [p=0.192, p=0.055, p=0.056 respectively] different between patients with IGHD and CPHD, although the latter two abnormalities were more common in patients with CPHD. However, abnormalities of the corpus callosum and pituitary stalk were both significantly greater in patients with CPHD as compared with IGHD [p=0.008, OR 6.13, 95% CI 1.37, 37.44; p=0.006, OR 2.84, 95% CI 1.33, 6.0 respectively]. Overall, prevalence of any one MFD was most significantly associated with CPHD occurring 5.5 times greater in patients with CPHD as compared with IGHD.

5.3.3 Identify abnormalities on MR imaging predictive of endocrine dysfunction in cohort of patients with ONH

ONH was present in 51 patients with hypopituitarism [group E] and 33 "controls". Male: female ratio [1.5:1] was not significantly different between both groups [Table 5.6, page 125]. Bilateral ONH was observed in the vast majority [n=72, 85.5%] of patients with no significant differences between group E (88%) and group B (82%). Within group E, IGHD was present in only 7 of 51 patients and the majority presented with CPHD [n=44]. Within patients in group E; GH, TSH, ACTH, gonadotrophin and AVP deficiencies were present in 47 [48 tested to date, 98%], 36 [71%], 42 [82%], 14 [34 tested to date, 45%] and 14 [27.5%] patients respectively. 3 patients have as yet not had GH provocation testing due to a normal growth pattern and normal serum IGF1 and IGFBP3 concentrations to date. ONH was confirmed on MR imaging in all patients. 34% of patients had ONH with structural H-P abnormalities, 10% had ONH with MFD and 56% patients had ONH, MFD and structural H-P abnormalities within the entire cohort. Prevalence of anterior pituitary hypoplasia [$p<0.0001$, OR 28.44, 95% CI 3.61, 224.27] and an undescended posterior pituitary was significantly greater [$p=0.0004$, OR 7.64, 95% CI 2.22, 26.31] in patients in group E as compared with group B [Table 5.7, page 126]. However, prevalence of septum pellucidum, corpus callosum or pituitary stalk abnormalities did not significantly differ between both groups. The pituitary stalk was absent in 23 patients and hypoplastic in 10 patients within group E, but no patient in group B demonstrated an absent stalk ($p<0.001$), although it was hypoplastic in 17 patients. All patients in group E demonstrated at least one H-P structural abnormality as compared with 76% of patients in group B and these differences are statistically significant [Table 5.7, page 126].

Table 5.2: Clinical characteristics of patients with hypopituitarism [group A] and normal endocrinology [group B].

	Group A	**Group B**	**Probability value (p)**
Number of patients	124	33	
Male: female ratio	2.1:1	1.2:1	0.158
Gestational age (weeks)	38.8 ± 3.1	37.9 ± 3.6	0.243
Assisted deliveries	34%	25%	0.879
Endocrinology			
GH deficiency	119/120 tested (99%)	0	
TSH deficiency	70 (56.5%)	0	
ACTH deficiency	70 (56.5%)	0	
Gonadotrophin deficiency	37/93 tested (40%)	0	
Diabetes insipidus	16 (13%)	0	
Serum prolactin (mU/L)	506 ± 569	376 ± 448	0.259

Table 5.3: Differences in magnetic resonance imaging in patients with hypopituitarism [group A] and normal endocrinology [group B].

	Group A	Group B	Probability value (p)	Odds ratio (Group A/Group B)	95% CI	
					Lower	Upper
Number of patients	124	33				
Undescended posterior pituitary	63 (51%)	1 (3%)	<0.0001	33.05	4.38	249.45
Anterior pituitary hypoplasia	109 (88%)	20 (61%)	0.0001	6.68	2.63	16.98
Absent / hypoplastic stalk	67 (54%)	16 (48.5%)	0.797	1.11	0.51	2.39
Absent septum pellucidum	25 (20%)	13 (39%)	0.222	0.39	0.17	0.89
Absent / hypoplastic corpus callosum	23 (18.5%)	15 (45.5%)	0.001	0.27	0.12	0.62
Any one hypothalamo-pituitary defect	124 (100%)	25 (76%)	<0.0001			
Any one midline forebrain defect	42 (34%)	20 (61%)	0.005	0.33	0.33	0.73

Table 5.4: Clinical characteristics of patients with isolated growth hormone deficiency [IGHD, group C] and combined pituitary hormone deficiency [CPHD, group D].

	Group C	Group D	Probability value (p)
Number of patients	44	93	
Male: female ratio	2.7:1	1.9:1	0.404
Age at diagnosis (years)	5.4 ± 3.4	2.7 ± 3.3	<0.0001
Gestational age (weeks)	39 ± 2	38 ± 4	0.309
Assisted deliveries	32%	34%	0.584
Optic nerve hypoplasia	7 (16%)	44 (47%)	0.0004
Endocrinology			
GH deficiency	44 (100%)	85/86 tested (99%)	
TSH deficiency	0	76 (82%)	
ACTH deficiency	0	73 (78.5%)	
Gonadotrophin deficiency	0	40/58 tested (69%)	
Diabetes insipidus	0	18 (19.4%)	
Serum prolactin (mU/L)	314 ± 272	579 ± 622	0.024

Table 5.5: Differences in magnetic resonance [MR] imaging in patients with isolated growth hormone deficiency [IGHD, group C] and combined pituitary hormone deficiency [CPHD, group D].

	Group C	Group D	Probability value (p)	Odds ratio (Group D/Group C)	95% CI	
					Lower	Upper
Number of patients	44	93				
Normal MR scan	5 (11%)	8 (9%)	0.607			
Absent / hypoplastic corpus callosum	2 (4.5%)	21 (23%)	0.008	6.125	1.367	37.436
Absent / hypoplastic stalk	14 (32%)	53 (57%)	0.006	2.839	1.334	6.045
Absent septum pellucidum	4 (9%)	21 (23%)	0.056	2.917	0.936	9.092
Undescended posterior pituitary	15 (34%)	48 (52%)	0.055	2.062	0.980	4.341
Anterior pituitary hypoplasia	38 (86%)	71 (76%)	0.192	0.499	0.173	1.439
Any one hypothalamo-pituitary defect	39 (89%)	85 (91%)	0.607	1.362	0.419	4.433
Any one midline forebrain defect	5 (11%)	37 (40%)	0.001	5.514	1.859	14.284

Table 5.6: Clinical characteristics in patients with optic nerve hypoplasia: group E, patients with hypopituitarism; group B, control group with normal endocrinology.

	Group E	Group B	Probability value (p)
Number of patients	51	33	
Male: female ratio	1.1:1	1.2:1	0.886
Age at diagnosis (years)	2.6 ± 3.0	1.8 ± 1.8	0.327
Gestational age (weeks)	39 ± 3.5	38 ± 3.6	0.399
Assisted deliveries	37.5%	25%	0.809
Bilateral ONH	45 (88%)	27 (82%)	0.412
Endocrinology			
GH deficiency	47/48 tested (98%)	0	
TSH deficiency	36 (71%)	0	
ACTH deficiency	42 (82%)	0	
Gonadotrophin deficiency	14/31 (45%)	0	
Diabetes insipidus	14 (27.5%)	0	
Serum prolactin (mU/L)	655 ± 626	376 ± 448	0.044

Table 5.7: Differences in magnetic resonance [MR] imaging in patients with optic nerve hypoplasia: group E, patients with hypopituitarism; group B, control group with normal endocrinology.

	Group E	Group B	Probability value (p)	Odds ratio (Group E/Group B)	95% CI	
					Lower	Upper
Number of patients	51	33				
Undescended posterior pituitary	24 (47%)	1 (3%)	<0.0001	28.444	3.608	224.272
Anterior pituitary hypoplasia	47 (92%)	20 (61%)	0.0004	7.638	2.217	26.308
Absent / hypoplastic stalk	33 (65%)	16 (48.5%)	0.229	1.725	0.707	4.211
Absent septum pellucidum	23 (45%)	13 (39%)	0.606	1.264	0.519	3.077
Absent / hypoplastic corpus callosum	18 (35%)	15 (45.5%)	0.352	0.655	0.268	1.600
Any one hypothalamo-pituitary defect	51 (100%)	25 (76%)	0.0002			
Any one midline forebrain defect	35 (69%)	20 (61%)	0.450	1.422	0.569	3.550

5.4 DISCUSSION

MR scanning was normal in only 13 of 137 patients with hypopituitarism. Abnormalities on MR imaging were not only restricted to patients with endocrinopathies but were also observed in patients with ONH without any endocrine dysfunction [group B], comparable with other reports (Stanhope et al. 1984; Traggiai and Stanhope 2002; Birkebaek et al. 2003). MR abnormalities noted included anterior pituitary hypoplasia [78%], an undescended posterior pituitary [38%], an abnormal [absent/hypoplastic] stalk [49%], an absent septum pellucidum [22%] and an abnormal [absent/hypoplastic] corpus callosum [22%]. Patients with ONH and normal endocrinology served as a "control" group to assess abnormalities on MR imaging associated with hypopituitarism. Although this is not ideal, it is not easy to identify a true control group from the general population for MR imaging and endocrine tests. All patients with ONH within this study demonstrated abnormalities of the H-P or MFS or both. This may suggest a selection-bias as the prevalence and incidence of MR abnormalities and endocrine problems depends on the referral rate from ophthalmologic and/or radiological departments.

In patients with abnormal imaging, there was a significantly greater prevalence of structural abnormalities within the H-P axis as opposed to MFD in patients with hypopituitarism on the whole [group A] and within the subgroup of patients with ONH [group E], as compared with controls with normal endocrinology [group B], in keeping with recent reports (Traggiai and Stanhope 2002; Birkebaek et al. 2003). Anterior pituitary hypoplasia occurred 6.7 and 7.6 times more commonly in patients with endocrine dysfunction in the large cohort of patients with hypopituitarism [group A] and in the subgroup of patients with ONH [group E] as compared with "controls". An undescended posterior pituitary was most likely to be

associated with endocrine dysfunction as it occurred 28-33 times more commonly in patients with hypopituitarism as compared with those with normal endocrinology.

60% of the patients with ONH within this study had evidence of endocrinopathies in keeping with other studies (Acers 1981; Costin and Murphree 1985; Zeki et al. 1992; Brodsky and Glasier 1993; Sorkin et al. 1996; Birkebaek et al. 2003). The frequency of abnormalities of septum pellucidum (43%), posterior pituitary (30%) and pituitary stalk (59%) in patients with ONH were also in keeping with other series (Brodsky and Glasier 1993; Birkebaek et al. 2003). 61% of ONH patients without endocrine dysfunction [group B] demonstrated anterior pituitary hypoplasia. As findings from this study indicate that anterior pituitary hypoplasia is associated with endocrine dysfunction, continued assessment and follow-up of these patients is necessary as evolving hypopituitarism is known in patients with ONH (Stanhope et al. 1984; Traggiai and Stanhope 2002).

Several previous studies have attempted to suggest risk factors in patients with hypopituitarism that help predict the development of CPHD over IGHD with conflicting results (Maghnie et al. 1999; Bozzola et al. 2000; Osorio et al. 2002; Maghnie et al. 2003). Evidence provided in this study suggests an increased severity of abnormalities on MR imaging in patients with CPHD. Although the association of an undescended posterior pituitary was two times greater in patients with CPHD as compared with IGHD, in keeping with similar observations from earlier reports (Pellini et al. 1990; Chen et al. 1999; Bozzola et al. 2000), the differences in prevalence of this abnormality were not statistically significant between groups. The association between abnormalities of the corpus callosum and of the pituitary stalk with CPHD was 6.13 times and 2.84 times greater than with IGHD

[p=0.008, OR 6.13, 95% CI 1.37, 37.44]. Anterior pituitary hypoplasia however was noted in a slightly greater number of patients with IGHD as compared with CPHD although differences were not statistically significant. Overall, MFD were 5.5 times more prevalent in patients with CPHD as compared with IGHD. In contrast to a recent report by Osorio et al, 2 patients with IGHD without ONH also demonstrated MFD (Osorio et al. 2002).

Birth trauma has been implicated in the development of pituitary stalk abnormalities leading to pituitary dysfunction (Craft et al. 1980). Earlier reports also suggest that transection of the pituitary stalk leads to an "ectopic" posterior pituitary (Campbell and Harris 1957; el Gammal et al. 1989; Osorio et al. 2002). The presence of an undescended posterior pituitary was observed with a relative risk of 1.76 in patients with an absent or hypoplastic pituitary stalk as compared with that of 0.48 in patients with a normal stalk with statistically significant [p=0.0001] differences. The incidence of assisted deliveries was not however significantly different in patients with hypopituitarism [37.5%] as compared with those with normal endocrinology [25%], both of whom demonstrated pituitary stalk abnormalities, suggesting that stalk abnormalities are more likely to be a developmental defect rather than traumatic or ischaemic injury, at least in some patients.

There was no correlation between the posterior pituitary position and DI. A total of 18 patients had DI; 10 with an undescended, 7 with a normal and one with an enlarged posterior pituitary. None of the 9 patients in whom the posterior pituitary was not visualized had DI although this association has been reported previously (Sorkin et al. 1996). 78% of patients with DI had ONH, and both, ACTH deficiency and DI were found to be more common in hypopituitary patients with ONH as compared with hypopituitary patients without ONH.

The present study found a greater male to female preponderance in patients with hypopituitarism on the whole [93:44] and significantly [p=0.004] greater in hypopituitary patients without ONH [66:20] as compared with hypopituitary patients with ONH [27:24]. In future studies, identification of mutations in genes such as *SOX3* leading to an X-linked inheritance of hypopituitarism (Solomon et al. 2002) may elucidate the genetic basis for this male preponderance in patients with presumed "idiopathic disease". A high prevalence of extra-pituitary malformations in these patients adds support to a genetic aetiology in the pathogenesis of hypopituitarism (Simon et al. 2006).

Data from this study suggest a good relation between structure and function with the H-P axis. Abnormalities of the anterior and posterior pituitary were most likely to be associated with endocrine dysfunction as they occurred 6.68 and 33.05 times more commonly in patients with endocrinopathies as compared with those without. These MR imaging abnormalities were also most likely to be associated with abnormal endocrine function in patients with ONH. In contrast, presence of MFD was most likely to be associated with CPHD in patients with hypopituitarism as they occurred 5.5 times more commonly in patients with CPHD as compared with IGHD.

CHAPTER 6

INFLUENCE OF THE ENDOCRINE PHENOTYPE AND NEUROANATOMY ON BIRTH SIZE AND EARLY GROWTH

6.1 INTRODUCTION

Fetal growth is largely influenced by maternal nutrition and placental function. Insulin and insulin-like growth factors I and II [IGFI and II] play important roles as final common mediators (Sara and Carlsson-Skwirut 1986; Milner and Hill 1989; Liu et al. 1993; Baker et al. 1993), as exemplified by patients with growth hormone [GH] resistance (Laron et al. 1993), IGF1 deficiency (Woods et al. 1996), and insulin insensitivity (Longo et al. 2002), all of whom have impaired intra-uterine growth. Although these factors have been implicated in regulation of early postnatal growth, mathematical modelling and clinical observations suggest that nutrition is the principal regulator of rapid weight gain and linear growth in infancy and this is independent of GH (Karlberg et al. 1987). This theory is further supported by studies that report normal birth size, weight and linear growth in infancy in children who later develop GH deficiency [GHD] (Lovinger et al. 1975; Karlberg and Albertsson-Wikland 1988). The suggestion that growth in infancy is independent of the classic postnatal endocrine growth factors is also supported by the observation that severe postnatal growth failure is observed in patients with abnormal hypothalamic neuroanatomy such as in Prader-Willi syndrome and diencephalic syndrome of infancy, despite normal or even elevated serum GH concentrations (Swaab et al. 1995; Swaab 1997; Poussaint et al. 1997).

Recent studies have challenged this assumption suggesting that congenital GHD is associated with impaired growth in-utero leading to reduced birth size and growth failure within the first year of life (Albertsson-Wikland et al. 1990; Gluckman et al. 1992; Wit and van Unen 1992; Niklasson et al. 1994; De Luca et al. 1995; Pena-Almazan et al. 2001). GH deficient patients with congenital midline forebrain defects [MFD] such as those observed within the phenotypic spectrum of septo-optic dysplasia [SOD] and holoprosencephaly [HPE] may have both pituitary and hypothalamic abnormalities (Fitz 1983; Lam et al. 1986; Yukizane et al. 1990; Fitz 1994), and this raises the possibility that these patients may demonstrate more severe growth abnormalities compared with GH deficient patients without widespread neuroanatomical defects. Patients with MFD are also known to exhibit an increased propensity for weight gain later in life (Hoyt et al. 1970; Ishihara 1983; Arslanian et al. 1984; Izenberg et al. 1984).

In this chapter, the effect of GH alone and the additional effects of other pituitary hormone deficiencies and neuroanatomical defects, on antenatal and postnatal growth were assessed retrospectively.

6.2 PATIENT SELECTION AND METHODS

The present study was performed retrospectively in patients with congenital GHD, in isolation or as combined pituitary hormone deficiencies [CPHD], who presented to a single endocrine centre, the London Centre for Paediatric Endocrinology [LCPE]. Patients were selected if sufficient growth data (at least three data points) were available from birth up to 2 years of age or onset of GH treatment, whichever was earlier. 44 of 268 patients recruited from LCPE met the selection criteria.

Patients [male: female ratio 1.4:1] were divided into 3 groups of increasing phenotypic complexity depending on the neuroanatomy and endocrinology:

- Group A, GHD patients with no other pituitary hormone deficiencies or midline defects [Appendix V-3],
- Group B, GHD patients with CPHD but with no midline defects [Appendix V-4],
- Group C, GHD patients with CPHD and midline defects [Appendix V-5].

Abnormalities of the optic nerves, septum pellucidum, corpus callosum and HPE were all included as midline defects in group C.

Parameters analysed were length, weight and body mass index [BMI, weight in kilograms ÷ (length in meters)^2]. Birth measurements were obtained from records of individual patients. Data regarding birth weight were available in all patients and birth length in 37/44 patients. Subsequent weights and lengths were measured by the same auxologist with an intra-observer co-efficient of variation of 0.15% and 0.1% at lengths of 75 cms and 100 cms respectively.

17 patients were started on GH treatment between the ages of 6 months and 2 years. In the statistical analysis of anthropometric data at each study point, patients who were already commenced on GH treatment were excluded from the cohort. Growth data at 6, 12, 18 and 24 months were therefore analysed in 44, 42, 33 and 27 patients respectively. There was no bias introduced by omitting those patients on treatment, as analysis of variance demonstrated no significant ($p=0.3$) differences in auxological terms between patients starting GH treatment earlier compared with those who received treatment at a later age.

6.3 RESULTS

6.3.1 Patient characteristics

12 patients (27%) belonged to group A, 10 patients (23%) were in group B and 22 patients (50%) were in group C. Patient details of the 3 groups are shown in Table 6.1. The overall male to female ratio was 59%: 41% and there were no statistically significant sex differences between groups. There were no significant differences in gestational ages of patients between 3 groups, ruling out errors in interpretation of birth length. Prevalence of assisted deliveries and of postnatal complications was significantly different between the 3 groups, being greatest in patients with CPHD [groups B and C]. No patient from Group A [with IGHD] presented with neonatal hypoglycemia.

Mean age at initial presentation was significantly ($p<0.05$) different between the 3 groups and earliest in group C. Thirty-two patients had evidence of CPHD [Groups B and C]. There were no significant differences in the prevalence of thyrotrophin [TSH] and corticotrophin [ACTH] deficiencies between these 2 groups [group B, TSH deficiency 90% ACTH deficiency 70%; group C, TSH deficiency 91% ACTH deficiency 91%]. The age at onset of GH treatment was significantly ($p<0.0001$) earlier in patients with CPHD [groups B and C] as compared with patients with IGHD. No patient in Group A received GH treatment within 2 years of age as compared with 40% of patients in Group B and 59% of patients in Group C.

Table 6.1: Clinical characteristics of cohort analysed to assess effects of GH, other pituitary hormone deficiencies and midline brain defects on birth size and growth in early infancy. Group A, isolated growth hormone deficiency and no midline defects; Group B, combined pituitary hormone deficiency [CPHD] but no midline defects; Group C, CPHD and midline defects.

Group	Male: female	Age at presentation	Assisted deliveries	Gestation	Postnatal complications	Neonatal hypoglycemia
A (n=12)	7:5	3.0 ± 1.6	16.7%	38.6 ± 2.0	8%	0%
B (n=10)	6:4	1.1 ± 1.0	70%	38.0 ± 2.4	80%	70%
C (n=22)	13:9	0.4 ± 0.6	36.4%	39.7 ± 2.5	73%	59%
p=	0.997	0.001	0.002	0.147	0.0003	0.001

p, probability value [ANOVA]

6.3.2 Serum growth hormone concentrations

GH provocation tests were performed in 38 of 44 patients. 6 patients with CPHD did not undergo provocation testing. One patient had a serum GH concentration of 3.0 mU/L at the time of spontaneous hypoglycemia and the remaining 5 patients had undetectable serum IGF1 and IGFBP3 concentrations with a poor growth velocity and CPHD. Serum GH concentrations did not show a significant correlation with birth length (r=-0.08, p=0.7), birth weight (r=-0.08, p=0.6) or age at induction of GH treatment (r=0.12, p=0.5). There were no significant differences between peak serum GH concentrations in patients of the 3 groups. Peak serum GH concentrations in patients with neonatal hypoglycemia (7.9 ± 5.3 mU/L) were not significantly different from those in patients without hypoglycemia (7.4 ± 5.3 mU/L; p=0.8). GH concentrations were not significantly lower in patients with (6.6 ± 5.4 mU/L) and without (10.3 ± 6.5 mU/L; p=0.08) anterior pituitary hypoplasia. Mean serum IGF1 and IGFBP3 concentrations (n=18) were –4.6 ± 2.9 SDS and -0.3 ± 1.0 SDS respectively.

6.3.3 Anthropometry

Mid-parental height was significantly (p=0.006) different between the 3 groups, shortest in group A patients. In spite of this, patients in group A had a greater birth length SDS than those in Group C although differences did not reach statistical significance [Table 6.2].

Length SDS decreased in all patients within 6 months of birth. The greatest decrease was observed in patients in group B as compared with those in group A or group C (p=0.03).

There were significant differences in birth weight SDS between the 3 groups. Patients in group C were significantly [p=0.009] heavier than those in group A [Table 6.3] and overall, patients with CPHD [groups B and C] were significantly heavier [p<0.05] than those with IGHD [Group A].

BMI SDS at birth was significantly greater in group C as compared with group A and group B [Table 6.4]. Patients in Group C continued to have significantly (p<0.05) greater weight and BMI SDS as compared with patients in Group A at 12, 18 and 24 months of age. Overall, BMI was significantly greater [p<0.05] at all study points in CPHD patients [Groups B and C] as compared with patients with IGHD.

Table 6.2: Comparison of length SDS at birth, 6, 12, 18, and 24 months. Group A, isolated growth hormone deficiency and no midline defects; Group B, combined pituitary hormone deficiency [CPHD] but no midline defects; Group C, CPHD and midline defects.

		Length (SDS) at months				
		0	**6**	**12**	**18**	**24**
Group A	n=	11	12	12	12	12
		-0.5 ± 1.3	-2.2 ± 1.3	-2.8 ± 1.4	-3.3 ± 1.6	-3.7 ± 1.6
Group B	n=	9	10	9	7	6
		-0.5 ± 1.3	-3.0 ± 1.8	-3.7 ± 2.2	-4.0 ± 2.2	-2.7 ± 3.6
Group C	n=	17	22	21	14	9
		-0.9 ± 1.3	-2.3 ± 1.8	-2.8 ± 2.0	-3.2 ± 1.8	-3.2 ± 2.2

Table 6.3:Comparison of weight SDS at birth, 6, 12, 18, and 24 months. Group A, isolated growth hormone deficiency and no midline defects; Group B, combined pituitary hormone deficiency [CPHD] but no midline defects; Group C, CPHD and midline defects.

		Weight (SDS) at months				
		0	**6**	**12**	**18**	**24**
Group A	n=	12	12	12	12	12
		-1.1 ± 0.8[1]	-2.6 ± 1.0	-2.7 ± 1.1[1]	-3.2 ± 1.2[1]	-3.3 ± 1.6[1]
Group B	n=	10	10	9	7	6
		-0.5 ± 1.4	-1.9 ± 1.5	-1.9 ± 2.3	-1.7 ± 2.9	-2.1 ± 3.2
Group C	n=	22	22	21	14	9
		-0.1 ± 0.9	-1.4 ± 2.3	-0.9 ± 2.4	-0.8 ± 2.4	-1.0 ± 2.5

[1] probability value, $p<0.05$ for group A compared with group C.

Table 6.4: Comparison of BMI SDS at birth, 6, 12, 18, and 24 months. Group A, isolated growth hormone deficiency and no midline defects; Group B, combined pituitary hormone deficiency [CPHD] but no midline defects; Group C, CPHD and midline defects.

		BMI (SDS) at months				
		0	**6**	**12**	**18**	**24**
Group A	n=	11	12	12	12	12
		-1.0 ± 1.1[1]	-1.8 ± 1.5[3]	-1.2 ± 1.3[1,3]	-1.3 ± 1.4[1,3]	-0.9 ± 1.5[1]
Group B	n=	9	10	9	7	6
		-0.4 ± 2.5[2]	-0.1 ± 1.5	+0.7 ± 2.2	+1.4 ± 2.1	+0.5 ± 2.3
Group C	n=	17	22	21	14	9
		+1.2 ± 1.3	-0.5 ± 2.2	+0.9 ± 1.6	+1.3 ± 2.0	+1.2 ± 1.9

[1] probability value, $p<0.05$ for [1] group A compared with group C; [2] group B compared with group C; [3] group A compared with group B

6.4 DISCUSSION

Mean birth weight, length and BMI SDS in this study population were –0.4, -0.9 and +0.1 SDS respectively (Mehta et al. 2005b). These observations were in agreement with other reports of reduced birth weight and length in GHD patients (Albertsson-Wikland et al. 1990; Gluckman et al. 1992; Niklasson et al. 1994; De Luca et al. 1995; Rappaport et al. 1997; Huet et al. 1999; Wasniewska et al. 2000). A comparative analysis of various studies on birth size in patients with congenital GHD is shown in Table 6.5.

CPHD patients [Groups B and C] were born with a lower length SDS as compared with IGHD patients although differences were not statistically significant. Birth weight was however significantly greater in patients with CPHD as compared with patients with IGHD. Although IGHD patients had significantly shorter parents than those of CPHD patients, they were born lighter but longer than the latter. It is possible that co-existing hormonal deficiencies may be contributory factors for the differences in birth size between patients with CPHD and IGHD. ACTH deficiency is known to result in an increased gestational age that may lead to differences either real, as weight gain continues, or artefactual, as comparison of birth size is made with postnatal standards of growth. However, there were no significant differences in the gestational ages of patients with [39.2 ± 2.6 weeks] and those without [38.6 ± 2.0 weeks] ACTH deficiency. Apart from the endocrine spectrum, neuroanatomic factors also appeared to influence birth size as demonstrated by CPHD patients with midline defects who were born with a lower length SDS but greater weight SDS and BMI SDS than CPHD patients without midline defects.

Table 6.5: Comparisons of birth size in various studies in patients with congenital growth hormone deficiency.

	Present study n=44	Niklasson et al (Niklasson et al. 1994) n=220	Wasniewska et al (Wasniewska et al. 2000) n=12	DeLuca et al (De Luca et al. 1995) n=16	Huet et al (Huet et al. 1999) n=59	Rappaport et al (Rappaport et al. 1997) n=49	Pena-Almazan et al (Pena-Almazan et al. 2001) n=46	Gluckman et al (Gluckman et al. 1992) n=52	Albertsson Wikland et al (Albertsson-Wikland et al. 1990) n=220
Weight	-0.4	-0.6	-1.0	-1.0		-0.3	0.18	-0.5	-0.6
Length	-0.9	-0.9	-2.0	-2.1	-0.9	-0.9	0.4	-0.8	-0.9

Reduced length SDS was evident within 6 months of age in all study groups with a greater effect on linear growth as compared with weight, in keeping with other studies confirming the role of GH in maintaining normal early growth (Albertsson-Wikland et al. 1990; Gluckman et al. 1992; De Luca et al. 1995; Pena-Almazan et al. 2001). There was a greater decrease in weight SDS as compared with length SDS in patients with IGHD resulting in a reduced BMI. These findings were surprising, given that GH is lipolytic, and GHD would therefore be expected to lead to relative obesity with an increase in BMI. A reduction in lean body mass could also contribute to the decreased weight SDS. Nutritional intake was not formally assessed in patients but it was reported to be normal. There was a greater reduction in length SDS observed in CPHD as compared with IGHD patients postnatally. Patients with CPHD continued to have a greater BMI postnatally in spite of optimization of serum thyroxine concentrations. 84.4% of CPHD patients also had ACTH deficiency. There were no significant differences in the prevalence of TSH and ACTH deficiencies in CPHD patients with or without midline defects. In spite of this, CPHD patients with midline defects [group C] had both greater weight and length SDS than those without these defects [group B].

Although the precise aetiology of pituitary dysfunction in conditions with midline defects such as HPE and SOD is unknown, abnormal hypothalamic neuroanatomy has been postulated in the latter (Huseman et al. 1978; Lam et al. 1986; Roessmann et al. 1987; Yukizane et al. 1990). Less severe effect on linear growth in these patients could be explained by an increased weight gain and hyperphagia secondary to a hypothalamic abnormality. It remains to be established whether the early increase in BMI in these patients in some way predisposes these patients to obesity in later life.

No patient with IGHD reported in this study presented with hypoglycemia, although this has been reported in a minority of cases (Esberg and Jacobsen 1996). Concomitant ACTH deficiency was evident in the majority of patients with CPHD who presented with hypoglycemia, although patients without ACTH deficiency also presented with low serum blood glucose concentrations implying a possible role for other pituitary hormones such as TSH apart from other metabolic factors in glucose homeostasis.

Present data suggest that GH has a minimal effect on birth size but is critical for maintenance of postnatal growth. Due to the evolving nature of hypopituitarism and the heterogeneity in the present cohort of patients, one cannot exclude some degree of overlap between the three groups. However, trends within these data suggest that growth during the intrauterine and infancy periods is influenced by the complexity of the hypopituitary phenotype as reflected by the presence of other pituitary hormone deficiencies and structural brain defects.

CHAPTER 7

GENOTYPE-PHENOTYPE CORRELATION IN PATIENTS WITH A MOLECULAR GENETIC CAUSE OF HYPOPITUITARISM

7.1 INTRODUCTION

Extrinsic and intrinsic signalling gradients determine the expression patterns of pituitary-specific factors in the developing anterior pituitary gland. Initially, pituitary gland commitment from the oral ectoderm occurs in response to inductive signals from the ventral diencephalon. Eventually, there is a sequential appearance of terminally differentiated cells, with gonadotrophs, thyrotrophs, somatotrophs, lactotrophs and corticotrophs located ventrally to dorsally.

Mutations in many intrinsic pituitary transcription factors have been identified in humans with hypopituitarism, with or without extra-pituitary manifestations, as shown earlier in Chapter 2, Table 2.1. These include *POU1F1* (Tatsumi et al. 1992) and *PROP1* (Wu et al. 1998), mutations of which result in non-syndromic combined pituitary hormone deficiency [CPHD]. Mutations within *PROP1* are associated with deficiencies of thyrotrophin [TSH], prolactin, growth hormone [GH], follicle stimulating hormone [FSH] and luteinising hormone [LH] (Duquesnoy et al. 1998; Wu et al. 1998; Deladoey et al. 1999; Vallette-Kasic et al. 2001) and have been reported to represent the commonest cause of familial CPHD. The phenotype is variable with respect to severity of hormone deficiency, age at the onset of puberty, adrenal function and even untreated final height (Arroyo et al. 2002). Evolving corticotrophin [ACTH] deficiency has been described in a number of patients (Agarwal et

al. 2000; Pernasetti et al. 2000), as well as anterior pituitary hyperplasia (Parks et al. 1999; Mendonca et al. 1999; Riepe et al. 2001; Voutetakis et al. 2004). *POU1F1* [murine orthologue P*it1*] was the first pituitary-specific transcription factor to be identified. In humans, mutations within *POU1F1* were first described in 1992 by three independent groups (Tatsumi et al. 1992; Ohta et al. 1992; Pfaffle et al. 1992; Radovick et al. 1992). Mutations within *POU1F1* are associated with GH, prolactin and TSH deficiencies, with variable pituitary hypoplasia. Deficiencies of GH and prolactin are generally complete. TSH deficiency is more variable although in the majority, hypothyroidism is early and profound.

Mutations in *LHX3* (Netchine et al. 2000) and *LHX4* (Machinis et al. 2001) result in CPHD with a similar endocrine phenotype as in patients with mutations in *PROP1*. Homozygous mutations in *LHX3* have currently been identified in 5 patients from 3 unrelated consanguineous families, all with a deficit in all anterior pituitary hormones except ACTH, as well as a short rigid, cervical spine with limited head rotation and trunk movement (Netchine et al. 2000; Sobrier et al. 2004; Bhangoo et al. 2006). Pituitary morphology was variable ranging from anterior pituitary hypoplasia with a normal posterior pituitary and normal midline forebrain structures [MFS] on MR imaging to an abnormality similar to that observed in patients with *PROP1* mutations characterized by an enlarged anterior pituitary (Netchine et al. 2000). One patient has been reported with a hypointense lesion within the anterior pituitary consistent with a microadenoma (Bhangoo et al. 2006). Mutations in *LHX4* have been reported in only one pedigree with GH, TSH and ACTH deficiencies (Machinis et al. 2001). MR scanning revealed anterior pituitary hypoplasia with an undescended posterior pituitary and an absent pituitary stalk. Additional manifestations included a poorly formed sella turcica and pointed cerebellar tonsils.

Mutations in *HESX1* in humans have been shown to present with varying phenotypes characterized by IGHD, CPHD and the syndrome of septo-optic dysplasia [SOD] (Dattani et al. 1998; Brickman et al. 2001; Thomas et al. 2001; Carvalho et al. 2003; Tajima et al. 2003; Cohen et al. 2003). The overall frequency of *HESX1* mutations in SOD is however low suggesting that mutations in other known or unknown genes contribute to this complex disorder. Recently a recessive mutation has been identified in two siblings from a consanguineous family who presented with a severe phenotype including CPHD, a coloboma of the right optic nerve with unilateral blindness and a left-sided diaphragmatic hernia and aortic coarctation (in one patient only) (Sobrier et al. 2005). Results of MR imaging are also highly variable with regards to H-P morphology and abnormalities of MFS.

More recently, mutations within *GLI2, GLI3, SOX3* and *SOX2* have been identified in patients with variable hypopituitarism. Mutations within *PITX2* or RIEG are found to be associated with Rieger syndrome, although none manifested pituitary hormone deficiencies (Cohen and Radovick 2002). Mutations within *GLI2* have been identified in 7 patients with holoprosencephaly (Roessler et al. 2003) with a variable phenotype. In all affected patients, pituitary gland function was abnormal accompanied by variable craniofacial abnormalities, post-axial polydactyly, single nares, single central incisor and partial agenesis of the corpus callosum. Mutations in *GLI3* result in Pallister-Hall syndrome. A number of pedigrees have been described with X-linked hypopituitarism involving duplications of Xq26-q27 containing the *SOX3* gene (Hamel et al. 1996; Hol et al. 2000; Solomon et al. 2002). The phenotype is of variable mental retardation and hypopituitarism. Further implication of *SOX3* in hypopituitarism came from identification of patients with expansion of a polyalanine tract within the gene (Laumonnier et al. 2002). Mutations in *SOX2* have recently

been reported resulting in a phenotype of anophthalmia, hypopituitarism, learning difficulties and esophageal atresia (Kelberman et al. 2006).

Approximately 5-30% of patients with IGHD have a presumed genetic basis to the disorder, with an affected first degree relative. Genes that have currently been implicated in the aetiology of GHD include *GH1, GHRHR, HESX1* and possibly mutations in other transcription factors [*PROP1, POU1F1]* that present with variable phenotypes. Mutations within *GH1* and *GHRHR* are usually associated with non-syndromic IGHD in the presence of a normal or hypoplastic anterior pituitary, with a normally sited posterior pituitary on MR scanning. However, Osorio *et al.* recently described a patient with *GH1* gene deletion with an undescended posterior pituitary (Osorio et al. 2002). Recent studies have also revealed novel insights into the aetiology and pathogenesis of autosomal dominant type II IGHD. In 8 out of 57 patients with type II IGHD, additional hormonal [partial TSH and/or ACTH] deficiencies were documented (Mullis et al. 2005). Mutations in *GHRHR* in patients with IGHD with extreme short stature suggest that *GHRHR* mutations may be a more common cause of IGHD than previously suspected.

Documentation of the detailed phenotype in patients with mutations in pituitary transcription factors, in conjunction with studies from mice with targeted disruption of these genes, will yield better insights into pituitary development. This part of the study investigated the prevalence of individual mutations in large numbers of patients with hypopituitarism, to document detailed endocrine and neuroradiological phenotypes and to investigate if genotype could predict the phenotype.

7.2 PATIENTS AND SELECTION

The broad phenotypes of patients screened for mutations within candidate genes is shown in Table 7.1 All 825 patients recruited into the study were screened for mutations in *HESX1* given the variability of phenotypes associated with these mutations. The remaining patients were selected for mutational analysis of appropriate candidate genes based on previous animal studies. Patients with IGHD were screened for mutations in *GH1* and *GHRHR*. Patients whose neuroimaging revealed abnormalities of MFS, the posterior pituitary or the pituitary stalk or those with anterior pituitary enlargement, were excluded from analysis for *GH1* and *GHRHR*. Patients with sporadic IGHD but without MFD were also screened for *PROP1, LHX3* and *POU1F1*. Patients with CPHD were screened for mutations within *POU1F1*, *PROP1*, *LHX3* and *LHX4*, depending on their phenotype based on studies from animal models. Patients with a X-linked inheritance of variable hypopituitarism were selected for *SOX3* screening. Mutation screening of *GLI2, SOX2, GLI3* or genes causing isolated hormone deficiency other than IGHD were not performed as a part of this study.

Mutations in *GH1, POU1F1, PROP1, HESX1* and *SOX3* were found in 48 patients (5.8%) within the entire cohort. No mutations were found in *LHX3, LHX4* or *GHRHR* within this study group.

Table 7.1: Broad endocrine spectrum of patients screened for mutations in pituitary transcription factors and genes causing developmental defects of the pituitary, eye and midline structures.

Gene	Number of patients screened	Familial cases	Number of patients with isolated deficiencies of				CPHD	Number of patients with normal endocrinology with	
			GH	TSH	Gn	AVP		Midline dysmorphism	Eye defects
HESX1	825	109	325	2	7	7	399	15	70
PROP1 / LHX3 / LHX4	233	44	79	1	0	0	153	0	0
POU1F1	129	24	48	1	0	0	80	0	0
SOX3	76	13	28	0	0	0	48	0	0
GH1 / GHRHR	103	32	103	0	0	0	0	0	0

GH, growth hormone; TSH, thyrotrophin; Gn, gonadotrophin; AVP, arginine vasopressin; CPHD, combined pituitary hormone deficiencies

7.3 MUTATIONS WITHIN *HESX1*

7.3.1 Patient phenotypes

Six patients [5 males, 1 female], from the cohort of 825 patients screened, were found to harbour mutations within *HESX1* yielding a frequency of 0.7%. There were 4 familial and 2 sporadic cases from 4 unrelated families [Table 7.2, page 152]. One patient was referred directly to LCPE whilst the remaining 5 patients were followed up at other endocrine centres within UK. Two patients [pedigree I] were of Asian origin and the others were Caucasian. Mean age at diagnosis of hypopituitarism was 4.3 ± 2.5 years. The reason for referral in all patients was poor growth and short stature with height SDS ranging from –2.8 to –3.9 SDS.

Sequencing of *HESX1* revealed a homozygous R120C mutation in both siblings from pedigree I. Sequencing of exon 4 from the parents, who were first cousins, revealed that they were heterozygous for the point mutation but did not exhibit any abnormal features consistent with an autosomal recessive mode of inheritance. A heterozygous S170L mutation in *HESX1* resulting in the substitution of a serine residue by leucine in a highly conserved region at the carboxy-terminal end of the homeodomain was identified in two brothers from pedigree II and in patient III. DNA binding studies confirmed a reduction in DNA binding by the mutant protein. A novel heterozygous missense mutation at a highly conserved residue (E149K) was identified in *HESX1* in patient IV. His mother, son and brother appear to be unaffected carriers of the mutation. Functional studies revealed that the mutation led to disruption of HESX1 function.

CPHD [GH, TSH, ACTH, gonadotrophin] was demonstrated in both siblings from pedigree I with the homozygous R120C mutation whilst the remaining 4 patients with heterozygous mutations had IGHD. These latter 4 patients [II.1, II.2, III, IV] demonstrated an evolving severity of GHD with serum GH concentrations at initial diagnosis of 14.5 mU/L (3.8 years), 20.4 mU/L (1.0 year), 1.1 mU/L (6.0 years) and 4.2 mU/L (6.3 years), and at repeat testing of 6.5mU/L (5.3 years), 14.5 mU/L (9.0 years), 0.9mU/L (18.0 years) and 1.2 mU/L (22 years). Longitudinal serum GH concentrations were not available in patients I.1 and I.2.

Evolution of other pituitary hormone deficiencies was not demonstrated in patients with IGHD. Patient IV with IGHD was initially suspected to have gonadotrophin deficiency in view of a hypoplastic scrotum and a small penis. A luteinising hormone releasing hormone test [LHRH] test performed at 12.5 years of age revealed peak serum FSH and LH concentrations of 2 U/L and 8 U/L. He was commenced on Sustanon replacement treatment until 17 years of age. At the end of puberty, however, his testicular sizes were 15-20 mls and a serum testosterone concentration off Sustanon replacement treatment was normal (13.4 nmol/L). He was subsequently proven fertile with the birth of a normal male child. Patient I.1 also had genital abnormalities with glandular hypospadias and gonadotrophin deficiency.

MR imaging was normal in both patients from pedigree II apart from the finding of kinked optic nerves in patient II.1 [Table 7.3, page 153]. The remaining 4 patients demonstrated several abnormalities on MR imaging including anterior pituitary hypoplasia (100%), hypoplastic pituitary sella (75%), undescended posterior pituitary (100%), abnormal corpus callosum (25%), absent septum pellucidum (50%), pituitary stalk abnormalities (100%) and ONH (50%). Neuroimaging revealed the diagnosis of SOD in both siblings from pedigree I

with all 3 features of the syndrome: optic nerve hypoplasia, an absent septum pellucidum and hypopituitarism with abnormal H-P morphology, although there were no visual abnormalities clinically [Figure 7.1 B and C]. Additionally patient I.1 demonstrated partial agenesis of the corpus callosum, whereas patient I.2 had hypogenesis of the corpus callosum. In contrast to patients from pedigree I, patient II.1 with normal H-P morphology, normal MFS but kinked optic nerves had clinical evidence of ONH. He also demonstrated extra-pituitary manifestations and was mildly dysmorphic with small hands and feet, simian creases and syndactyly of the 2nd and 3rd toes bilaterally. Patients III [Figure 6.1 D] and IV did not reveal abnormalities of the MFS or optic nerves on imaging. Digital abnormalities were however observed in Patient IV who had small bilateral supernumerary digits requiring excision.

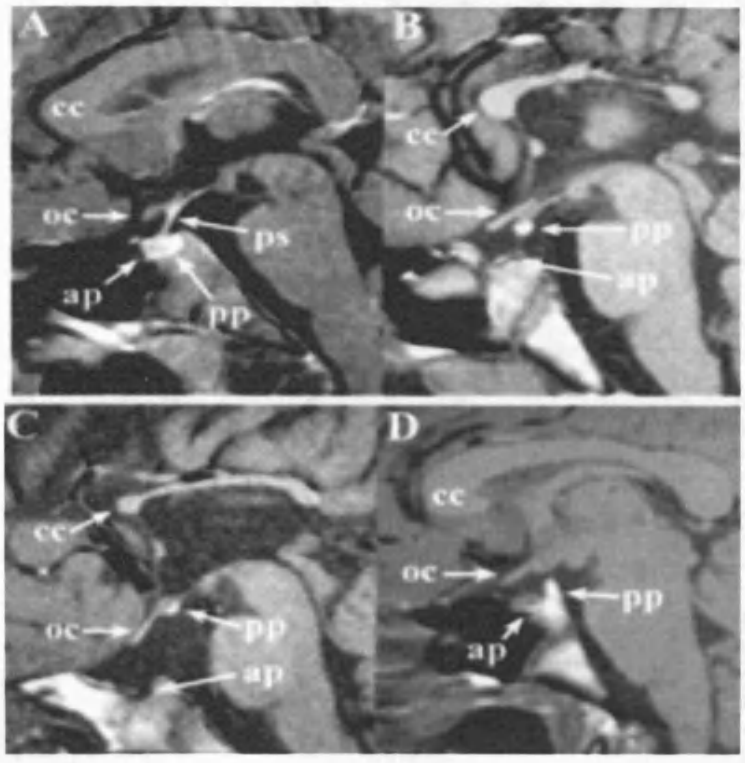


Figure 7.1:
Magentic resonance imaging in patients with mutations in *HESX1*.

A, normal scan;
B, patient I.1;
C, patient I.2;
D, patient III.

Table 7.2: Clinical and endocrine data of patients with mutations in *HESX1*

	Ethnic origin	Sex	At Diagnosis		Endocrinology							Mutation
					Peak				FT4	Unstimulated		
			Age (years)	Height (SDS)	GH (mU/L)	Cortisol (nmol/L)	LH (U/L)	FSH (U/L)	(pmol/L)	TSH (mU/L)	PRL (mU/L)	
I.1	Asian	M	N/A	N/A	3.0	40	UND	UND	4.7	UND	N/A	R120C
I.2	Asian	F	N/A	N/A	UND	13	UND	UND	<5	0.2	N/A	R120C
II.1	Caucasian	M	3.8	-3.9	14.5	1052	N/A	N/A	105[1]	4.4	155	S170L
II.2	Caucasian	M	1.0	-3.6	20.4	N	N	N	N	N	N	S170L
III	Caucasian	M	6.0	-2.8	1.1	920	N	N	17.1	2.7	252	S170L
IV	Caucasian	M	6.3	-3.7	4.2	N	8.0	2.0	N	N	N	E149K

M, male; F, female; GH, growth hormone; TSH, thyrotrophin; FSH, follicle stimulating hormone; LH, luteinising hormone; AP, anterior pituitary; PP posterior pituitary; SP, septum pellucidum; CC, corpus callosum; ON, optic nerves; N/A, not available; D, deficient; H, hypoplastic; N, normal; U, undescended; P, partial; A, absent; K, kinked; [1] total thyroxine, UND, undetectable

Table 7.3: Magnetic resonance [MR] imaging in patients with mutations in *HESX1*

	MR Imaging						
	Sella	**Anterior pituitary**	**Posterior pituitary**	**Septum pellucidum**	**Corpus callosum**	**Stalk**	**Optic nerves**
I.1	Hypoplastic	Hypoplastic	Undescended	Absent	Partial	Hypoplastic	Hypoplastic
I.2	Normal	Hypoplastic	Undescended	Absent	Hypoplastic	Hypoplastic	Hypoplastic
II.1	Normal	Normal	Normal	Normal	Normal	Normal	Kinked
II.2	Normal	Normal	Normal	Normal	Normal	Normal	Normal
III	Hypoplastic	Hypoplastic	Undescended	Normal	Normal	Hypoplastic	Normal
IV	Hypoplastic	Hypoplastic	Undescended	Normal	Normal	Hypoplastic	Normal

7.3.2 Discussion

The R120C mutation found in patients 1.I and 1.II was the first reported mutation in *HESX1* and was implicated in the etiology of SOD in humans (Dattani et al. 1998). Since then both, homozygous and heterozygous mutations in *HESX1* have been associated with markedly variable phenotypes in humans. Although reported heterozygous *HESX1* mutations S170L and T181A are, in general, associated with milder phenotypes and incomplete penetrance with most affected individuals inheriting the mutation from one of their unaffected parents (Thomas et al. 2001), two further heterozygous mutations [a deletion at nucleotide position 1684 (Cohen et al. 2003) and a *de novo* 2bp insertion (Tajima et al. 2003)] have been identified in patients presenting with a more severe phenotype of SOD with GH deficiency, hypoplasia of the anterior pituitary and optic nerves and midline forebrain abnormalities. The patient with the insertion mutation also exhibited evidence of gonadotrophin deficiency and hypothyroidism. A similar variability has been observed in patients with homozygous *HESX1* mutations. Apart from the first report by Dattani at al (Dattani et al. 1998), 5 patients have been described with 4 different homozygous mutations. One patient presented with a milder phenotype of evolving CPHD, anterior pituitary hypoplasia and no optic nerve abnormalities (Carvalho et al. 2003), and 2 sibs were reported with CPHD, aplasia of the anterior pituitary, coloboma of the right optic nerve and left-sided diaphragmatic hernia. One sib also had aortic coarctation (Sobrier et al. 2005). More recently 2 unrelated patients have been described with a life-threatening neonatal condition associated with anterior pituitary aplasia, panhypopituitarism, a normal posterior pituitary and no optic nerve abnormalities (Sobrier at al. 2006).

There is a poor genotype-phenotype correlation in patients with mutations in *HESX1* within this study cohort as well. The brothers from pedigree II presented with peak serum GH concentrations of 14.5 mU/L and 20.5 mU/L with an evolving severity of GHD and gradually decreasing concentrations of GH with age. Patient III however had severe GHD at diagnosis (1.1 mU/L) with repeat concentrations at end of his growth of 0.9 mU/L. MR imaging in these patients, with the same genotype, was also variable. Patients II.1 and II.2 essentially demonstrated a normal MR scan apart from patient II.1 demonstrating kinked optic nerves. Patient III, however, demonstrated a hypoplastic pituitary sella and anterior pituitary, an undescended posterior pituitary and a hypoplastic stalk. There was also a poor correlation between the genotype and the neuroanatomical phenotype within families with the same mutation. The pituitary sella was hypoplastic with partial agenesis of the corpus callosum in patient I.1 but normal with hypogenesis of the corpus callosum in patient I.2. Similarly, kinked optic nerves revealed on MR imaging in Patient II.1 were not demonstrated in patient II.2.

MR imaging did not reveal MFD in patients with heterozygous mutations unlike previous reports (Cohen et al. 2003; Tajima et al. 2003). However, these abnormalities have not been reported in some patients with homozygous *HESX1* mutations as well. Patients with homozygous mutations present with a more severe endocrine phenotype as compared with those with heterozygous mutations, with evolving CPHD (Carvalho et al. 2003; Sobrier et al. 2005) or complete panhypopituitarism with pituitary aplasia (Sobrier et al. 2006).

Patient II.1, with a heterozygous S170L mutation had clinical optic nerve dysplasia and kinked optic nerves on MR imaging, a phenotype similar to but milder than that observed in patients I.1 and I.2 with SOD. Patient II.1and patient IV, with IGHD, also had digital anomalies as have been previously observed in patients with SOD (Pagon and Stephan 1984; Orrico et al. 2002; Harrison et al. 2004).

A novel E149K heterozygous missense mutation that led to functional compromise was identified in patient IV [McNay et al, 2006, in press]. While both the patient and his brother have inherited this mutation from their mother, they inherited different paternal haplotypes. His brother, mother and a son are unaffected carriers of the mutation. It is possible that this may represent variable penetrance or the patient may have inherited an as yet unidentified *HESX1* mutation from his father resulting in a phenotype due to compound heterozygosity.

7.4 MUTATIONS WITHIN *PROP1*

7.4.1 Patient phenotypes

Of 233 patients screened, mutations in *PROP*1 were detected in 15 patients [12 males, 3 females] from 8 pedigrees yielding a frequency of 6.4% [Table 7.4, page 162]. In addition, a further patient from pedigree IV who presented with a height of –4.5 SDS at 60 years of age, was found to have GH, TSH and gonadotrophin deficiencies, but had died of ischaemic heart disease shortly after presentation. DNA was not available from this patient. Patients from pedigrees III, IV, V, VI, VII and VIII were siblings.

There was a wide range in the age at initial presentation. The mean age at diagnosis of hypopituitarism was made at 17.5 ± 17.0 years. 5 patients [II, IV.1, IV.2, VII.1, VII.2] were diagnosed late with CPHD, at ages 29.0 years, 50 years, 55 years, 26.3 years and 22.3 years. Short stature (mean height SDS –6.6 ± 1.8 SDS) was the reason for presentation in all 15 patients. Patient IV.1 diagnosed at 50 years of age also had hypoglycaemic seizures at presentation. 8/15 patients (patients I, II, V.1, V2, VI.1, VI.2, VII.1, VII.2), all with the same mutation, a homozygous 13bp deletion (112-124Δ), were of Asian origin (6 Indian, 2 Pakistani). The remaining patients were Caucasian; siblings from pedigree III harbouring a homozygous 1bp deletion (150ΔA) were from Russia, brothers from pedigree IV and those from pedigree VIII harbouring the common 2-bp deletion within exon 2 (301-302ΔAG) were from the UK and Poland respectively. Mode of inheritance was autosomal recessive in all patients.

All 15 patients had CPHD, evolving in the case of patient VIII.3 and possibly in patient II. GHD was present in all patients (precise GH concentrations unavailable in 2 patients) with a mean serum GH concentration of 1.7 ± 1.3 mU/L. GHD was evident in 14/15 patients at initial presentation. Patient VIII.3, when tested in infancy, had normal pituitary function tests at one year of age but was found to be GHD at 5 years of age. Patient II was initially investigated in Afghanistan and details of investigations performed were not available. He received thyroxine treatment but continued to be severely growth retarded until the diagnosis of GHD at the age of 29 years.

TSH deficiency was present in 14/15 (93%) patients. The mean serum TSH concentration in patients with hypothyroidism was 3.0 ± 1.5 mU/L. Patient VII.1 with no clear evidence of TSH deficiency, had a free thyroxine concentration at the lower end of normal at 13.1 pmol/L with a serum TSH concentration of 2.9 mU/L at the age of 26.3 years. Similar to GH, TSH deficiency was evolving in Patient VIII.3. Patient V.1 had a marginally elevated serum TSH concentration of 6.1 mU/L at diagnosis.

The serum prolactin concentrations were normal (100-500 mU/L) in 8/15 patients (chronological age range 11.0-58.0 years), although it was in the lower range of normal in 50% of them (100 –182 mU/L). Seven of 15 (47%) patients were prolactin deficient, evolving in patient VIII.3, 9 years after the diagnosis of GH and TSH deficiencies.

Siblings from pedigree III have not had testing for gonadotrophin deficiency. 4/10 males with confirmed gonadotrophin deficiency had evidence of undescended testes. Patient II also had micropenis and gynaecomastia, brothers from pedigree IV had genitalia that were near

ambiguous and patient VIII.1 had needed bilateral orchidopexies in the past. Although gonadotrophin deficiency has not been confirmed in patient I in view of his age, he presented with a micropenis and bilaterally undescended testes and preliminary investigations revealed peak serum FSH and LH concentrations of 0.7 U/L and 0.2 U/L [9 years of age].

Hypocortisolemia was documented in 3/15 patients (20%). Both brothers from pedigree IV presented with hypocortisolaemia at initial presentation at ages of 50 years and 55 years. Patient V.2 had normal cortisol secretion initially but was found to be deficient at the age of 17 years after a tonic clonic seizure at the time of dental extraction. 7/12 patients without cortisol deficiency are now adults and continue to have normal cortisol secretion.

Results of MR imaging, particularly with respect to anterior pituitary morphology, were variable among 11 patients in whom it was performed [Table 7.5, page 163]. The posterior pituitary, pituitary stalk, septum pellucidum, corpus callosum and optic nerves were normal in all patients apart from patient I in whom, although morphologically normal, the pituitary stalk was anteriorly displaced, and the posterior pituitary appeared compressed. Abnormalities of the anterior pituitary and pituitary sella were demonstrated in 100% and 45.5% of patients. MR imaging in patient I revealed an enlarged sella turcica with a markedly enlarged anterior pituitary (8.6 mm) (Argyropoulou et al. 1991), with enhancing lesions suggestive of possible haemorrhage [Figure 7.2 (i) a]. Repeat MR imaging performed 4, 12 and 21 months after the first scan showed significant waxing and waning in the size of the anterior pituitary mass at 6.8 mm, 8.3 mm and 4.2 mm respectively [Figure 7.2 (i) b, (i) c, (i) d].

Figure 7.2: Magnetic resonance imaging in two patients with a 13 bp deletion in *PROP1* illustrating variability between genotype and phenotype between pedigrees.
(i) Patient 1 (ii) Patient 2.

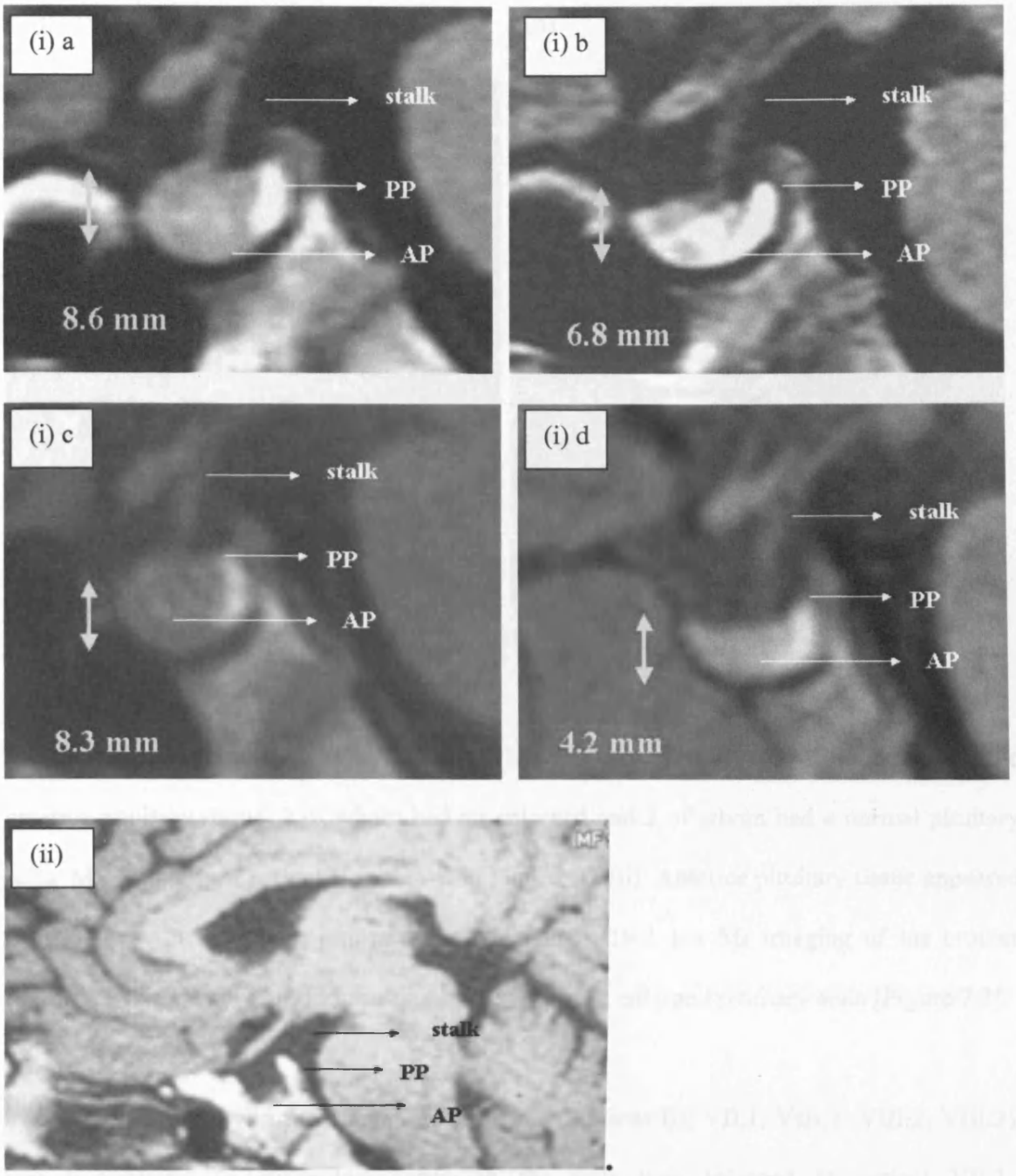
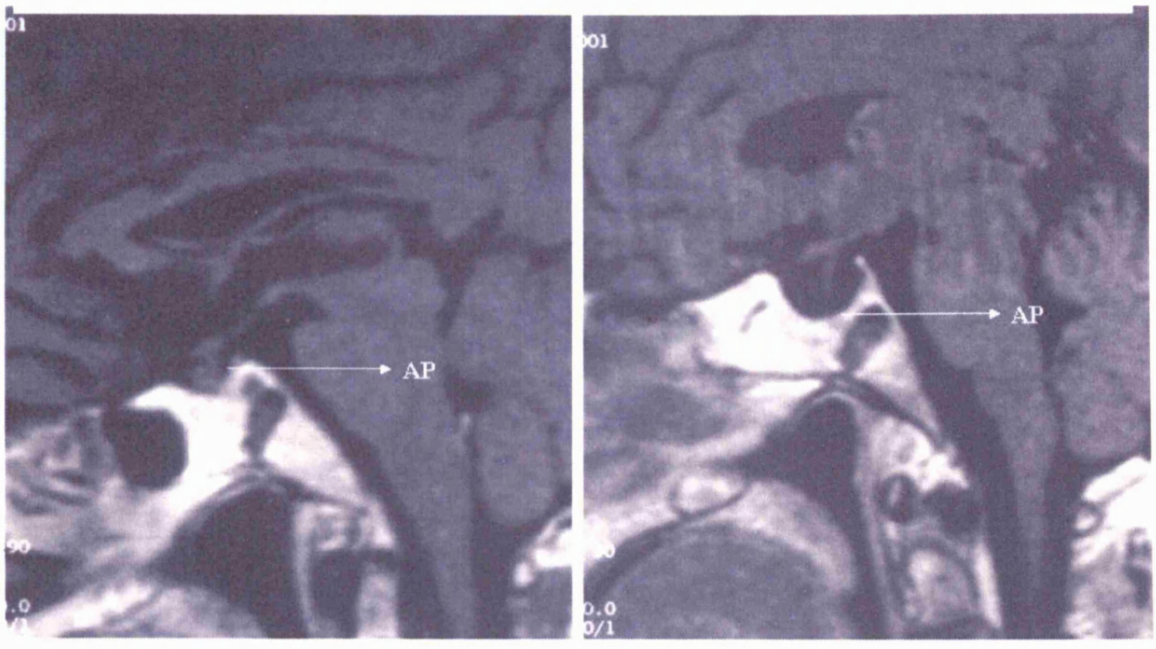


Figure 7.3: Magnetic resonance imaging in siblings IV.1 and IV.2 with 301-302ΔAG deletion in *PROP1* illustrating variability between genotype and phenotype within pedigrees. (i) Patient IV.1 (ii) Patient IV.2.

(i) (ii)



Four patients [patients II, IV.2, V.2 and VII.2] had a very thin rim of barely discernible anterior pituitary tissue, 2 of whom had an enlarged and 2 of whom had a normal pituitary sella. MR imaging of patient II is shown in Figure 7.2 (ii). Anterior pituitary tissue appeared dysgenetic with a shallow pituitary fossa in patient IV.1 but Mr imaging of his brother revealed a thin rim of anterior pituitary tissue within an enlarged pituitary sella [Figure 7.3].

The anterior pituitary in the remaining 5 patients (patients III, VII.1, VIII.1, VIII.2, VIII.3) was hypoplastic in appearance, although the sella was enlarged in patient VII.1.

Table 7.4: Clinical and endocrine data of patients with mutations in *PROP1*

	Ethnic origin	Sex	At diagnosis		Endocrinology								Mutation
			Height (SDS)	Age (years)	Peak GH (mU/L)	FT4 (pmol/L)	TSH (mU/L)	PRL (mU/L)	Cortisol (nmol/L)		Peak (U/L)		
									Basal	Peak	LH	FSH	
I	Asian	M	-4.5	9	2.1	6.7	2.2	322	393	693	0.7	0.2	112-124Δ
II	Asian	M	-7.3	29	0.6	5.3	1.6	136	499	997	0.2	0.5	112-124Δ
III.1	Caucasian	F	-6.0	6.6	1.5	Low	Low	78	379	N/A	N/A	N/A	150ΔA
III.2	Caucasian	M	N/A	N/A	Low	L	L	L	N	N	N/A	N/A	150ΔA
IV.1	Caucasian	M	-6.0	50	1.5	9.3	1.7	182	308	352	1.0	0.3	301-302ΔAG
IV.2	Caucasian	M	-6.2	55	D	5.3	1.3	126	L	138	2.5	0.9	301-302ΔAG
V.1	Asian	F	-5.1	10.5	2.4	L	6.1	100	N	N	2.3	3.0	112-124Δ
V.2	Asian	M	-5.7	7.4	1.2	L	4.7	72	59	352	0.1	0.2	112-124Δ
VI.1	Asian	M	-6.7	8.0	2.4	62*	4.0	N	N	N	L	L	112-124Δ
VI.2	Asian	F	-6.5	6.0	1.8	52*	3.0	N	N	N	L	L	112-124Δ
VII.1	Asian	M	-5.5	26.3	1.5	13.1	2.9	40	368	737	1.2	1.5	112-124Δ
VII.2	Asian	M	-6.2	22.3	1.5	8.0	3.1	38	158	598	0.4	0.5	112-124Δ
VIII.1	Caucasian	M	N/A	8.3	1.8	55.9*	1.9	36	269	585	0.6	0.6	301-302ΔAG
VIII.2	Caucasian	M	N/A	1.0	3.3	62.4*	0.8	256	490	740	0.6	0.1	301-302ΔAG
VIII.3	Caucasian	M	N/A	5.0	5.7	6.7	2.5	60	366	909	N/A	N/A	301-302ΔAG

N/A not available; * total thyroxine; M, male; F, female; GH, growth hormone; TSH, thyrotrophin; FSH, follicle stimulating hormone; LH, luteinising hormone; PRL, prolactin; L, low.

Table 7.5: Magnetic resonance [MR] imaging in patients with mutations in *PROP1*

	MR Imaging			
	Sella	**Anterior pituitary**	**Posterior pituitary**	**Pituitary stalk**
I	Enlarged	Enlarged	Normal	Normal
II	Enlarged	Thin rim	Normal	Normal
III.1	Normal	Hypoplastic	Normal	Normal
III.2	N/A	N/A	N/A	N/A
IV.1	Hypoplastic	Hypoplastic/dysgenetic	Normal	Normal
IV.2	Enlarged	Thin rim	Normal	Normal
V.1	N/A	N/A	N/A	N/A
V.2	Normal	Thin rim	Normal	Normal
VI.1	N/A	N/A	N/A	N/A
VI.2	N/A	N/A	N/A	N/A
VII.1	Enlarged	Hypoplastic	Normal	Normal
VII.2	Normal	Thin rim	Normal	Normal
VIII.1	Normal	Hypoplastic	Normal	Normal
VIII.2	Normal	Hypoplastic	Normal	Normal
VIII.3	Normal	Hypoplastic	Normal	Normal

N/A not available

7.4.2 Discussion

Mutations within *PROP1* are reported to be the commonest genetic cause of CPHD with incidence rates quoted between 50-100% in familial cases of CPHD (Deladoey et al. 1999; Mody et al. 2002). *PROP1* mutations were identified in 15 patients with hypopituitarism in this study cohort (6.4%) (Turton et al. 2005a). No mutations were identified in patients screened with IGHD or in those with morphological abnormalities of the posterior pituitary and/or pituitary stalk. The true prevalence of mutations in *PROP1* in patients with CPHD without posterior pituitary and pituitary stalk abnormalities was 11/37 (30%), as 37/233 patients screened had CPHD and MR available that did not demonstrate pituitary stalk or posterior pituitary abnormalities. Of 15 patients in whom mutations were identified, 13 patients would be classified as familial cases. As the cohort screened included 44 familial cases, the prevalence of *PROP1* mutations in sporadic hypopituitarism was only 1.1% (2/189) and increased to 29.5% (13/44) in familial hypopituitarism. The reason for the discrepancy in incidence of mutations between various reports is unclear. All mutations identified in the present study were homozygous, in keeping with other reports (Wu et al. 1998; Agarwal et al. 2000; Riepe et al. 2001). Eight patients from 5 pedigrees of Indian/Pakistani origin harboured an identical mutation (112-124Δ) suggesting the presence of a possible founder mutation in *PROP1* within the Indian subcontinent (Turton et al. 2005a).

The present findings, like other previously published studies, show marked phenotypic variability in patients with *PROP1* mutations. For example, patient VII.I displayed a borderline free thyroxine concentration with a normal serum TSH concentration despite all other patients demonstrating central hypothyroidism. Patients I, II, IV.1, IV.2 and VIII.1

showed probable early-onset gonadotrophin deficiency demonstrated by genital abnormalities at birth. Prolactin concentrations were highly variable ranging from 38–322 mU/L. Patients III.1, III.2, V.II, VII.I, VII.2, VIII.I and VIII.3 demonstrate prolactin deficiency with variable concentrations whilst patients 1, II, IV.1, IV.2, V.1, VI.1, VI.2, VIII.2 maintain normal concentrations to date. Three patients, V.2 on repeated assessment and two older patients, IV.1 and 1V.2, manifested frank cortisol deficiency as observed in some other patients with mutations in *PROP1* (Agarwal et al. 2000; Pernasetti et al. 2000; Vallette-Kasic et al. 2001). No other patient within this cohort harbouring the same 13 bp mutation as detected in patient V.2 demonstrated hypocortisolemia although it has been reported previously (Agarwal et al. 2000). The endocrine phenotype within families with the same mutation was also variable, as observed in pedigree VIII where only patient VIII.3 revealed evolving hormonal deficiency. There was a poor correlation between the genotype and neuroradiological phenotype between patients and within families with an identical mutation in *PROP1*. MR imaging of patient I revealed an enlarged pituitary gland that waxed and waned in size, not reported previously, before undergoing virtual complete involution 20 months after the first scan. Patients II and VII.1, with the same mutation as patient I.1, also had an enlarged sella but with a hypoplastic rim of anterior pituitary tissue that may possibly be explained by prior pituitary enlargement. Although evolving ACTH deficiency during a period of pituitary involution has been suggested (Pernasetti et al. 2000), ACTH deficiency was not evident in these 3 patients. Of 3 patients with ACTH deficiency, patient IV.1 had a hypoplastic anterior pituitary but an enlarged pituitary sella, which could be explained by prior pituitary enlargement and subsequent involution. The other 2 patients, however, demonstrated hypoplastic sellae.

7.5 MUTATIONS WITHIN *POU1F1*

7.5.1 Patient phenotypes

Eight patients [4 males, 4 females] belonging to 6 unrelated families, from a cohort of 129 patients screened, were found to harbour mutations in *POU1F1* yielding a frequency of 6.2% [Table 7.6, page 169]. Pedigrees I, II and III were Maltese in origin, patient IV and patients from pedigree V were from the UK and patient VI was Russian. The mean age at diagnosis [0.6 ± 0.8 years] was significantly [<0.05] earlier than that for patients with mutations in *PROP1*. Short stature [mean height -5.2 ± 1.9 SDS], poor growth and failure to thrive were the reasons for seeking medical attention in 4 patients [II, III, IV, V.2] while patients I.1, V.1 and VI were diagnosed due to prolonged unconjugated hyperbilirubinaemia. Patient I.2 was asymptomatic but was investigated at birth in view of the diagnosis of CPHD in his older brother.

A homozygous E230K mutation [substitution of a glutamate residue by lysine at position 230] was found in patients II and III. Both brothers from pedigree I were compound heterozygotes for two mutations: E230K inherited from the mother and R172Q [substitution of an arginine residue by glutamine at position 172] inherited from the father. Mutational analysis of *POU1F1* in patient VI also revealed compound heterozygosity: the E230K mutation [inherited from the mother and maternal grandmother] and insA791 in exon 6 of the gene, the latter either inherited from the father [paternal DNA not available] or a de novo mutation. Patients IV, V.1 and V.2 were heterozygous for a R271W point mutation.

CPHD was present in all 8 patients. Profound GHD was diagnosed in all 7 patients tested, with a mean peak serum GH concentration of 1.4 ± 1.2 mU/L. Patient I.2 has not, as yet, had GH provocation testing. The diagnosis of GHD was made within 3 years in all 7 patients, and within the first year of life in 3/7 patients.

TSH deficiency manifested itself earlier than GHD in 4 patients. Compared with patients with mutations in *PROP1* [2.8 ± 1.5 mU/L], the mean serum TSH concentration in patients with mutations in *POU1F1* [0.8 ± 0.9 mU/L] was significantly lower [p=0.002]. TSH deficiency was present in 7/8 patients and had manifested itself within the first year of life in all 7 patients. Patient II had a borderline free thyroxine [FT4] when investigated for failure to thrive at 4 months of age. Although diagnosed with profound GH deficiency at the age of 2.4 years, her TRH test was normal. Repeat dynamic pituitary function testing at 16.3 years off all treatment confirmed GH, partial TSH and prolactin deficiencies. She is now aged 20.5 years with a serum FT4 concentration that is normal at 15.6 pmol/L without any thyroxine treatment.

No patient was confirmed to have cortisol or gonadotrophin deficiencies although 2 patients received hydrocortisone replacement treatment and one patient had puberty induced, temporarily. Patient IV demonstrated borderline hypocortisolaemia [unstimulated and peak serum cortisol concentrations of 335 nmol/L and 426 nmol/L respectively] at the age of 2 years when the diagnosis of severe GH and prolactin deficiencies was made. Hydrocortisone replacement was commenced at the age of 6.5 years in view of symptoms of fatigue in conjunction with previously documented borderline cortisol insufficiency. He also had bilaterally undescended testes and was presumed to be gonadotrophin deficient and puberty

was induced at 11 years of age with Sustanon treatment. However, when re-investigated off all replacement treatment at the end of his statural growth, GH, TSH and prolactin deficiencies were re-confirmed but he mounted a satisfactory serum cortisol response to insulin-induced hypoglycaemia [626 nmol/L] and a normal gonadotrophin response to LHRH [LH 15.1 U/L, FSH 5.8 U/L] with a serum testosterone concentration of 15 nmol/L. Patient V.2 also demonstrated a partial cortisol response to metyrapone at 11 years of age that resulted in substitution with cortisone acetate treatment. When full dynamic pituitary testing was repeated as an adult off all replacement treatment, GH and TSH deficiencies were re-confirmed but cortisol secretion was normal.

The mean serum prolactin concentration in patients was 48.5 ± 48 mU/L and was again significantly lower [p=0.05] than that of patients with mutations in *PROP1* [120.5 ± 91 mU/L]. Patients III and V.2 had borderline low unstimulated serum prolactin concentrations. In spite of that patient V.2 presented with failure of lactation and an absent prolactin response to stimulation with a TRH test confirming prolactin deficiency.

Results of MR imaging were available in 7/8 patients [Table 7.7, page 170]. Neuroimaging revealed normal posterior pituitary, pituitary stalk, septum pellucidum, corpus callosum and optic nerve morphology in all 7 patients. The anterior pituitary was hypoplastic in 6/7 patients and normal in patient V.1.

Table 7.6: Clinical and endocrine data of patients with mutations in *POU1F1*.

	Ethnic origin	Sex	Presentation		Endocrinology								Mutation
			Height (SDS)	Age (years)	Peak GH (mU/L)	FT4 (pmol/L)	Unstimulated		Cortisol (nmol/L)		Peak (U/L)		
							TSH (mU/L)	PRL (mU/L)	Basal	Peak	LH	FSH	
I.1	Caucasian	M	-3.6	0.1	3.9	5.2	0.1	14	675	1380	29.8	3.9	E230K / R172Q
I.2	Caucasian	M	-2.3	0.1	N/T	2.6	0.1	10	N/T	N/T	N/T	N/T	E230K / R172Q
II	Caucasian	F	-5.8	2.4	0.3	15.6	0.3	14	578	745	40.1	6.1	E230K
III	Caucasian	M	-5.6	1.0	2.1	7.8	2.7	120	543	895	N/T	N/T	E230K
IV	Caucasian	M	-7.8	0.5	0.9	37.7*	1.0	44	464	626	15.1	5.8	R271W
V.1	Caucasian	F	N/A	0.3	0.9	9.1	0.9	50	300	1150	12	30	R271W
V.2	Caucasian	F	N/A	0.2	0.9	20.5*	1.0	124	N/A	725	30	5.0	R271W
VI	Caucasian	F	-5.9	0.3	<0.7	Low	0.1	12	430	N/A	N/A	N/A	E230K / insA778

N/A not available; * total thyroxine; M, male; F, female; GH, growth hormone; TSH, thyrotrophin; FSH, follicle stimulating hormone; LH, luteinising hormone; PRL, prolactin; AP, anterior pituitary; PP posterior pituitary; H, hypoplastic; N, normal

Table 7.7: Magnetic resonance [MR] imaging in patients with mutations in *POU1F1*.

	MR Imaging		
	Anterior pituitary	**Posterior pituitary**	**Pituitary stalk**
I.1	Hypoplastic	Normal	Normal
I.2	N/A	N/A	N/A
II	Hypoplastic	Normal	Normal
III	Hypoplastic	Normal	Normal
IV	Hypoplastic	Normal	Normal
V.1	Normal	Normal	Normal
V.2	Hypoplastic	Normal	Normal
VI	Hypoplastic	Normal	Normal

N/A not available

7.5.2 Discussion

The incidence of *POU1F1* mutations in patients with GH, TSH and prolactin deficiencies may be as high as 50% (Brown et al. 1998). The present study suggests that the prevalence of mutations in *POU1F1* in patients with sporadic hypopituitarism is 3.8% [4 of 105] but increased to 16.7% [4 of 24] in familial cases of CPHD as 24 of 129 patients screened had evidence of familial disease. No mutation was identified in patients with IGHD or those with abnormalities of the posterior pituitary and pituitary stalk.

A novel mutational "hot-spot" within *POUIF1* [E230K] was identified in 5 individuals from 4 different pedigrees. Of these, 3 pedigrees originated from Malta, suggesting that a founder effect cannot be excluded. Two novel mutations [ins778A and R172Q] were also identified in compound heterozygosity with E230K (Turton et al. 2005b).

Phenotypic variability in patients with *POU1F1* mutations, as with mutations in *PROP1*, was present mainly with respect to the presence of central hypothyroidism and prolactin deficiency. Deficiencies of GH, TSH and prolactin were more severe in patients with mutations in *POU1F1* as compared with mutations in *PROP1* as was the age at diagnosis. All patients with *POU1F1* mutations in the present study tested for GHD showed profound GH deficiency. Previous reports have suggested that TSH deficiency is invariably associated with mutations within *POU1F1* (Pfaffle et al. 1999). Patient II with an identical genotype to Patient III [E230K] had preserved thyroxine secretion until 20.5 years of age unlike Patient III who developed TSH deficiency at 1.0 year of age. Prolactin deficiency was not complete in all patients. Six patients demonstrated severe prolactin deficiency while the remaining 2

patients had a serum prolactin concentration in the lower range of normal at 120 mU/L and 124 mU/L. The latter patient, however, presented with failure of lactation illustrating a possible role of hormone-receptor interaction resulting in this inter-individual variation in phenotype. In contrast to patients with mutations within *PROP1*, patients with mutations within *POU1F1* did not manifest gonadotrophin and cortisol deficiency.

Whereas MR scanning revealed a small, normal or enlarged anterior pituitary in patients with mutations within *PROP1,* the anterior pituitary was either hypoplastic or normal in patients with mutations within *POU1F1*. Two patients were successfully weaned off hydrocortisone treatment following mutational screening. These findings illustrate the importance of careful phenotypic characterization and genetic analysis in patients with hypopituitarism.

7.6 MUTATIONS WITHIN *GH1*

7.6.1 Patient phenotypes

14 patients [11 males, 3 females] from 7 unrelated families, of 103 patients screened, were found to have mutations within the *GH1* gene yielding a frequency of 13.6% [Table 7.8, page 175]. Apart from patient IV, all patients had an affected family member.

Three brothers from pedigree II, from Pakistan, harboured a 6.7 Kb homozygous deletion in the *GH1* gene indicating an autosomal recessive mode of inheritance. Mutational analysis revealed heterozygous intronic point mutations, predicted to result in abnormal splicing, in the majority of the remaining patients: IVS3 +2nt in patient I.1 and her son I.2, IVS3 +6nt in patient III.1 and her father III.2, and IVS3 +1nt in patient IV, VI.1 and his son VI.2 , VII.1 and his mother VII.2. Patient V.1 and his son V.2 were found to harbour a heterozygous point mutation [A>C] resulting in substitution of a glutamate residue by alanine at the 2nd base of exon 3, in an exon splice enhancer, likely to result in aberrant splicing as well. These heterozygous mutations indicated an autosomal dominant mode of inheritance resulting in type II IGHD. All patients with these splice site mutations, apart from pedigree I from Denmark, were from the UK.

All 14 patients presented with short stature and poor growth, at a mean age of 4.7 ± 3.8 years with a mean height of –6.2 ± 1.7 SDS. Patient VI.1 was diagnosed with IGHD in childhood for which he received GH treatment from the age of 7.5 years until 18 years of age. He continued to be GHD as an adult with low serum IGF1 concentrations and an elevated serum cholesterol. However, he was more recently found to develop CPHD with

infertility secondary to gonadotrophin deficiency [low basal serum gonadotrophin and serum testosterone concentrations], a borderline cortisol response and prolactin deficiency suggesting evolving hormonal deficiencies. No other patient has demonstrated abnormalities of any other pituitary hormones and five patients have reached adulthood.

Patient V.1 was diagnosed with IGHD at the age of 7.3 years with a peak serum GH concentration of 4.2 mU/L. He has since demonstrated fluctuating serum GH concentrations on longitudinal testing. He was treated with pituitary-derived hGH between the ages of 8.3 years to 9.7 years, however, given the association between Creutzfeldt-Jacob disease with pituitary-derived GH, the latter was discontinued. He was re-commenced on recombinant hGH at 10.1 years of age until 16.2 years of age. Repeat GH provocation testing at 16.6 years, 19.9 years and 29 years revealed GH concentrations of 8.4 mU/L, 24.6 mU/L and < 0.8 mU/L. His serum IGF1 concentrations have however been very low. Similar to his endocrine findings, results of his neuroimaging have been conflicting. A CT scan at 7.3 years of age was reportedly normal. A repeat CT scan performed at 16.2 years of age revealed anterior pituitary hypoplasia and a MR scan performed at 25 years of age was reported to be normal.

Apart from patient V.1 with the unusual MR imaging and patient IV whose MR imaging revealed an empty pituitary sella with extension of chiasmatic cistern into the sella, the anterior pituitary in the remaining patients was either hypoplastic [patients III.1, III.2, V.2, VI.1, VI.2 and VII.1] or normal [Patient I.1, Table 7.8, page 175]. There were no abnormalities within the posterior pituitary, pituitary stalk, septum pellucidum, corpus callosum or optic nerves.

Table 7.8: Clinical features, endocrinology and magnetic resonance imaging in patients with mutations in *GH1*.

	Sex	Ethnic origin	Presentation			Endocrinology								Anterior pituitary on imaging	Mutation
			Age (yrs)	Height (SDS)	Weight (SDS)	Peak GH (mU/L)	Basal TSH (mU/L)	FT4 (pmol/L)	PRL (mU/L)	Cortisol (nmol/L)		Peak (U/L)			
										Basal	Peak	LH	FSH		
I.1	M	Caucasian	1	-4.6	N/A	0.7	N	N	N	N	N	N/T	N/T	N	IVS3+2nt
I.2	F	Caucasian	8	N/A	N/A	D	N	N	N	N	N	N	N	N/A	IVS3+2nt
II.1	M	Asian	4.0	-8.5	-6.6	D	N	N	N	N	N	N/T	N/T	N/A	6.7Kb deletion
II.2	M	Asian	6.0	-8.6	-8.5	D	N	N	N	N	N	N/T	N/T	N/A	6.7Kb deletion
II.3	M	Asian	3.0	-8.1	-6.2	D	N	N	N	N	N	N/T	N/Y	N/A	6.7Kb deletion
III.1	F	Caucasian	1.25	-6.1	-5.0	<0.3	2.3	101*	N	155	718	N	N	H	IVS3+6nt
III.2	M	Caucasian	14.8	-7.2	-3.3	9.2	2.5	13.3	106	N	831	9.3	24.4	H	IVS3+6nt
IV	M	Caucasian	1.6	-5.4	-5.0	3.9	1.5	16	260	474	1194	N	N	A	IVS3+1nt
V.1	M	Caucasian	7.3	-4.3	-3.7	4.2	N	N	700	N	N	N	N	N	E58A/E32A
V.2	M	Caucasian	5.7	-5.0	N/A	4.2	N	N	N	N	N	N/T	N/T	H	E58A/E32A
VI.1	M	Caucasian	2.3	-4.4	-3.2	<0.6	0.9	16	90	388	504	L	L	H	IVS3 +1nt
VI.2	M	Caucasian	7.5	N/A	N/A	2.1	N	N	N	N	N	N	N	H	IVS3 +1nt
VII.1	M	Caucasian	1.5	N/a	N/a	5.4	N	N	N	N	N	N/T	N/T	H	IVS3 +1 nt
VII.2	F	Caucasian	2.5	N/A	N/A	D	N	N	N	N	N	N	N	N/A	IVS3 +1 nt

N/A not available; N/T, not tested; * total thyroxine; M, male; F, female; GH, growth hormone; TSH, thyrotrophin; FSH, follicle stimulating hormone; LH, luteinising hormone; PRL, prolactin; D, deficient; N, normal; H, hypoplastic; A, absent.

7.6.2 Discussion

IGHD may be inherited in an autosomal recessive [type I GHD], autosomal dominant [type II GHD] or X-linked recessive [type III GHD] manner. Type 1A, type II and rarely, type 1B can be due to mutations in *GH1* gene. Type 1B, is however, more commonly due to mutations in *GHRHR*. Some forms of the X-linked type of GHD are now believed to be due to mutations in *SOX3*. Heterozygous mutations in *HESX1* can also lead to IGHD (Brickman et al. 2001; Thomas et al. 2001).

14 patients from a cohort of 103 patients with IGHD were found to harbour mutations in *GH1* resulting in a prevalence of 13.6%. 32/103 patients had a history of familial GHD and hence the prevalence of *GH1* mutations in familial GHD increased to 40.6%. No patients with IGHD within the present study were found to harbour mutations in *GHRHR*. Splice site mutations in *GH1* were the commonest [64.3% of all mutations] resulting in type II autosomal dominant GHD. Additionally both patients in pedigree V harboured a heterozygous point mutation (A>C) resulting in the substitution of a glutamate residue by alanine at the 2nd base of exon 3. Although this potentially results in the substitution of an amino acid, it is more likely that the phenotype is due to aberrant splicing. Splice site and exon splice enhancer mutations within exon 3 can lead to a total skipping of exon 3 and loss of amino acids 32-71 from the mature 22kD GH protein. This leads to a truncated 17.5kDa product which has a dominant negative effect preventing the production, storage and release of the normal wild-type 22kDa hGH from the other allele with a consequent deleterious effect on pituitary somatotrophs. The 17.5kDa isoform is retained in the endoplasmic reticulum, disrupts the Golgi apparatus and reduces the stability of the 22kDa isoform,

which becomes unavailable for exocytic release (Hayashi et al. 1999; Graves et al. 2001). This phenomenon is further accelerated by the GHRH drive, macrophage response to the dying defective somatotrophs and the type of mutation, most effective in the IVS3+1 splice site mutation. Patients with this type of IGHD show variably reduced serum GH concentrations but usually respond well to exogenous GH therapy. Patient V.I demonstrated variable concentrations of GH at different times of provocation testing. This could be as a result of this dominant negative effect, which is accentuated when the patient is off-GH treatment. The assay would pick up greater concentrations of the mutant 17.5kDa GH molecules along with reduced concentrations of the normal 22kD protein, provided that the 17.5kDa form of hGH is secreted from the pituitary gland and this remains to be proven. The variability in the ratios of 17.5kDa to 22kDA GH proteins produced could also be an important contributor to the extent of pituitary gland damage and hence the variability observed in the anterior pituitary size in these patients.

The phenomenon of evolving CPHD in patients with type II IGHD was evident in patient VI.1 [Turton et al., in press] and it has been recently documented in a murine model as well as other human patients (McGuinness et al. 2003; Mullis et al. 2005; Salemi et al. 2005). The phenomenon of evolving CPHD could be attributed to an invasion by activated macrophages leading to a significant bystander endocrine cell killing, which in time compromises cellular repletion of other cell lineages and ultimately to additional endocrine deficits, as observed in transgenic mice. Early treatment with rhGH in these patients may prevent the progressive dysfunction of the somatotrophs and possibly other cell lines by suppressing the GHRH drive and hence production of the mutant 17.5 kD protein, although this remains to be proven.

7.7 MUTATIONS WITHIN *SOX3*

7.7.1 Patient phenotypes

Mutations in *SOX3* were found in 5.3% of selected males with hypopituitarism. Inheritance was X-linked recessive. A submicroscopic duplication of Xq27.1 including the *SOX3* gene, the smallest reported to date (685.6 Kb), was found in one pedigree from Finland, which 2 half-brothers had inherited from their unaffected mother. An expansion by 7 alanines of the first polyalanine tract was identified in a consanguineous Asian family from Qatar with 3 affected males ($A_{(7)}$240^241 ins) leading to reduced transcriptional activation and impaired nuclear localisation of the mutant protein.

Pedigree I: Two half-brothers, patients I.1 and I.2, had a history of hypoglycaemia at birth. Patient I.1 then remained asymptomatic until 7 years of age when he presented with short stature [[Table 7.9, page 181]. He was diagnosed with GHD, borderline serum FT4 concentrations [6.6-13.3 pmol/L] with a serum TSH concentration of 2.8 mU/L, but normal serum prolactin and cortisol concentrations. He underwent spontaneous puberty. His half-brother patient I.2 was found to have severe cortisol, TSH, GH and gonadotrophin deficiency at 2 months of age [height –3.8 SDS]. He had hypoplastic genitalia with undescended testes and a micropenis. The anterior pituitary was hypoplastic in both patients on MR imaging and the posterior pituitary was undescended in patient I.2 and partially descended in patient I.1 [Table 7.10, page 182]. The pituitary stalk was hypoplastic in its lower half in patient I.1 but absent in patient I.2. A cyst was noted within the splenium of the corpus callosum only in the patient I.1 while the septum pellucidum was normal in both [Figure 7.4]. Psychomotor development, apart from hyperactivity in patient I.2, was normal.

Pedigree II: These siblings were born to first-degree consanguineous Qatari parents. All 3 brothers, patients II.1, II.2, and II.3, presented with short stature at 3.0, 4.5 and 2.7 years with heights of -2.5 SDS, –2.5 SDS and –1.3 SDS [Table 7.9, page 181]. All 3 brothers were found to have undescended testes; the younger 2 patients also had a micropenis. All 3 patients had GH, TSH, ACTH and gonadotrophin deficiencies. An evolving hormone deficiency was observed only in patient II.1 with GHD diagnosed at presentation, followed by TSH deficiency at 6.2 years and gonadotrophin and ACTH deficiencies at the age of 15 years. Patient II.2, however, was diagnosed with GH, ACTH and TSH deficiencies at 6.0 years of age. A 3-month course of Sustanon led to an increase in the size of the phallus but he required pubertal induction at 13.5 years of age in view of gonadotrophin deficiency. Patient II.3 was found to have GH, TSH and ACTH deficiencies at initial investigations. Although the testes descended into the scrotum in response to 3 weeks of human chorionic gonadotrophin treatment, the serum testosterone response was poor. He too required pubertal induction. Neurodevelopment of all brothers is normal. A CT scan was reported as being normal in patient II.1. MR imaging of the younger brothers, patients II.2 [Figure 7.4 (iii)] and II.3 [Figure 7.4 (iv)] revealed a hypoplastic anterior pituitary in the sella that was severely attenuated in II.2, a hypoplastic stalk that was particularly difficult to visualize in II.3 and an undescended posterior pituitary in both [Table 7.10, page 182].

Their heterozygous mother is of normal height. A further male sibling presented at 9 years of age with GHD [peak GH 8.4 mU/L] and probable gonadotrophin deficiency [peak serum LH and FSH < 0.5 IU/L]. Serum FT4 [13.3 pmol/L], prolactin [170 mU/L] and basal cortisol [335 nmol/L] concentrations were normal. DNA was unavailable from this child.

Figure 7.4: Variability in magnetic resonance imaging in patients with abnormalities within *SOX3*. (i) Patient 1.I (ii) patient 1.II (iii) patient 2.II (iv) patient 2.III

(i)

(ii)

(iii)

(iv)

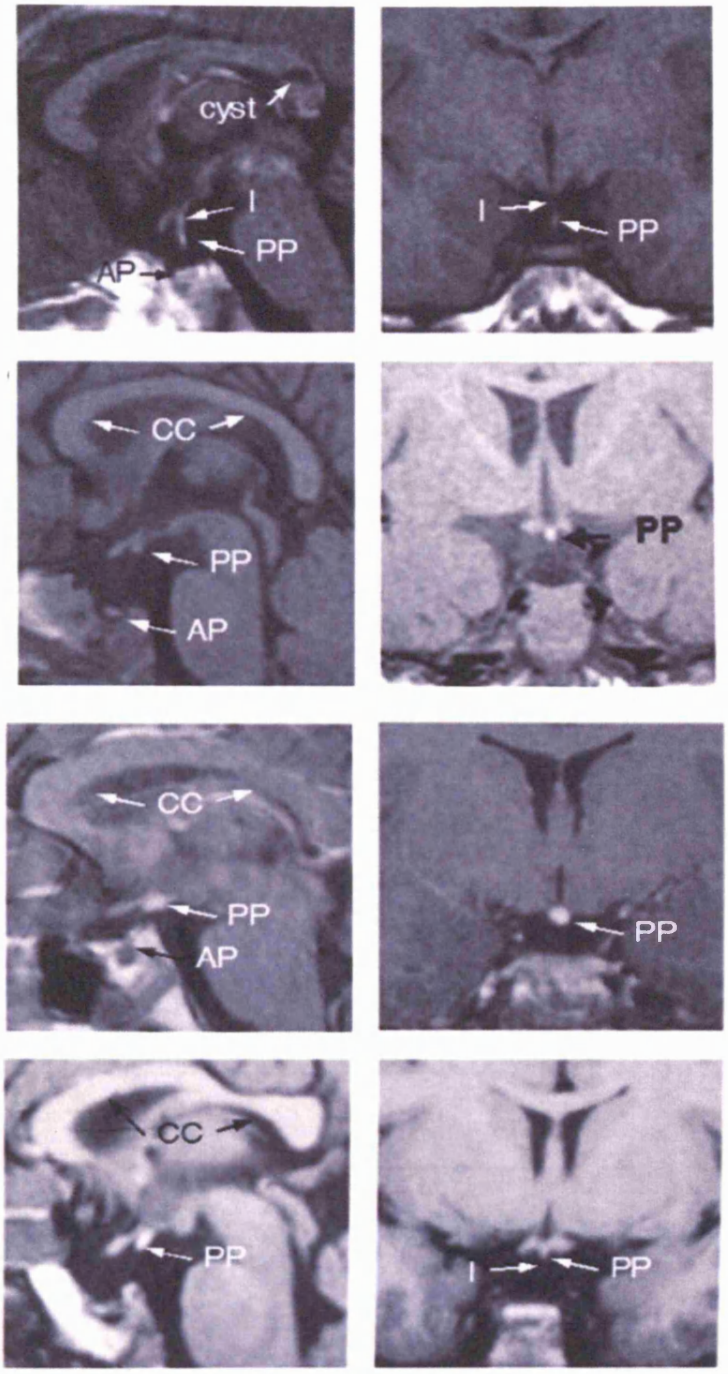


Table 7.9: Clinical and endocrine data of patients with abnormalities within *SOX3*.

	Sex	Ethnic origin	Presentation			Endocrinology							Mutation
			Age (yrs)	Height SDS	Weight SDS	Peak GH mU/L	FT4 pmol/L	Basal		Peak			
								TSH mU/L	PRL mU/L	Cortisol nmol/L	LH U/L	FSH U/L	
I.1	M	Caucasian	7.0	-2.8	-0.9	3.0	13.3	2.8	124	934	5.0	4.5	685.6 Kb Xq27.1duplication
I.2	M	Caucasian	0.2	-3.8	-1.5	3.9	8	0.01	1710	70	0.8	0.5	685.6 Kb Xq27.1duplication
II.1	M	Asian	3.0	-2.5	N/A	5.1	6.6	0.08	N/A	55	L	L	$A_{(7)}$240^241 ins
II.2	M	Asian	4.5	-2.5	N/A	2.1	9.3	3.3	146	108	0.5	0.5	$A_{(7)}$240^241 ins
II.3	M	Asian	2.7	-1.3	0.7	2.1	6.6	2.8	N/A	88	1	<1	$A_{(7)}$240^241 ins

M, male; F, female; GH, growth hormone; TSH, thyrotrophin; PRL, prolactin; FSH, follicle stimulating hormone; LH, luteinising hormone; AP, anterior pituitary; PP posterior pituitary; SP, septum pellucidum; CC, corpus callosum; N/A, not available; L, low; H, hypoplastic; N, normal; U, undescended; P, partial; A, absent;

Table 7.10: Magnetic resonance imaging in patients with abnormalities within *SOX3*.

	Anterior pituitary	Posterior pituitary	Pituitary stalk	Corpus callosum	Septum pellucidum
I.1	Hypoplastic	Partially undescended	Partially hypoplastic	Cyst in the splenium	Normal
I.2	Hypoplastic	Undescended	Absent	Normal	Normal
II.1	N/A	N/A	N/A	N/A	N/A
II.2	Hypoplastic	Undescended	Hypoplastic	Normal	Normal
II.3	Hypoplastic	Undescended	Hypoplastic	Normal	Normal

N/A not available

7.7.1 Discussion

X-linked hypopituitarism is a rare and variable condition. Recent studies implicated duplications at Xq26-27 in the aetiology of this form of hypopituitarism (Hamel et al. 1996; Hol et al. 2000; Solomon et al. 2002). The duplication found in pedigree I refines the critical interval to a 685.6 Kb region containing *SOX3* and two other transcripts not expressed in the infundibulum (Woods et al. 2005). Overdosage as a result of duplications, and underdosage as a result of a polyalanine expansion, within *SOX3,* are both associated with similar phenotypes, predominantly that of infundibular hypoplasia with variable effects on the corpus callosum suggesting that gene dosage of *SOX3* is critical for the normal development of the diencephalon and the infundibulum in humans.

Five patients [from 2 pedigrees] of 78 patients screened were found to have mutations in *SOX3,* indicating a mutation frequency of 5.3%. The underlying mechanism whereby both under- and over-dosage of *SOX3* can lead to a disease phenotype remains, as yet, unknown. Mice deleted for *Sox3* are affected by a variable reduction in growth rate, pituitary concentrations of GH, TSH, LH and FSH and display craniofacial defects with dysgenesis of the corpus callosum (Rizzoti et al. 2004). The similarity between the null mutant mice and phenotype of patients described suggest that, both, the polyalanine expansion and duplication in *SOX3* results in a loss of function, though a gain of function effect cannot be excluded in the latter case. Anterior pituitary hypoplasia and an undescended posterior pituitary possibly reflect the effects of infundibular hypoplasia.

There was variability in both the endocrine phenotype and findings on MR imaging in patients and within families with abnormalities of *SOX3,* although the variability in phenotype between the two half-brothers in pedigree I may reflect differences in genetic background from their different fathers. Patient I.1 had a milder phenotype with a later age at onset of hypopituitarism in contrast to his brother. Patient I.1 also had variable thyroxine concentrations that may highlight the possibility of evolving TSH deficiency. The genotype - phenotype correlation in pedigree II was more complete, possibly related to the genetic homogeneity of the consanguineous union of their parents.

None of the patients manifested any craniofacial defects or evidence of mental retardation as previously reported in a pedigree with a longer polyalanine expansion (Laumonnier et al. 2002). The lack of learning difficulties in patients from the present study could be explained by differences in the genotype or alternatively may reflect variation in the penetrance of the central nervous system defects as shown in the murine model (Rizzoti et al. 2004).

CHAPTER 8

EVALUATION OF PITUITARY HORMONE SECRETION

8.1 INTRODUCTION

Evaluation of pituitary hormone secretion is essential in the following settings:

- in a patient with known pituitary dysfunction of one hormone abnormality to evaluate rest of the pituitary function,
- in the follow-up of a patient with combined pituitary hormone deficiency [CPHD] to assess for evolving endocrine disease,
- in a new patient suspected of having hypopituitarism and/or
- in a patient "at-risk" of hypopituitarism with abnormalities such as optic nerve hypoplasia [ONH].

Secretion of hormones is a complicated process in which the hypothalamo-pituitary [H-P] - target gland axis is subject to complex feedback mechanisms. The diagnosis of hypopituitarism in patients is not straightforward and a simple assessment of unstimulated hormonal secretion is often not adequate to make a precise diagnosis. The H-P-target gland axis can be activated by various pharmacological and physiological stimuli that have formed the basis of tests used in the evaluation of hormone secretion and have been extensively studied. Previous studies have attempted to differentiate patients as having "pituitary" disease strictly implying a quantitative abnormality associated with low pituitary hormone secretion and an absent or blunted response to external stimulation, with "hypothalamic" disease postulated to be a qualitative abnormality due to a prolonged lack of hypothalamic

releasing hormones resulting in reduced pituitary cell content and a delayed rise in pituitary hormone concentration on stimulation (Milner and Herber 1983). However, separating patients based on this cellular differentiation is not always possible. "Provocative" tests have numerous other limitations as well. The tests are expensive, complicated to perform, uncomfortable to the patient, require supervision in specialized endocrine centres with trained personnel, detailed protocols and require good laboratory facilities. Interpretation of hormone concentrations following stimulation needs to be made in light of the hormonal assay in use and endogenous hormonal rhythmicity. Some provocative agents used act at the upper end of the dose response curve and are influenced by external factors altering hormone response. Provocative testing with some agents reflects a non-physiological milieu that may bear no resemblance to the endogenous state of the patient. Finally, there is a lack of a "gold standard" test in detecting several hormone deficiency states resulting in difficulties with comparison of test performance.

The diagnosis of growth hormone deficiency [GHD] in children has always been fraught with problems. The GH-insulin like growth factor 1 [IGF1] axis can be stimulated with various provocative agents such as insulin, glucagon, arginine, and clonidine. Various cut-off's have been used to define GHD generally at a value of 20 mU/L based on test performance and likelihood of detecting serious deficiency. Some provocative tests have poor specificity but overall the insulin induced hypoglycaemia [IIH] test is considered as the "gold standard" for assessing both the GH-IGF1 axis and the H-P-adrenal axis. However, the test can be extremely unsafe in infants and young children (Shah et al. 1992; Hurel et al. 1996). Glucagon stimulation induces GH and cortisol release although the exact mechanism remains obscure and may be related to a glucagon-induced catecholamine release (Goodwin

et al. 1976) or an indirect effect via the hypothalamus. A good correlation has been reported previously between peak serum GH and cortisol concentrations in response to stimulation with glucagon and IIH (Spathis et al. 1974; Orme et al. 1996). Chanoine et al reported asymptomatic hypoglycemia at least once between 120 and 180 minutes in 11% of infants who underwent glucagon provocation and the elevation in serum GH and cortisol concentrations may therefore reflect response to hypoglycaemia (Chanoine et al. 1995). However, the glucagon test is also known to be potentially hazardous and should be performed with caution (Shah et al. 1992). To resolve some of the controversies surrounding the diagnosis of GHD, the Growth Hormone Research Society and National Institute for Clinical Excellence have both set guidelines for its diagnosis.

In contrast to the diagnosis of GHD, there is no overall consensus for the diagnosis of thyrotrophin [TSH], corticotrophin [ACTH] and gonadotrophin deficiency and the diagnosis can become particularly difficult in patients with borderline results. TSH releasing hormone [TRH] releases preformed TSH from the pituitary into the circulation and also increases TSH synthesis. Administration of TRH in normal individuals produces a consistent rise in serum TSH concentration with a peak concentration at 20 minutes followed by a decrease in measured concentrations at 60 minutes. The test has been used as an adjunct in the diagnosis of central hypothyroidism [CH]. An absent or impaired response is believed to be indicative of primary pituitary disease and a prolonged lack of TRH in patients with hypothalamic disease has been postulated to result in a delayed rise in serum TSH concentration. The optimal method for establishing the diagnosis of ACTH deficiency also remains unclear inspite of its potentially serious consequences such as hypoglycaemia, consequent brain damage and even death particularly in newborns and infants. The standard Synacthen test

(SST) was first used in clinical practice as a direct measure of adrenal function (Wood et al. 1965) It has since, by implication, been used to evaluate H-P-adrenal axis integrity in patients with H-P disorders, with good correlation between peak serum cortisol concentrations at 30 minutes after Synacthen and those achieved in response to the IIH test, considered to be the "gold-standard" (Kehlet et al. 1976; Nelson and Tindall, Jr. 1978; Lindholm et al. 1978; Lindholm and Kehlet 1987; Stewart et al. 1988; Jackson et al. 1994; Hurel et al. 1996; Abdu et al. 1999; Gonzalbez et al. 2000). Unlike the IIH test, the SST is safe and relatively easy to perform in young infants, although it has been reported to generate normal responses in patients with subtle adrenal insufficiency (Cunningham et al. 1983; Broide et al. 1995). Previous data have suggested that unstimulated serum luteinising hormone [LH] and follicle stimulating hormone [FSH] concentrations are not reliable parameters to test pituitary gonadotrophin reserve particularly in children (Lovrencic et al. 1975; Dickerman et al. 1979; Kletter et al. 1996). Pituitary stimulation with LH releasing hormone (LHRH) is an established method of assessing gonadotrophin reserve in adults with hypopituitarism and at puberty, although conflicting results have been well documented in distinguishing adolescents with hypogonadotrophic hypogonadism [HH] and constitutional delay of puberty (Job et al. 1977; Ehrmann et al. 1989; Ghai et al. 1995; Kauschansky et al. 2002). Post-LHRH measurement of serum reproductive hormones in infants with H-P disorders may help to identify those with gonadotrophin deficiency at an early stage.

This part of the study was undertaken in order to assess the usefulness of currently used tests to diagnose hormone deficiency.

8.2 THYROTROPHIN [TSH] DEFICIENCY

Serum TSH and thyroxine [T4] concentrations of 54 patients with central hypothyroidism [CH] were analysed retrospectively. In order to determine whether TRH tests have a role to play in supporting the diagnosis and in differentiating between "pituitary" and "hypothalamic" CH, serum TSH responses to stimulation with TRH were retrospectively analysed in 30 of 54 patients.

8.2.1 Patient Selection

Diagnosis of CH was based on subnormal serum T4 concentrations [free T4 (FT4) <12.0 pmol/L or total T4 (TT4) <65 nmol/L] with either an inappropriately low serum TSH concentration [<5 mU/L], thereby ruling out primary hypothyroidism, or biochemical evidence of other pituitary hormone deficiencies.

Based on findings on their MR scans, they were divided into 2 groups:

- Group A (n=24); patients with no midline forebrain defects [MFD; Appendix V-6],
- Group B (n=30); patients with MFD [Appendix V-7].

The TRH test was performed according to the standardised protocol within the department [See Appendix IV].

8.2.2 Methods and controls

Unstimulated serum TSH concentrations of patients were compared with that of:

(1) 93 short normal children [mean age of 9.9 (± 3.0) years (M:F 1:1)] who were investigated for short stature between 1985-1992 with normal results. These children were followed-up until adulthood to ensure normal growth and spontaneous puberty.

(2) 38 consecutive newborns with congenital primary hypothyroidism.

30 of 93 normal children underwent a TRH test. The 10^{th} and the 90^{th} centiles of the ΔTSH [difference between the peak stimulated and unstimulated serum TSH concentrations] in these children were 4.5 mU/L and 17.8 mU/L respectively and were arbitrarily selected to delineate a "normal" range.

Based on the "normal" range, serum TSH responses of patients were grouped as:

- absent or blunted response (ΔTSH< 4.5 mU/L) with peak serum TSH at 20 minutes
- normal response (ΔTSH 4.5 – 17.8 mU/L) with peak serum TSH at 20 minutes
- exaggerated response (ΔTSH > 17.8 mU/L) and peak serum TSH at 20 minutes
- delayed response with peak serum TSH concentration at 60 minutes.

Table 8.1: Clinical characteristics of patients with central hypothyroidism [CH].

Group A, patients with CH and no midline forebrain defects (MFD); Group B, patients with CH and MFD.

	n=	M:F ratio	Age at first presentation (yrs)	Age at diagnosis of CH (yrs)	Patients with evolving CH (n=)	FT4 (n=44, pmol/L)	TT4 (n=10, nmol/L)	Unstimulated TSH (mU/L)	Number of patients with deficiencies of			
									GH	ACTH	Gn	AVP
Group A	24	3:1	2.9 ± 3.2	3.4 ± 4.1	2	7.7 ± 1.8	53.6 ± 9.8	2.5 ± 1.5	20	19	10	0
Group B	30	3:2	1.2 ± 2.0	2.3 ± 3.7	6	8.6 ± 1.5	50.8 ± 9.1	3.1 ± 2.5	28	23	15	7
p			0.02	0.31	-	0.07	0.65	0.31	0.7	0.7	0.6	0.04

M, males; F, females; [1] Chronological age at first presentation; [2] Chronological age at diagnosis of CH; p, probability value; FT4, free thyroxine; TT4, total thyroxine; TSH, thyrotrophin; GH, growth hormone; ACTH, corticotrophin; GN, gonadotrophin; AVP, arginine vasopressin

8.2.3 Results

8.2.3.1 Patient characteristics

Clinical characteristics of patients from both groups are shown in Table 8.1. Two patients had isolated TSH deficiency. Patient 16, group A, presented with growth failure in infancy and apart from CH, he had normal anterior pituitary function. He responded to thyroxine treatment and continued to have profound TSH deficiency on retesting in adulthood. MR scan was normal. Patient 34, group B, had holoprosencephaly [HPE] and has not developed any other hormone deficiency. 40% of group B patients were diagnosed with CH and other hormone deficiencies within the first month of life as compared with 12.5% in group A ($p=0.05$). Evolving TSH deficiency was evident in 8 individuals, patients 13 and 18 from group A and patients 25, 29, 38, 46, 49, 53 from group B, up to 11.8 years after initial presentation.

8.2.3.2 Basal serum TSH concentrations

There were no significant differences between the serum concentrations of FT4, TT4 and unstimulated serum TSH concentrations of both groups [See Table 8.1]. Although the majority (90.7%) of patients had unstimulated serum TSH concentrations less than 5 mU/L, elevated concentrations were observed in 5 patients (patients 22, 32, 40, 52, 54), four with SOD [Figure 8.1]. The relationship between serum FT4 and unstimulated serum TSH was shifted to the left as compared with euthyroid individuals [Figure 8.2]. A similar relationship was observed between serum TT4 and basal serum TSH concentration (data not shown). Patients with primary hypothyroidism had a markedly elevated serum TSH concentration at diagnosis.

Figure 8.1: Unstimulated serum thyrotrophin concentrations in patients with central hypothyroidism. Horizontal bar represents cut-off at 5 mU/L

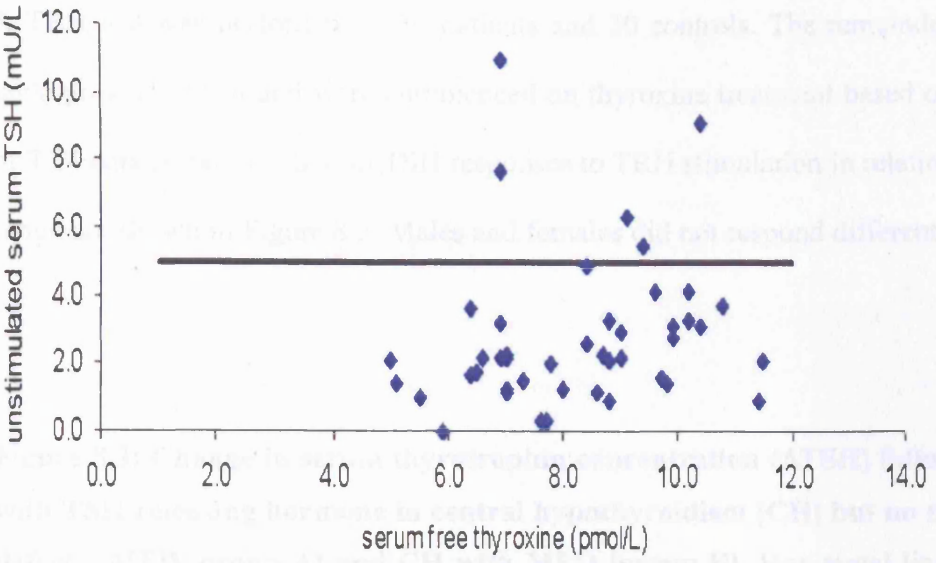
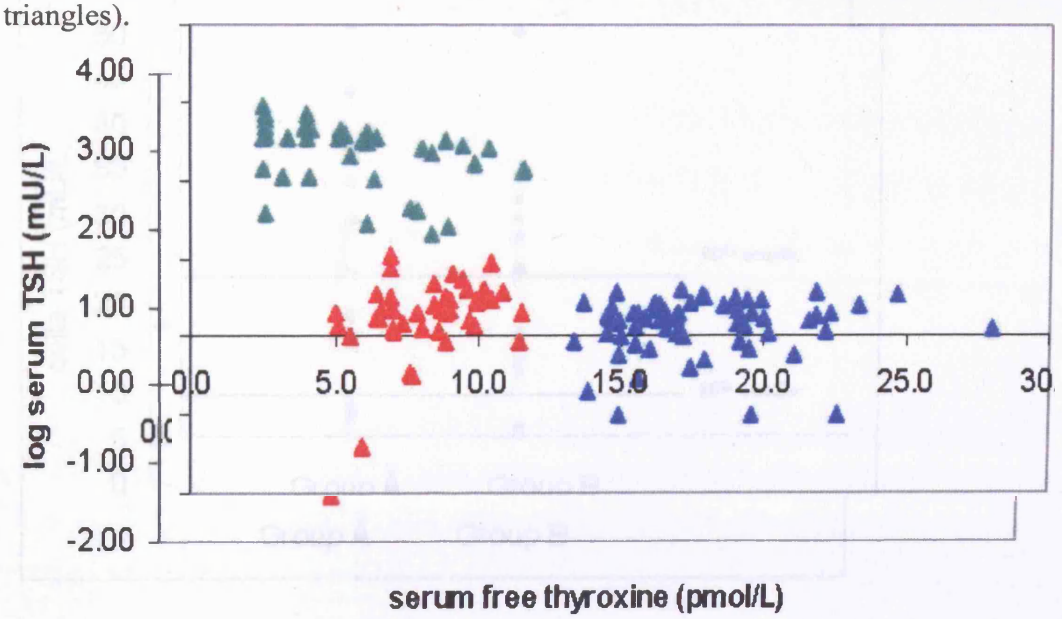


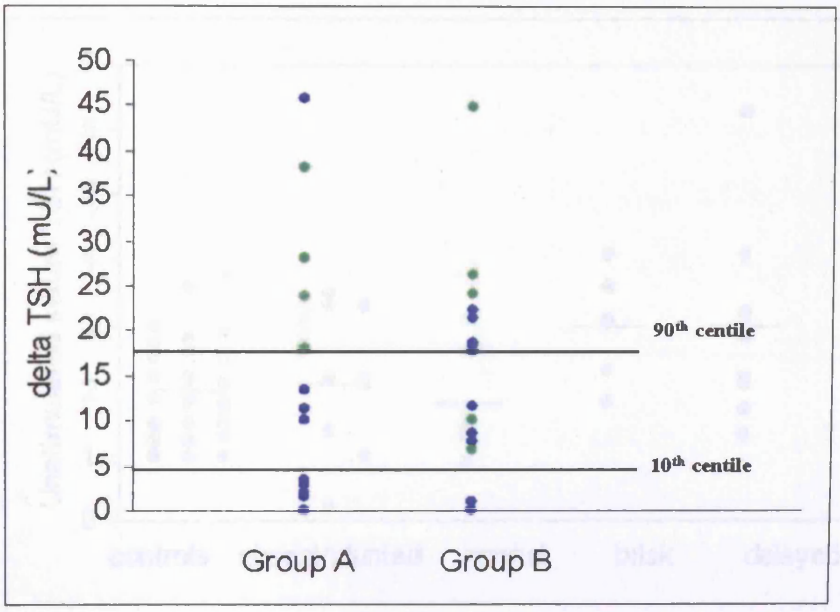
Figure 8.2: Relationship between serum free thyroxine and unstimulated serum thyrotrophin concentrations in patients with central hypothyroidism (CH, n=44, red triangles)**, primary hypothyroidism** (n=38, green triangles) **and controls** (n=63, blue triangles).



8.2.3.3 *Thyrotrophin releasing hormone stimulated serum TSH responses*

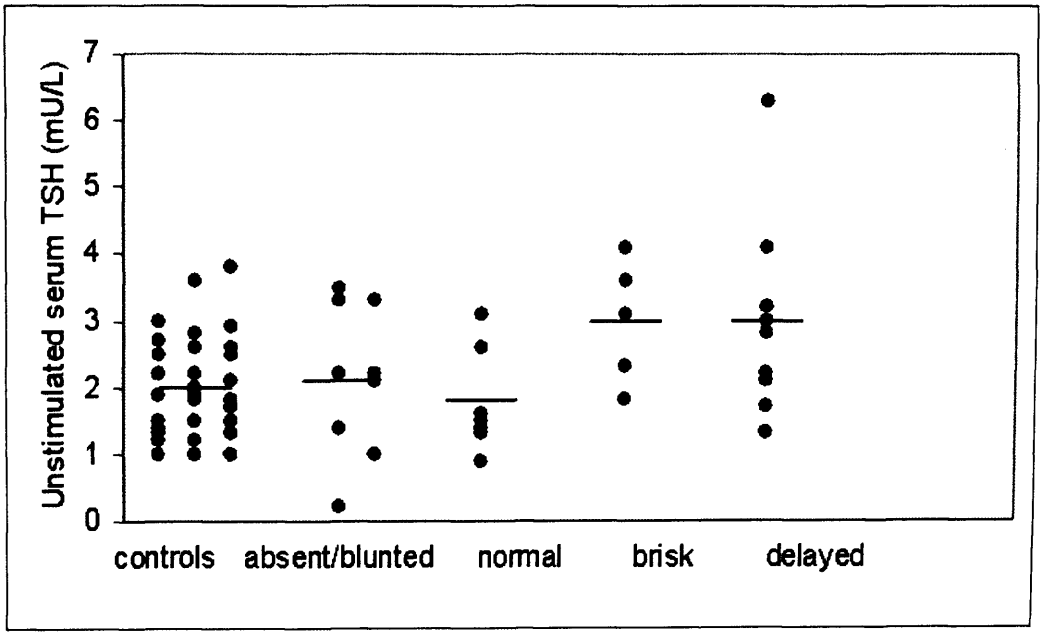
A TRH test was performed in 30 patients and 30 controls. The remainder (24/54) did not undergo a TRH test and were commenced on thyroxine treatment based on low serum FT4 or TT4 concentrations. Serum TSH responses to TRH stimulation in relation to the "normal" range are shown in Figure 8.3. Males and females did not respond differently to stimulation.

Figure 8.3: Change in serum thyrotrophin concentration (ΔTSH) following stimulation with TSH releasing hormone in central hypothyroidism [CH] but no midline forebrain defects [MFD, group A] and CH with MFD [group B]. Horizontal lines depict the 10th and 90th percentiles of the change in serum TSH concentration in controls. Blue circles represent patients with peak serum TSH concentration at 20 minutes and green circles represent patients with peak serum TSH concentration at 60 minutes.



Patients with exaggerated or delayed responses showed significantly higher unstimulated serum TSH concentrations as compared with those who had an absent/blunted response (p=0.02), a normal response (p=0.02) or controls (p=0.003) [Figure 8.4]. A normal TRH test was observed in 7 patients in spite of subnormal serum thyroxine concentrations in patients from both groups (patients 4, 6, 19, 34, 36, 47, 53). Seven patients (patients 12, 13, 16, 18, 20, 21, 24) in Group A demonstrated an absent/blunted response as compared with two patients from group B (patients 25, 42). Five patients had an exaggerated response. Of these, the majority (80%) were in group B (patients 28, 46, 48, 51). A delayed response was observed in significantly greater number of patients [30%] compared with controls [6.7%].

Figure 8.4: Unstimulated serum thyrotrophin concentrations in patients and controls who underwent a thyrotrophin releasing hormone test (horizontal line represents the mean for each group).



8.2.3.4 Differentiation between "pituitary" and "hypothalamic" CH

MR imaging was normal in 3 patients, patient 16 with idiopathic isolated TSH deficiency and patients 10 and 15 with CPHD. Abnormalities in the remaining patients included anterior pituitary hypoplasia (n=39), an undescended posterior pituitary (n=24), an absent septum pellucidum or absent/hypoplastic corpus callosum (n=22), an absent/hypoplastic stalk (n=21), ONH (n=23) and HPE (n=2).

The unstimulated serum TSH concentration or the ΔTSH did not differ significantly between patients with anterior pituitary hypoplasia or a normal anterior pituitary. Only 50% of the patients with anterior pituitary hypoplasia and no midline or stalk abnormalities demonstrated an absent / blunted serum TSH response. A delayed serum TSH rise was demonstrated in 41.7% of cases with stalk abnormalities but also seen in 22.2% of patients who had a normal stalk. This difference was not statistically significant.

The mean serum prolactin concentration was 1088 ± 830 mU/L in those with an absent / thin stalk as compared with 501 ± 390 mU/L in those with a normal stalk but again this difference did not reach statistical significance (p=0.06). Only 7.7% [n=13] of patients with MFD demonstrated an absent/blunted serum TSH response as compared with 47% [n=17] of patients without such a defect (p=0.05).

8.2.4 Discussion

Only 30% of patients in this study demonstrated an absent or blunted serum TSH response characteristic of "pituitary" disease. A further 30% of cases had a delayed "hypothalamic" response and an exaggerated serum TSH response was present in 16.7% of patients. The majority (77.8%) of patients who demonstrated a delayed serum TSH peak had exaggerated concentrations on stimulation (ΔTSH>17.8 mU/L). Only 50% of patients with anterior pituitary hypoplasia, and no stalk or midline defects, demonstrated an absent or blunted response. Stalk disruption suggestive of H-P disconnection and hence "hypothalamic" disease was evident in a total of 12/30 patients who underwent the TRH test. Of these, a delayed response was observed in only 41.7% and such a response was also observed in 22.2% of patients who did not have stalk disruption. These data suggest that differentiation between "pituitary" and "hypothalamic" disease is difficult to discern, at least based on current assessments. This observation is supported by Cohen et al (Cohen et al. 1995) who reported a patient with CH due to a mutations in *POU1F1* who demonstrated a delayed TSH rise after TRH stimulation. A similar response was reported in a patient with a mutation in *PROP1* (Vieira et al. 2003). Both *PIT1* and *PROP1* are pituitary-specific transcription factors and are not associated with a hypothalamic phenotype. Hence, a pituitary cause of CH cannot be ruled out on the basis of a delayed serum TSH response.

Unstimulated serum TSH concentrations ranged from 0.04-11.0 mU/L in this study as compared with 1.0-3.8 mU/L in controls. Of 5 patients with elevated unstimulated serum TSH concentrations, an absent pituitary stalk suggestive of "hypothalamic" disease was documented in only one patient. The exact mechanism underlying elevated unstimulated

serum TSH concentrations in CH remains unknown, although some studies have postulated that the TSH measured in these patients may be biologically inactive (Horimoto et al. 1995). This explanation may also apply in 7 patients who demonstrated a "normal" serum TSH response in spite of developing CH. Four of 5 patients (80%) with elevated unstimulated serum TSH concentrations at diagnosis had SOD, a complex phenotype that is highly variable, evolving and with hypothalamic dysfunction. A TRH test was performed in 14 patients with SOD within this cohort. Of 4 patients with elevated unstimulated serum TSH concentrations, only one had been investigated using a TRH test, the response to which was delayed. Patients with SOD who had abnormalities of the septum pellucidum and/or corpus callosum were less likely to demonstrate an absent/blunted response (7.7%) as compared with those without these abnormalities (47%). Evolving hypothyroidism was more common in patients with SOD.

Isolated TSH deficiency was rare in the cohort. The high incidence of CPHD in patients with CH highlights the need to investigate the H-P axis carefully for other endocrine abnormalities. It is clear from the present data that a normal TRH test, observed in 23.3% of patients with CH does not exclude abnormalities of the H-P-thyroid axis. Regular evaluation of the serum FT4 (or TT4) concentrations can often reveal the diagnosis. The TRH test is of limited use in patients in whom other biochemical or neuroradiological H-P abnormalities have been demonstrated. There is considerable overlap between patients with "pituitary" disease and those with "hypothalamic" disease in terms of the serum TSH response to TRH (Mehta et al. 2003).

8.3 CORTICOTROPHIN DEFICIENCY

This section of the study was a prospective analysis undertaken to ascertain the usefulness of the short Synacthen test [SST] as a marker of endogenous cortisol secretion. Serum cortisol concentrations obtained after Synacthen stimulation were compared with those obtained on spontaneous secretory profiles in children with and "at-risk" of hypopituitarism [including disorders of eye, forebrain and pituitary]. Sub-optimal endogenous cortisol secretion was accepted as an indirect marker of corticotrophin [ACTH] deficiency in these patients as it is a true reflection of the physiological milieu.

8.3.1 Patient selection and methods

28 consecutive patients [male: female ratio 0.9:1; age range 0-5.1 years (mean 1.0 ± 1.0 years)] investigated for ACTH deficiency from March 2002 until June 2004 were selected for analysis. All patients had evidence of ONH, MFD or other pituitary hormone deficiencies. The procedures for collection of blood for analysis of spontaneously secreted cortisol and performing the SST were based on standardized protocols within the department and are shown in Appendix IV.

Previously published data in 28 short normal children reported a mean [average concentration from the twelve 2-hourly samples obtained] spontaneous serum cortisol concentration of 289 ± 72 nmol/L (Charmandari et al. 2002). Based on these studies, a "cut-off" of 145 nmol/L or -2SD from the mean in normal children was taken as a normal

spontaneous serum cortisol concentration for the purpose of this analysis. Patients were defined as ACTH deficient and ACTH sufficient if they demonstrated mean spontaneous serum cortisol concentrations < 145 nmol/L and equal or > 145 nmol/L respectively. Patients with a mean spontaneous serum cortisol concentration > 3SD below the mean at 73nmol/L were considered to have severe ACTH deficiency.

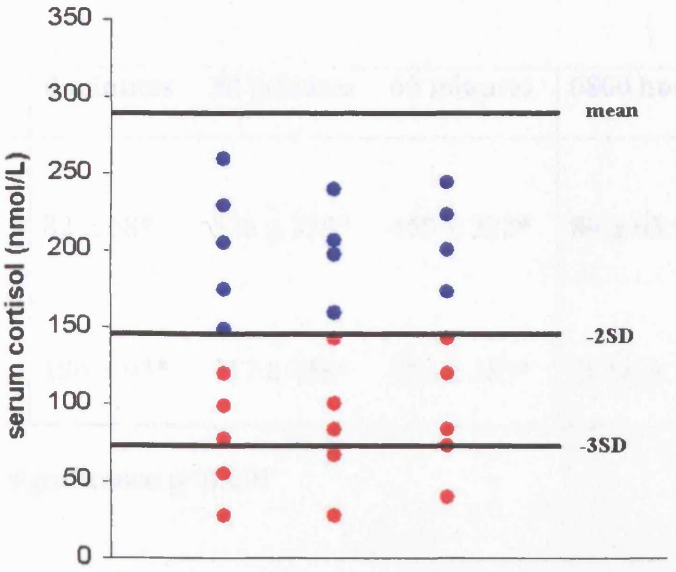
A normal SST was defined as a 30-minute [min] serum cortisol concentration greater than 540 nmol/L (Weintrob et al. 1998), since the 30-min sample has been standardised against the IIH test.

8.3.2 Results

8.3.2.1 Mean spontaneous serum cortisol concentrations

Of 28 patients, none had a mean spontaneous serum cortisol concentration greater than the mean documented for normal children [289 nmol/L]. There were no significant gender- or age-related differences in mean spontaneous serum cortisol concentrations. 15 patients were ACTH deficient [Appendix V-8; Figure 8.5]. Of these, five were severely deficient [-3SD]. The majority of these patients had SOD [patients 3-11, 12, 14, 15]; patient 13 had isolated ONH and patients 1 and 2 had "idiopathic" H-P disease. CPHD was documented in 13 patients with ACTH deficiency. Of 13 patients with ACTH sufficiency, 4 patients [patients 16, 23, 25, 27] had other pituitary hormone deficiencies.

Figure 8.5: Mean spontaneous serum cortisol concentration in study patients compared with 28 short normal children. Blue circles, patients with ACTH sufficiency; red circles, patients with ACTH deficiency.



8.3.2.2 Efficacy of the short Synacthen test

13 patients passed the SST, all of whom also achieved a 30-min increment > 200 nmol/L. Of the 15 patients who failed the SST, 9 patients achieved a 30-min increment > 200 nmol/L despite the 30-min absolute serum cortisol < 540 nmol/L. 26/28 patients demonstrated peak serum cortisol concentrations at 60 min. Basal, 30-min and 60-min serum cortisol concentrations were significantly lower in patients with ACTH deficiency as compared with those without [Table 8.2]. There was a significant correlation ($r=0.7$, $p<0.0001$) between the 30-min SST and the mean spontaneous serum cortisol concentrations.

Table 8.2: Short Synacthen test and 0800 hour serum cortisol concentrations in patients with corticotrophin sufficiency and deficiency.

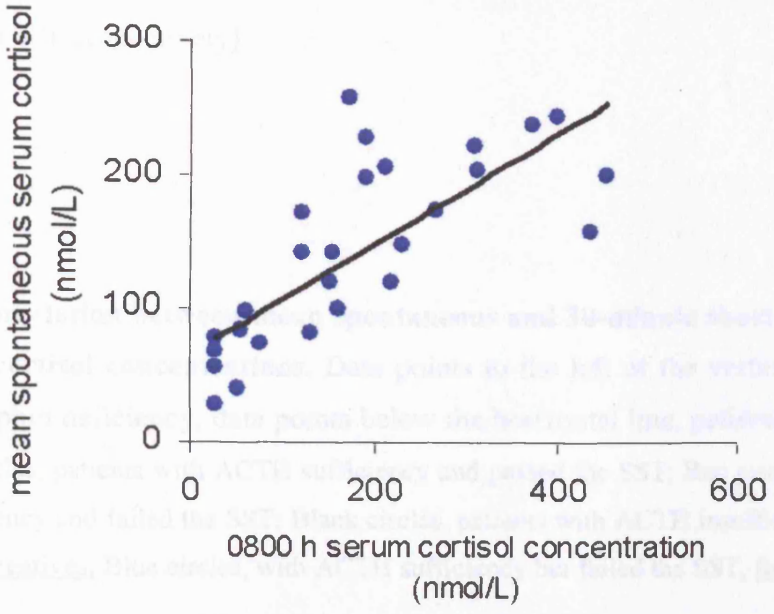
	Serum cortisol concentration (nmol/L) at			
	0 minutes	**30 minutes**	**60 minutes**	**0800 hour**
ACTH deficient	82 ± 68*	376 ± 239*	468 ± 322*	89 ± 63.9*
ACTH sufficient	196 ± 93*	717 ± 156*	865 ± 183*	283.6 ± 108.4*

* Level of statistical significance $p<0.001$

8.3.2.3 0800-hour serum cortisol concentrations

There was a significant correlation between the 0800-hour [h] and the mean spontaneous serum cortisol concentration [$r=0.8$, $p<0.01$, Figure 8.6]. A significant correlation was also observed between the 0800h serum cortisol concentration and the 0-min SST ($r=0.7$, $p<0.0001$), 30-min SST ($r=0.5$, $p=0.003$) and 60-min SST ($r=0.5$, $p<0.0001$) serum cortisol concentrations [Data not illustrated]. Mean 0800h serum cortisol concentrations were significantly lower in patients with ACTH deficiency [See Table 8.2].

Figure 8.6: Relationship between 0800-hour and mean spontaneous serum cortisol concentrations (r=0.8, p<0.01)



8.3.2.4 Comparison of Test Performance

Using spontaneous cortisol secretion, an indicator of the endogenous cortisol milieu as an indirect marker of ACTH secretion, sensitivity and specificity of the SST in diagnosing ACTH deficiency was 80% and 76.9% respectively [Figure 8.7]. The test detected all 5 patients with severe ACTH deficiency. 60-min SST serum cortisol concentration, although highly specific (100%), had a reduced sensitivity (66.7%). Of 3 patients [patients 10, 12, 15] with a false negative SST, patients 10 and 12 were symptomatic with fatigue, poor growth and recurrent hypoglycemia, and hydrocortisone treatment resulted in amelioration of their symptoms. 3 patients [patients 16, 25, 27], with CPHD, had a false positive SST. Mean

spontaneous serum cortisol in patient 16 was borderline [147 nmol/L]. Although patients 25 (SOD) and 27 ("idiopathic" hypopituitarism) failed the SST, the 60-min concentrations were 548 nmol/L and 645 nmol/L respectively. Both also achieved good 30-min increments [366 nmol/L, 233 nmol/L respectively].

Figure 8.7: Correlation between mean spontaneous and 30-minute short Synacthen test [SST] serum cortisol concentrations. Data points to the left of the vertical line, patients with corticotrophin deficiency; data points below the horizontal line, patients who failed the SST. Green circles, patients with ACTH sufficiency and passed the SST; Red circles, patients with ACTH insufficiency and failed the SST; Black circles, patients with ACTH insufficiency but passed the SST, false negatives: Blue circles, with ACTH sufficiency but failed the SST, false positives.

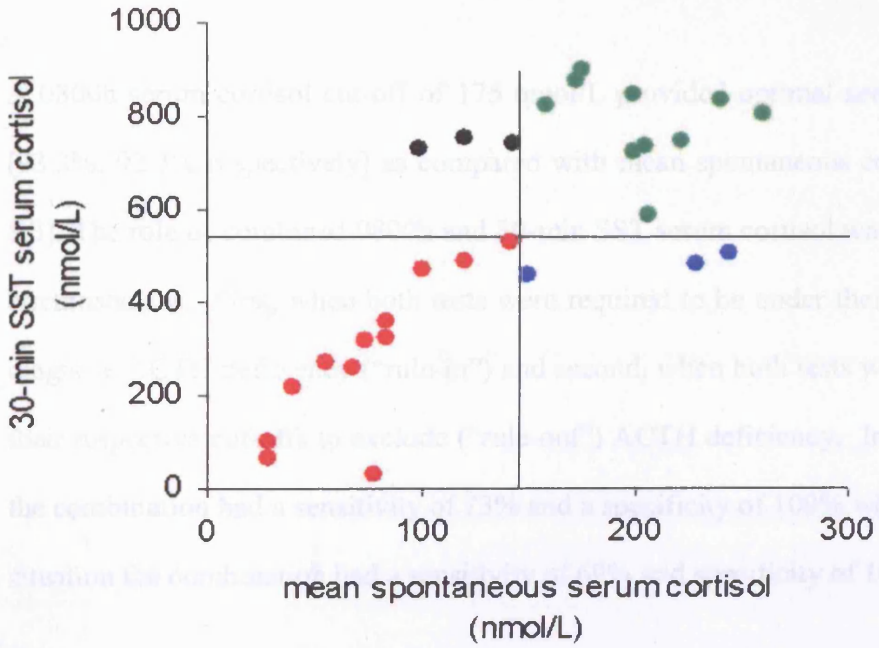
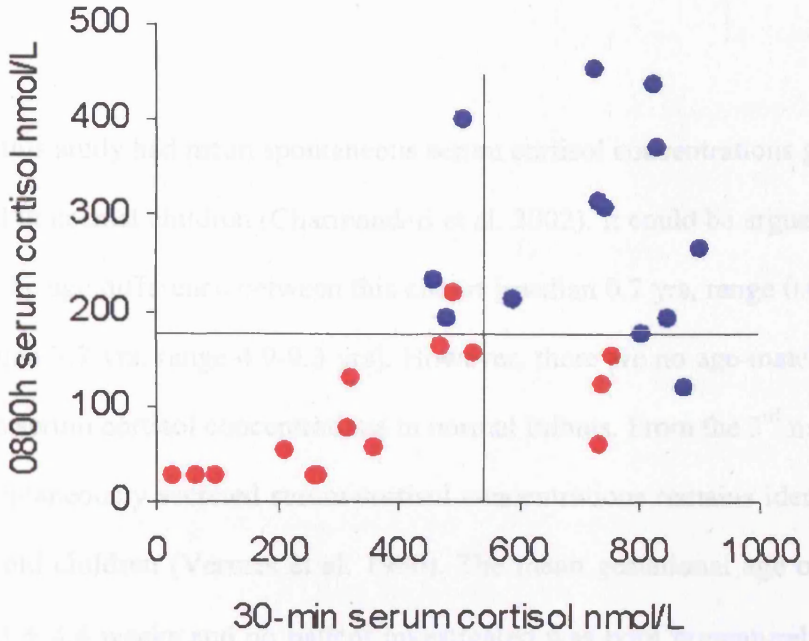


Table 8.3: Cut-off values for 0800-hour serum cortisol concentration

0800 h serum cortisol concentration	Sensitivity %	Specificity %
100	60	100
125	66.7	92.3
150	73.3	92.3
175	93.3	92.3
200	93.3	69.2
225	100	61.5

A 0800h serum cortisol cut-off of 175 nmol/L provided optimal sensitivity and specificity [93.3%, 92.3% respectively] as compared with mean spontaneous cortisol secretion [Table 8.3]. The role of combined 0800h and 30-min SST serum cortisol was considered under two circumstances. First, when both tests were required to be under their respective cut-offs to diagnose ACTH deficiency ("rule-in") and second, when both tests were required to be over their respective cut-offs to exclude ("rule-out") ACTH deficiency. In the "rule-in" scenario the combination had a sensitivity of 73% and a specificity of 100% whereas in the "rule-out" situation the combination had a sensitivity of 69% and specificity of 100% [Figure 8.8].

Figure 8.8: Combination of 0800-hour and 30-minute serum cortisol concentration after Synacthen (r=0.5, p=0.003). Red circles, patients with corticotrophin deficiency; blue circles, patients with corticotrophin sufficiency.



8.3.3 Discussion

The sensitivity and specificity of the SST as compared with assessment of mean spontaneous cortisol secretion were 80% and 76.9% respectively (Mehta et al. 2005a). Several different "cut-off" values have been applied to the 30-min SST serum cortisol concentration. This study used a "cut-off" value of 540 nmol/L since this has been shown to perform well against the IIH test in children (Weintrob et al. 1998). Raising this "cut-off" value to 600 nmol/L did not improve either the sensitivity or the specificity of the test.

Employing an increment at 30-min of 200 nmol/L or using the 60-min SST as a measure of normal cortisol secretion increased specificity (100% in both) at the expense of a reduction in sensitivity (40%, 66.7% respectively). Using more stringent criteria of both 30-min absolute and increment in serum cortisol concentration did not improve sensitivity or specificity.

No patient in this study had mean spontaneous serum cortisol concentrations greater than the mean reported in normal children (Charmandari et al. 2002). It could be argued that this may be a result of the age difference between this cohort [median 0.7 yrs, range 0.03-5.1 yrs] and controls [median 7.7 yrs, range 4.9-9.3 yrs]. However, there are no age-matched data on 24 h spontaneous serum cortisol concentrations in normal infants. From the 3rd month of life the rhythm of spontaneously secreted serum cortisol concentrations remains identical to that of 1 and 3-year old children (Vermes et al. 1980). The mean gestational age of our cohort at birth was 38.3 ± 4.4 weeks and no patient investigated was born prematurely (<36 weeks). Additionally, the present data did not suggest significant gender- or age-related changes in spontaneous or stimulated serum cortisol concentrations, both in the cohort as a whole and in the subgroup of patients with normal cortisol responses.

Ten patients passed both tests and 12 patients failed both tests. Of the latter, 10 patients with CPHD and abnormal H-P imaging had a 0800h serum cortisol concentrations < 175 nmol/L, one patient with TSH deficiency and normal MR imaging had a 0800h serum cortisol concentration of 28 nmol/L and one patient with ONH, no other pituitary hormone abnormality and normal MR imaging failed both tests. Of 10 patients who passed both tests, 6 patients [with ONH] demonstrated abnormal MR imaging but had no evidence of any

pituitary hormone deficiency to date, 3 patients [with ONH] had both normal MR imaging and no other hormone deficiency and patient 23 had ONH with deficiencies of all other pituitary hormones. The 30-min absolute, incremental and 0800 serum cortisol concentrations were 587 nmol/L, 440 nmol/L and 205 nmol/L respectively in patient 23. He has no symptoms to date suggestive of adrenal insufficiency and remains well on replacement treatment with recombinant human GH, thyroxine and desmopressin. However, regular re-evaluation of the H-P- adrenal axis will be required in order to diagnose an evolving endocrinopathy at an early stage (Stanhope et al. 1984).

Three patients with ACTH deficiency, defined by spontaneous cortisol secretion, passed the SST [false negatives]. All had 0800h serum cortisol concentrations < 175 nmol/L. Symptoms of fatigue, poor growth and recurrent hypoglycemia in 2/3 patients resolved on treatment with hydrocortisone. A further 3 patients with ACTH sufficiency, defined as a mean spontaneous serum cortisol concentration > 145 nmol/L, failed to respond to the SST [false positives]. All 3 patients had CPHD. Mean spontaneous serum cortisol concentration in one of the patients (Patient 16) with SOD was only 147 nmol/L and this may reflect incipient ACTH deficiency. The other 2 patients (patients 25, 27) failed the SST based on 30-min absolute serum cortisol concentration but had 60-min serum cortisol concentrations greater than 540 nmol/L, a 30-min rise greater than 200 nmol/L and 0800h serum cortisol concentrations greater than 175 nmol/L.

Although the circadian rhythm does not appear until 3 months of age (Zurbrugg 1976; Vermes et al. 1980), an 0800h serum cortisol concentration of 175 nmol/L had a sensitivity and specificity of 93% and 92.3% respectively as compared with spontaneous cortisol

secretion. "Cut-off" values for young children are unknown, although data from adults suggest that values < 100 nmol/L are indicative of hypocortisolaemia.(Hagg et al. 1987; le Roux et al. 2002) However, there was a wide range of mean spontaneous serum cortisol concentrations associated with a given 0800h value and the use of the latter alone.

Combining the 0800h serum cortisol concentration with the 30-min SST serum cortisol response produced a "rule-in", both tests failing to exceed their respective cut-offs, with a sensitivity of 73% and a specificity of 100%. In "rule-out" mode when both tests must exceed their respective cut-offs, the sensitivity was 69% and specificity 100%. A combination approach has the advantage that it includes a marker of physiological secretion with a measure of cortisol reserve and be therefore, considered a suitable overall assessment of the H-P- adrenal axis. Data from this study indicates that no patient with ACTH sufficiency demonstrated both a 30-min SST serum cortisol concentration < 540 nmol/L and an 0800h serum cortisol concentration < 175 nmol/L. Conversely, no patient with ACTH deficiency achieved both a 30-min SST-stimulated serum cortisol concentration > 540 nmol/L and an 0800h serum cortisol concentration equal or > 175 nmol/L.

8.4 GONADOTROPHIN DEFICIENCY

The role of the postnatal surge in reproductive hormone secretion remains unclear. This increase is gender-specific. Previous data have suggested that unstimulated serum LH and FSH concentrations are not reliable parameters to test pituitary gonadotrophin reserve, particularly in children. The present study was undertaken to investigate if luteinising hormone releasing hormone [LHRH] stimulated serum LH and FSH responses continue to be gender-specific, to compare the rise in reproductive hormone secretion to those of normal pre-pubertal and pubertal children and to assess a possible role of the LHRH test in infancy.

8.4.1 Patient Selection and methods

30 consecutive patients [Table 8.4 and Appendix V-9] at LCPE who underwent a LHRH test within the first 18 months of life were selected for this study [mean age 7.3 ± 5.2 months, age range 0.3-17.4 months, male to female ratio 1.3:1]. The LHRH test was performed in view of other pituitary hormone deficiencies and/or abnormal H-P structures or MFD on neuroimaging and hence risk of gonadotrophin deficiency. The LHRH test was carried out in accordance with the set protocol used within the department as shown in Appendix 9.7. There was no significant correlation between age at testing with serum LH or serum FSH concentrations at 0 minutes [r=-0.2, p=0.3; r=-0.1, p=0.4], 20 minutes [r=-0.1, p=0.6; r=0.01, p=1.0] or 60 minutes [r=-0.1, p=0.5; r=0.01, p=1.0] ruling out a bias due to the age at which the test was performed.

Table 8.4: Clinical data, endocrinology and neuroimaging in patients who underwent the luteinising hormone releasing hormone test

	Males	Females
Number of patients (n)	17	13
Gestational age	39 ± 3 weeks	39 ± 4 weeks
Birth weight	2.9 ± 0.9 kgs	3.0 ± 0.9 kgs
ONH	47%	69%
Endocrinology: % of patients with deficiencies of		
GH	75%	78%
TSH	47%	69%
ACTH	41%	61%
AVP	12%	15%
Serum prolactin concentration	754 ± 1554 mU/L	997 ± 767 mU/L
MR imaging		
H-P abnormality	71%	92%
MFD	59%	85%

ONH, optic nerve hypoplasia; GH, growth hormone; TSH, thyrotrophin; GH, growth hormone; ACTH, corticotrophin; AVP, arginine vasopressin; MR, magnetic resonance; MFD, midline forebrain defect; H-P, hypothalamo-pituitary

8.4.2 Controls

LHRH-stimulated serum LH and FSH responses of 2 groups were used as controls: (i) 18 pre-pubertal normal children [male: female 2:1, age 8.3±2.0 years] (ii) 9 pubertal normal children [male: female 8:1, age 12.7±2.0 years]. There were significant ($p<0.05$) differences between serum LH concentrations [unstimulated and stimulated] and unstimulated serum FSH concentrations between pre-pubertal and pubertal children. Post-LHRH serum FSH concentrations, however, did not significantly differ between both control groups. Peak serum FSH concentrations were reached at 60 minutes in both groups of controls.

8.4.3 Results

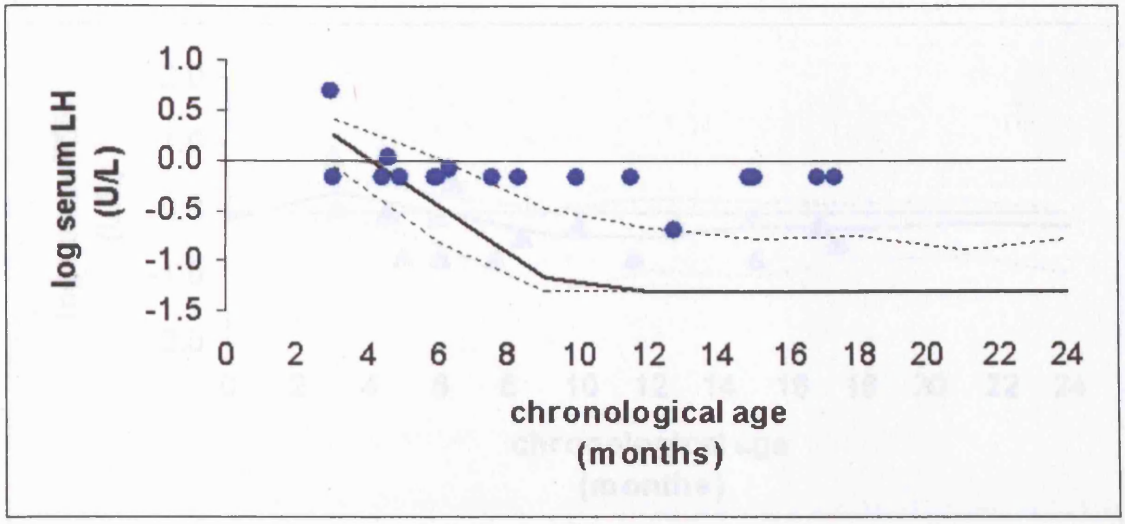
8.4.3.1 Gender differences in serum gonadotrophin concentrations

Ten patients had normal endocrinology to date and the remaining 20 patients had evidence of other pituitary hormone deficiencies, 55% of whom demonstrated complete anterior pituitary hormone dysfunction. Unstimulated gonadotrophin concentrations however, did not significantly differ between patients with other pituitary hormone deficiencies [LH 0.9 ± 1.0 IU/L; FSH 2.4 ± 4.7 IU/L] as compared with those without [LH 0.7 ± 0.03; FSH 4.0 ± 4.9]. Unstimulated serum LH and FSH concentrations did not differ significantly from age-matched controls (Andersson et al, 1998). There was a marked inter-individual variation in unstimulated serum FSH concentrations in both sexes [Figures 8.9 and 8.10].

Eight male patients were born with bilaterally undescended testes and a micropenis. Mean unstimulated serum LH and FSH concentrations were not significantly (p=0.3) different in patients with genital abnormalities at birth [LH 0.7; FSH 0.6 IU/L] as compared with those with normal genitalia [LH 1.2; FSH 1.4 IU/L respectively]. Females demonstrated significantly greater unstimulated serum FSH concentrations as compared with males [Table 8.5]. Differences between serum LH concentrations were however not statistically significant between sexes. Both serum LH and FSH responses were absent in 16.7% [3 males, 2 females] of patients suggestive of gonadotrophin deficiency, all of whom had other pituitary hormone deficiencies. Two of the 3 male patients were born with bilaterally undescended testes and a micropenis. Females demonstrated significantly greater serum FSH responses following LHRH administration as compared with males. There were no significant sex-related differences in LHRH-stimulated serum LH responses [Table 8.5].

Figure 8.9: Unstimulated serum luteinising hormone [LH] concentrations in patients compared with those from normative longitudinal data. Solid line, mean concentration; dotted lines, range (Andersson et al, 1998).
(i) Serum LH in males; (ii) Serum LH in females

(i)



(ii)

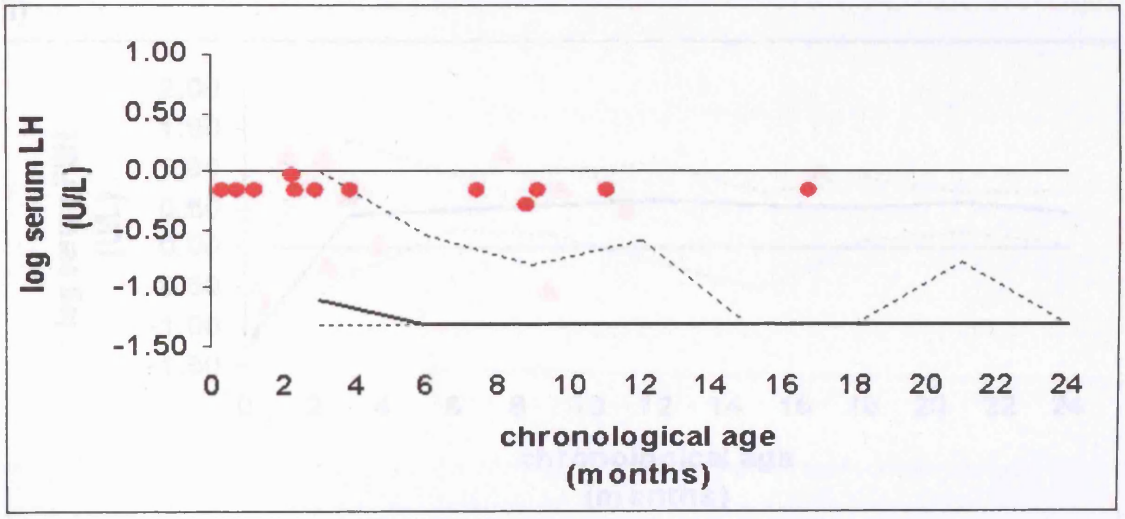
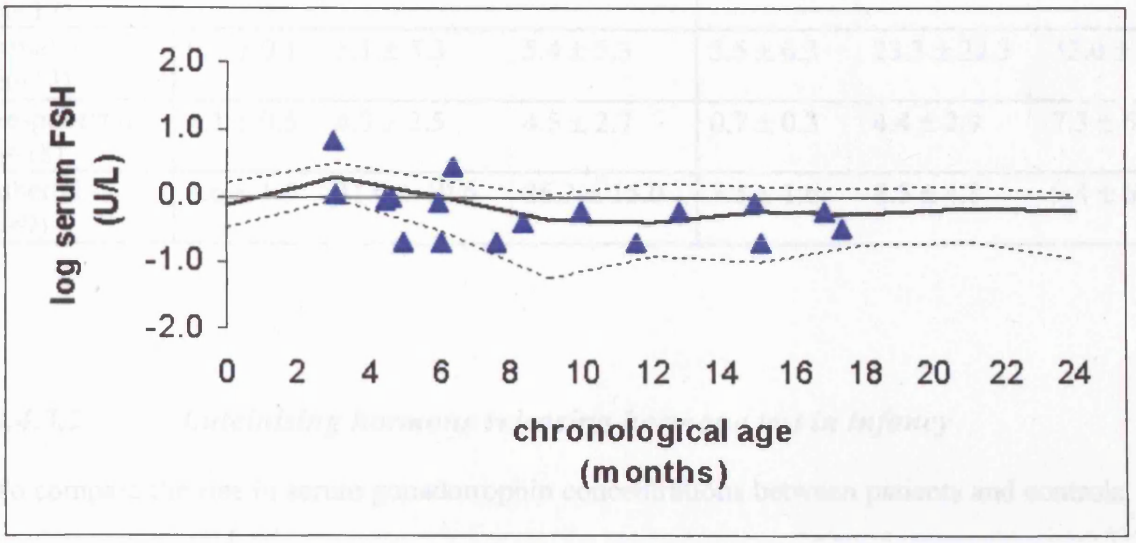


Figure 8.10: Unstimulated serum follicle stimulating hormone [FSH] concentrations in patients compared with those from normative longitudinal data. Solid line, mean concentration; dotted lines, range (Andersson et al, 1998).
(i) Serum FSH in males; (ii) Serum FSH in females

(i)



(ii)

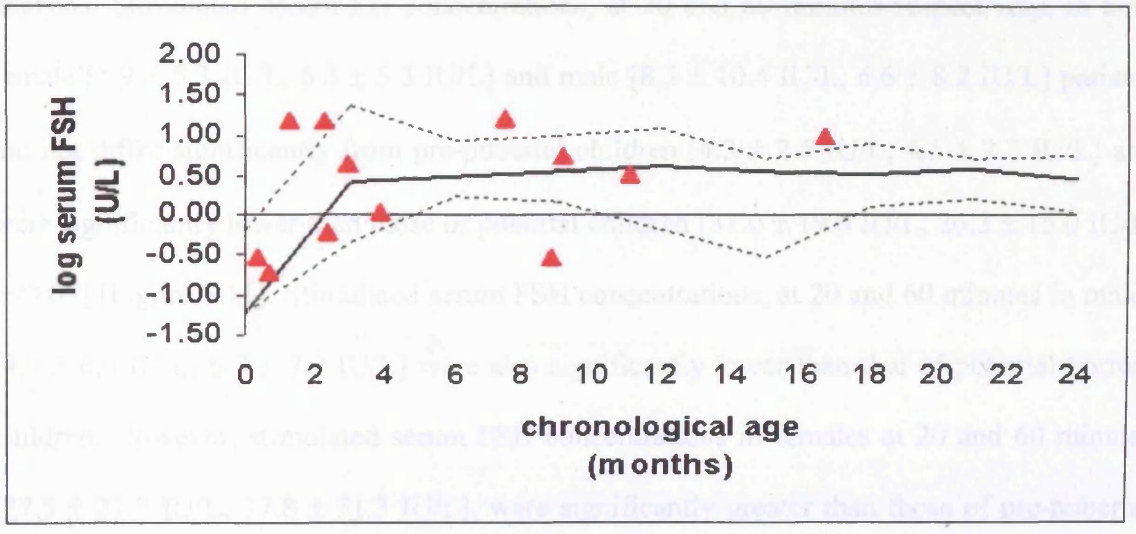


Table 8.5: Serum gonadotrophin concentrations following stimulation with luteinising hormone releasing hormone in patients, pre-pubertal and pubertal controls.

	Luteinising hormone (IU/L)			Follicle stimulating hormone (IU/L)		
	minutes			minutes		
	0	20	60	0	20	60
Males (n=17)	1.0 ± 1.1	7.0 ± 9.8	5.5 ± 7.7	1.0 ± 1.6	4.0 ± 5.7	5.5 ± 7.5
Females (n=13)	0.7 ± 0.1	5.1 ± 5.3	5.4 ± 5.3	5.5 ± 6.3	23.3 ± 22.3	32.0 ± 31.9
Pre-pubertal (n=18)	1.1 ± 0.5	4.3 ± 2.5	4.5 ± 2.7	0.7 ± 0.3	4.4 ± 2.9	7.3 ± 6.1
Pubertal (n=9)	3.6 ± 1.5	31.0 ± 19.6	26.3 ± 15.0	3.5 ± 1.6	8.5 ± 4.8	9.4 ± 6.3

8.4.3.2 Luteinising hormone releasing hormone test in infancy

To compare the rise in serum gonadotrophin concentrations between patients and controls, 5 patients who demonstrated absent serum LH and FSH responses were excluded from further analysis. Stimulated serum LH concentrations, at 20 and 60 minutes respectively, in both female [5.9 ± 5.3 IU/L, 6.3 ± 5.3 IU/L] and male [8.3 ± 10.4 IU/L, 6.6 ± 8.2 IU/L] patients did not differ significantly from pre-pubertal children [4.3 ± 2.5 IU/L, 4.5 ± 2.7 IU/L] and were significantly lower than those of pubertal children [31.0 ± 19.6 IU/L, 26.3 ± 15.0 IU/L; $p<0.01$] [Figure 8.11]. Stimulated serum FSH concentrations, at 20 and 60 minutes in males [4.9 ± 6.0 IU/L, 6.7 ± 7.9 IU/L] were also significantly lower than that of pubertal normal children. However, stimulated serum FSH concentrations in females at 20 and 60 minutes [27.5 ± 21.7 IU/L, 37.8 ± 31.3 IU/L], were significantly greater than those of pre-pubertal [4.4 ± 2.9 IU/L, 7.3 ± 6.1 IU/L; $p<0.0005$] and even pubertal [8.5 ± 4.8 IU/L, 9.4 ± 6.3 IU/L; $p<0.05$] individuals [Table 8.5 and Figure 8.12].

Figure 8.11: Serum luteining hormone [LH] responses following LH releasing hormone [LHRH] stimulation. Male patients, blue circles; female patients, red circles; mean post-LHRH serum LH response in pubertal children, solid line; prepubertal children, dotted line.

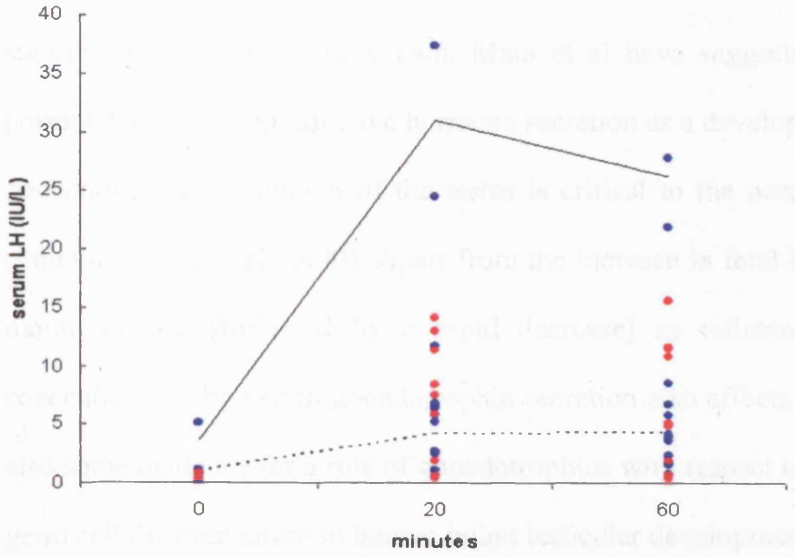
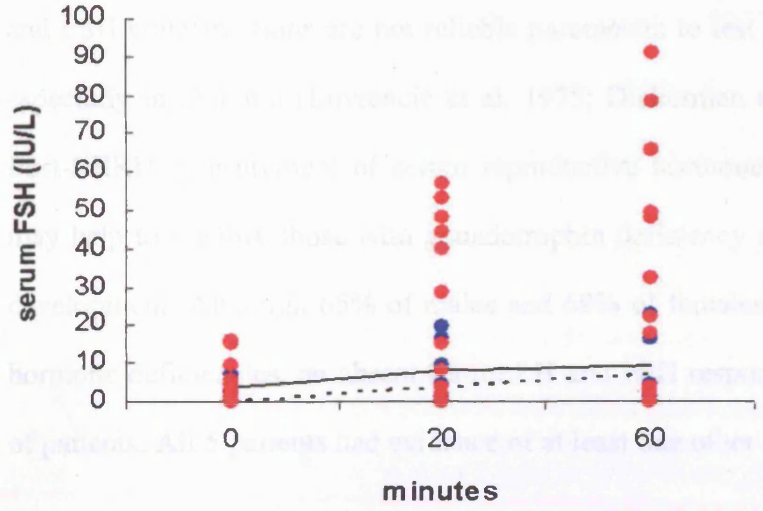


Figure 8.12: Serum follicle stimulating hormone [FSH] responses following LHRH stimulation. Male patients, blue circles; female patients, red circles; mean post-LHRH serum LH response in pubertal children, solid line; prepubertal children, dotted line.



8.4.4 Discussion

Fetal gonadotrophin secretion first becomes established during the 12th week of intrauterine life. During early infancy, there is an increase in both FSH and LH although the exact significance of this is not known. Main et al have suggested a significant role for this postnatal surge in reproductive hormone secretion as a developmental window during which gonadotrophin stimulation of the testes is critical to the normal development of the male genitalia (Main et al. 2000). Apart from the increase in fetal Leydig cell function in the 3rd month of life [followed by a rapid decrease] as reflected in the serum testosterone concentration, the rise in gonadotrophin secretion also affects Sertoli cell function. There is also some evidence for a role of gonadotrophins with respect to Leydig cell proliferation and germ cell differentiation in human infant testicular development.

The increase in reproductive hormones is gender-specific in normal infants (Forest et al. 1974; Winter et al. 1975; Forest et al. 1976; Winter et al. 1976; Forest et al. 1980; Burger et al. 1991; Andersson et al. 1998). Previous data have suggested that unstimulated serum LH and FSH concentrations are not reliable parameters to test pituitary gonadotrophin reserve, especially in children (Lovrencic et al. 1975; Dickerman et al. 1979; Kletter et al. 1996). Post-LHRH measurement of serum reproductive hormones in infants with H-P disorders may help to identify those with gonadotrophin deficiency and provide clues about gonadal development. Although 65% of males and 69% of females had evidence of other pituitary hormone deficiencies, an absent serum LH and FSH response was observed in only 16.7% of patients. All 5 patients had evidence of at least one other hormone abnormality. 2/3 males

with absent LH and FSH responses were born with undescended testes and a micropenis at birth.

Females demonstrated significantly higher unstimulated serum FSH concentrations compared with males in keeping with other studies (Job et al. 1977; Andersson et al. 1998). Similar gender-specific differences were not observed in unstimulated serum LH concentrations. Inhibin B produced mainly by Sertoli cells of the testis in males and by granulosa cells in females plays an important role in negative feedback regulation of the H-P-gonadal axis. Inhibin concentrations in males in early infancy have been shown to be unexpectedly high, into the supra-adult range and persist longer than in the female and longer than the rise in serum gonadotrophins (Andersson et al. 1998). Gender differences in serum FSH responses between males and female in the present study may reflect this physiological difference. LHRH stimulated serum FSH responses were markedly exaggerated in females with concentrations significantly greater than those observed in pre-pubertal and even pubertal children. Such serum FSH responses have been reported in normal females although the concentrations did not exceed 20 U/L (Job et al. 1977). There was a marked inter-individual variation in the serum FSH responses in females but not in males, possibly indicative of a cyclic regulation (Chellakooty et al. 2003).

Reduced prenatal growth has been associated with FSH hypersecretion and reduced gonadal size in adolescent boys and girls. Ibanez et al have recently reported a 2- to 4- fold increase in serum unstimulated FSH concentrations in children born small for gestation age than those born of a normal size, in the 1st year of life (Ibanez et al. 2002). Longitudinal growth assessment was not undertaken in this group of patients and no patient was born premature

or small for gestation age. However, a postnatal growth restraint due to CPHD resulting in an augmented serum FSH drive to maintain inhibin B requirements cannot be excluded, although, this alone cannot explain the gender-specific serum FSH responses.

The present study has several drawbacks including the small size of the cohort and lack of proper control data at this age group. However, these preliminary data provide an interesting observation, particularly with regard to exaggerated FSH responses in females who need detailed evaluation and follow-up at puberty to investigate for gonadotrophin deficiency and an assessment of fertility at that stage. Based on further data from this study, early treatment with gonadotrophins in future patients may induce testicular and penile growth in males (Main et al. 2002). Whether early treatment may also prove beneficial in the long term towards adult male sexual and reproductive function in both sexes remains to be seen.

CHAPTER 9

CONCLUSIONS

9.1 HETEROGENEITY OF CONGENITAL HYPOPITUITARISM

The clinical presentation of pituitary dysfunction varies with the age of the patient and the endocrine spectrum that ranges from growth hormone deficiency [GHD] in isolation through to combined pituitary hormone deficiency [CPHD]. Documentation of a deficiency in one pituitary hormone requires evaluation of the others. Briefly, evaluation consists of a good history, thorough clinical examination and assessment of the hypothalamo-pituitary [H-P] axis, both its function and morphology. With the advent of magnetic resonance [MR] imaging, it is now possible to relate neuroanatomy in patients with hormone deficiencies to the likely underlying pathophysiological process. MR imaging also helps identification of patients with more complex phenotypes such as septo-optic dysplasia [SOD] and holoprosencephaly [HPE]. In recent years, considerable progress has been made in the understanding of pituitary gland development and the critical cascade of transcription factors responsible for the differentiation of pituitary cell types. Pituitary development is intricately linked to that of the eye and forebrain and rodent pituitary development has provided a useful model to understand the pathophysiology in patients with hypopituitarism and in linking it to defects of the forebrain and the eye. Each cell type of the anterior pituitary gland is characterized by the secretion of one or more trophic hormones that regulates a diverse range of important biological processes in response to signals from the hypothalamus and peripheral organs. The H-P axis is constantly subject to positive and negative feedback mechanisms and the ability of an individual to respond to a variety of

suppressive and stimulatory agents forms the basis of several dynamic pituitary tests that are used to assess integrity of the axis. The diagnosis of hypopituitarism, in spite of the availability of a multitude of tests, is fraught with problems. Difficulties with diagnosis are compounded by variability in phenotype, evolving hormonal deficiencies and lack of "gold standards" with which to compare test performance.

This study was undertaken in sub-groups from a cohort of 825 patients with variable hypopituitarism and developmental defects of the eye and forebrain to test the hypothesis that the phenotype of a patient with hypopituitarism is influenced by the position of the abnormal gene within the pituitary developmental cascade and that this temporospatial positioning influences the morphology of the H-P and midline forebrain structures [MFS] and the endocrine status of the individual. The aims of the study were to define possible structure – function relationships within the H-P axis in order to determine risk factors associated with hypopituitarism, to investigate the influence of neuroanatomy on birth size and early growth, to ascertain the optimal tests for assessing hormone deficiency and to establish genotype - phenotype correlations in patients with a genetic hypopituitarism.

Data from this study demonstrated a good relation between structure and function within the H-P axis and MFS. Overall, abnormal H-P morphology was a reasonable indicator of endocrine dysfunction as all patients with endocrine dysfunction demonstrated at least one H-P structural defect as compared with 76% of patients without endocrine dysfunction. In particular, the presence of an undescended posterior pituitary offered the strongest association with hypopituitarism with an odds ratio of 33.05 [95% CI 4.38, 249.45] in patients with hypopituitarism as compared to those without. Anterior pituitary hypoplasia

also occurred 6.7 [95% CI 2.63, 16.98] times more commonly in patients with endocrinopathies as compared with those without. Amongst patients with hypopituitarism, MFD and abnormalities of the pituitary stalk were significantly associated with the presence of CPHD over IGHD. This association was particularly significant for abnormalities of the corpus callosum as they were observed in 23% of patients with CPHD as compared with 4.5% of patients with IGHD with an odds ratio of 6.13 [95% CI 1.37, 37.44]. Patients with optic nerve hypoplasia [ONH] who demonstrate anterior pituitary hypoplasia and an undescended posterior pituitary were found to be significantly at risk of endocrine dysfunction as compared with children with ONH and normal endocrinology. Although MFD occurred with an odds ratio of 1.42 [95% CI 0.57, 3.55] in ONH patients with endocrine dysfunction as compared with ONH patients with normal endocrinology, the differences did not reach statistical significance. The endocrine spectrum in hypopituitary patients with ONH included a significantly greater prevalence of ACTH and AVP deficiencies as compared with hypopituitary patients without ONH [presumed "idiopathic" hypopituitarism]. There was a significant male to female preponderance in patients with "idiopathic" hypopituitarism as compared with hypopituitary patients with ONH suggesting the possibility of a role for SOX3 or other unknown genes with an X-linked inheritance as the etiology in those patients currently labelled as "idiopathic".

Birth size and growth in infancy in patients with congenital GHD with or without other pituitary hormones was also influenced by the neuroanatomical phenotype. Although birth size in patients with GHD, on the whole, was not reduced, patients with the mildest phenotype of IGHD were born with a greater birth length SDS than those with the most severe phenotype characterized by CPHD and midline defects. Differences in weight and

body mass index [BMI] were particularly significant as patients with a severe phenotype [CPHD + midline defects] were born heaviest, with greatest BMI, than those with the mildest phenotype with IGHD. GH appeared to be critical for early postnatal growth as length SDS decreased in all patients within 6 months of birth. Trends within data from this study suggest that this growth during the infancy period was influenced in part by the complexity of the hypopituitary phenotype reflected by the presence of CPHD and midline defects. Patients with CPHD had a greater decrease in length as compared with patients with IGHD. Patients with CPHD and midline defects were longer, heavier and with greater BMI than CPHD patients without midline defects.

A retrospective evaluation of serum TSH response to stimulation with TRH in patients with central hypothyroidism revealed that responses remained normal in 23.3% of patients limiting the role of the TRH test as a diagnostic tool in central hypothyroidism. There was considerable overlap between stimulated serum TSH responses in patients with "pituitary" and those with "hypothalamic" disease, and a distinction between the two pathologies is not easy based on this test. In patients in whom other biochemical or neuroradiological H-P abnormalities have been demonstrated, regular evaluation of the serum thyroxine concentrations alone may reveal the diagnosis of TSH deficiency which is best made in the presence of a low serum thyroxine concentration in the face of a low, normal or even modestly elevated unstimulated serum TSH concentration. It is important to establish the diagnosis of ACTH deficiency quickly in order to avoid the risk of hypoglycemia, adrenal crisis and mortality. A combination of the short Synacthen test and measurement of 0800-hour serum cortisol concentration, both of which are safe and relatively easy to perform in young children, represented the optimal method of investigation for ACTH deficiency.

Patients who have a serum cortisol concentration < 540 nmol/L at 30 minutes following stimulation with Synacthen and < 175 nmol/L at 0800 hours should receive glucocorticoid replacement. A reassessment of all individuals' cortisol status should be performed at a later stage to identify those normal individuals who may have received treatment inappropriately as the sensitivity of the test remains below 85%. A high index of clinical suspicion should be exercised even in patients with normal test results in order to identify evolving H-P disease with later onset of ACTH deficiency. Preliminary data on serum LH and FSH responses following stimulation with LHRH demonstrated that responses are gender specific with exaggerated serum FSH responses in females in infancy, with concentrations significantly greater than those in prepubertal and pubertal normal children.

Mutations in pituitary transcription factors were found in 0.7%, 6.4%, 6.2%, 13.6% and 5.3% of hypopituitary patients screened for mutations in *HESX1, PROP1, POU1F1, GH1* and *SOX3* respectively. On the whole, they were rare in sporadic hypopituitarism. As there was a significant increase in the male to female ratio in patients with "idiopathic" hypopituitarism as compared with that in hypopituitary patients with ONH, identification of mutations in *SOX3* and other unknown genes leading to an X-linked inheritance of hypopituitarism may elucidate the genetic basis of this male preponderance in patients thought to have idiopathic disease. There was a poor genotype phenotype correlation in patients with genetic hypopituitarism both between patients and within families with the same mutation. Variability in phenotype was most obvious in patients with mutations in *HESX1,* where the endocrine spectrum ranged from patients with IGHD, with and without

ONH to SOD. Neuroanatomical phenotype ranged from normal MR imaging to that with complex abnormalities of the H-P axis and MFS. There was a similar variability in patients with mutations in *SOX3,* an endocrine phenotype ranging from IGHD to evolving CPHD with variable MR imaging abnormalities. Patients with mutations in *PROP1* also showed considerable variability in phenotype particularly with respect to ACTH and prolactin deficiencies and the size of the anterior pituitary gland. A pituitary mass identified in one patient waxed and waned in size prior to its involution. An evolving nature of hormone deficiencies in patients with *PROP1* mutations suggests a progressive decline in the anterior pituitary axis indicating a need for continual monitoring of patients for the development of hormone deficiency that may not be apparent at initial presentation. TSH and prolactin deficiencies were not complete in all patients with mutations in *POU1F1.* IGHD in patients with type II autosomal dominant GHD may also evolve leading to CPHD. Early treatment with recombinant human GH [rhGH] and avoidance of precipitate withdrawal of treatment following attainment of adult height in these patients may be important in maintaining pituitary function by providing a feedback signal to reduce the GHRH drive, reduce somatotroph proliferation and reduce the rate of cell destruction. This could potentially reduce pituitary damage and hence loss of other endocrine cells types. However, this hypothesis remains to be proven.

It is however important to note that our knowledge of the field of pituitary development is in its embryonic stages and further research in the areas of clinical and molecular medicine will better clarify the ambiguities in the spectrum of hypopituitarism.

9.2 SHORTCOMINGS OF THE PRESENT STUDY

A few criticisms could be made from these preliminary studies. The study was performed retrospectively and hence data collection was not systematic. Although patients were selected systematically, there is likely to be an inherent variability in the anthropometry, biochemical testing and hormone assays between patients within the LCPE and between national and international centres. The population of children studied was highly selective, selecting only those with apparent pituitary pathology reflecting the nature of referrals to the LCPE and possibly to other centres. There was also a lack of true age-related control data as it was not ethically possible to undertake pituitary function testing and MR imaging in normal children. Short normal children who had undergone investigations as part of their diagnostic assessment and found to have no pathology were used as controls for the studies evaluating pituitary hormone function. These children have been followed up until adulthood to ensure normal growth and puberty. Children with ONH without endocrine dysfunction were used as controls in the study identifying MR abnormalities associated with endocrine dysfunction. Not all patients were analysed longitudinally and hence an accurate assessment of evolving endocrine dysfunction was not possible. Although functional studies have provided insight into the effects of genetic mutations, it is difficult to assess its impact on the general population. Although this study is one of the largest documenting the detailed phenotype and prevalence of mutations in pituitary transcription factors in patients with hypopituitarism, it is still relatively small given the rarity of these conditions and it is therefore difficult to make definitive conclusions. Long-term follow-up is essential to determine the likelihood of evolving endocrinopathy and the long-term outcome of these patients.

9.3 CLINICAL IMPACT OF PRESENT RESEARCH

This study has led to the implementation of a number of recommendations for clinical practice at the London Centre fpr Paediatric Endocrinology LCPE]. As structural abnormalities on magnetic resonance [MR] imaging significantly helped predict the risk of hypopituitarism and the likelihood of developing combined pituitary hormone deficiency over isolated growth hormone deficiency, MR imaging is recommended in all patients with hypopituitarism and those "at-risk" of hypopituitarism. Patients with optic nerve hypoplasia and abnormal MR imaging should receive long-term follow up as they may demonstrate evolving endocrine dysfunction.

Analysis of tests used in evaluating pituitary hormone secretion revealed several shortcomings, particularly in infants and young childen, in whom some endocrine tests may prove hazardous and interpretation of results is often difficult. As the TRH test was found to be normal in several patients with central hypothyroidism, it was not deemed necessary for its diagnosis. Regular evaluation of free thyroxine concentration is recommended in patients who demonstrate other pituitary hormone and/or structural brain abnormalities. A combination approach of measurement of 0800-hour serum cortisol concentration, a marker of physiological secretion, with measurement of post-Synacthen serum cortisol concentrations, a measure of cortisol reserve, was considered a suitable overall assessment of the H-P- adrenal axis in infants and young children. Patients who have a serum cortisol concentration < 540 nmol/L at 30 minutes following stimulation with Synacthen and < 175 nmol/L at 0800 hours should receive glucocorticoid replacement with a reassessment of all

individuals' cortisol status at a later stage. This study also illustrates that interpretation of the LHRH test in infancy is difficult, and long-term follow up of hypopituitary patients with "abnormal" LHRH tests is necessary to correlate the biochemistry with the clinical phenotype. The results of LHRH testing were particularly variable in female patients who demonstrated exaggerated serum FSH responses following stimulation.

Data from this study indicates that growth failure in infancy, as early as 6 months of age, may be attributed to congenital GHD. In patients with congenital GHD, identification of other pituitary hormone deficiencies and MFD on MR imaging helped predict those who would present earlier with more severe growth deceleration and those with a propensity for early weight gain respectively.

Although data from this study suggest a lack of complete genotype-phenotype correlation in patients with a molecular genetic cause for hypopituitarism, molecular analysis led to several benefits. Given the difficulties with interpretation of current endocrine tests and the evolving nature of hypopituitarism, DNA analysis was found not only to be useful in a better understanding of H-P development, but also helpful in making a correct clinical diagnosis as observed in patients with a mutation in *POU1F1* misdiagnosed with ACTH deficiency, in a patient with a "pituitary tumour" who was found to harbour a mutation in *PROP1,* and in diagnosing evolving CPHD in patients with IGHD who harbour a splice site mutation in *GH1.*

9.4 IDEAS FOR FUTURE RESEARCH

This study demonstrates the striking phenotypic heterogeneity in patients with hypopituitarism. A vast proportion of patients with hypopituitarism did not have a known genetic cause, many of whom had extra-pituitary malformations, suggesting that unknown genes may not only be implicated in H-P but other organ development as well. Using mice expression and transgenic data, the next few years may see the identification of such novel genes. In some patients with a proven genetic cause, the genotype alone could not explain the variabilities in the phenotype and identification of genetic or environmental modifiers may in the future explain this phenotypic variability. Although good structure-function relationships are demonstrated within the H-P axis following MR imaging, several questions remain unanswered. In particular, the contribution of hypothalamic versus pituitary dysfunction to the phenotye needs to be examined further. It is possible that the arrival of higher resolution MR scanners providing thinner cuts in the future, may identify structures currently reported as "absent", providing a better explanation for the phenotypic ambiguity observed in some patients e.g. those with an "absent" pituitary stalk who did not demonstrate panhypopituitarism, those with an "absent" posterior pituitary who did not develop diabetes insipidus or those with anterior pituitary hypoplasia who have normal pituitary function to date. Future longitudinal follow-up studies will prove beneficial in identification of risk factors associated with evolution of the endocrinopathy and in determining outcomes of morbidity in these patients. Detailed phenotyping, genotyping and epidemiological data in these groups of patients may shed further light on the etiology, particularly with respect to the contribution of environmental and genetic factors leading to improved investigation and treatment of these patients.

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APPENDIX

I. CLINICAL DETAILS PROFORMA

PATIENT DNA SAMPLE NO.	
NAME	SURNAME
DOB	SEX
HOSPITAL / CONSULTANT	HOSPITAL NO.
ANTENATAL HISTORY	
MATERNAL AGE	
MATERNAL SMOKING	YES / NO
MATERNAL ALCOHOL	YES / NO
CONSANGUINITY	YES / NO
FAMILY HISTORY	
GESTATION AT BIRTH	DELIVERY
BIRTH WEIGHT	BIRTH LENGTH
NEONATAL COMPLICATIONS	
AGE AT DIAGNOSIS	
WEIGHT ON PRESENTATION	HEIGHT ON PRESENTATION
DEVELOPMENT	
MID PARENTAL HEIGHT	FINAL HEIGHT
SPONTANEOUS PUBERTY	YES / NO
VISUAL ACUITY RIGHT EYE	VISUAL ACUITY LEFT EYE
RIGHT OPTIC DISC	LEFT OPTIC DISC
ERG	VER
OTHER OPHTHALMOLOGICAL FEATURES	
FREE THYROXINE	
BASAL TSH	PEAK TSH
BASAL PROLACTIN	PEAK PROLACTIN
GH PROVOCATION TEST TYPE	
PEAK GH	
BASAL CORTISOL	PEAK CORTISOL
BASAL LH	PEAK LH
BASAL FSH	PEAK FSH
DIABETES INSIPIDUS	YES / NO
HYDROCORTISONE TREATMENT (AGE)	
THYROXINE TREATMENT (AGE)	
GH TREATMENT (AGE)	
DDAVP TREATMENT (AGE)	
SEX STEROIDS (AGE)	
NEUROIMAGING RESULT	
OTHER ASSOCIATED FEATURES	
CANDIDATE GENES TO BE TESTED	
DIAGNOSIS	

II. PATIENT / PARENT INFORMATION SHEET

Title of Project: A genetic analysis of children with forebrain, eye and/or pituitary defects

We would like to ask your permission to include your child in this project.

Investigator: Dr. M.T. Dattani

Introduction: The pituitary gland produces a number of hormones that are essential for growth and normal pubertal development of children. In some children who suffer from growth failure, the pituitary gland may be unable to produce growth hormone (GH) and/or other hormones. The gland may be very small or even absent. These children would need to be treated with the hormones that are missing. Occasionally, the small pituitary gland may be associated with developmental abnormalities affecting the eyes and the midline structures of the brain, when the condition is called septo-optic dysplasia or SOD. The reason for this association of developmental abnormalities lies in the close relationship between normal pituitary, forebrain and eye development in man. A number of genes are implicated in controlling the development of these structures, and these include *PIT1*, *PROP1*, *LHX3*, *LHX4* and *HESX1*.

The aim of the study: In this study, we aim to analyse the genes controlling the development of the pituitary gland. We have recently found that changes in a new gene called *HESX1* are associated with SOD, pituitary hypoplasia and GH insufficiency or deficiency in man. We now wish to screen children for abnormalities in this and other genes important for the normal development of the forebrain, eye and pituitary gland in man and mouse.

Why is the study being done? This study will help us to understand why children develop abnormalities of the forebrain, eyes and pituitary gland. Additionally, we will gain further insights into the complex development of these structures. Finally, knowledge of the exact genetic mechanism for the underlying disease will enable us to make an earlier diagnosis in further affected children from the same family, and also to offer antenatal diagnosis. This will reduce the complications that could potentially arise in children with these disorders.

How is the study being done? We need to take a small blood sample (2 teaspoonfuls) from your child. To minimise discomfort, we will perform the blood test using local anaesthetic cream prior to collection of blood. Full genetic analysis will be performed on these samples. This investigation will be performed by a member of the Paediatric Endocrinology team at Great Ormond Street Children's Hospital during one of your visits to the hospital. Only a single sample of blood is required for the study. Where possible, the blood sample will be taken at the same time as other tests of pituitary gland function which may need to be performed on your child from time to time in the out-patient clinic.

What are the risks and discomfort? No risk to the child can be foreseen. There is discomfort from a single needle prick for blood sampling, but this will be minimised by using local anaesthetic cream.

What are the potential benefits? This study may help us to understand why your child has developed their medical condition. Additionally, we may be able to make the diagnosis earlier, and where one child in the family already has the disorder, we may be alerted so that an earlier diagnosis is made in future children.

Who will have access to the case/research records? Only the researcher and a representative of the Research Ethics Committee will have access to the data collected during the study.

What are the arrangements for compensation? The project has been approved by an independent research ethics committee who believe that it is of minimal risk to you. However, research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this study. Only the work of the academic staff on this project is covered by a compensation scheme, which may apply in the event of any significant harm occurring to your child as a result of taking part in this study. Under this scheme, it would not be necessary for you to prove fault. You might also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

Do I have to take part in this study? No. If you decide, now or at a later stage, that you do not wish to participate in this research project, that is entirely your right, and will not in any way prejudice any present or future treatment.

Who do I speak to if problems arise? Please contact Dr. Dattani or Dr Mehta directly with any problems relating to the study. If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee, by post via the Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, or if urgent, by telephone on 020 7242 9879 ext. 2620, and the Committee administration will put you in contact with him.

III. CONSENT FORM

Great Ormond Street Hospital for Children NHS Trust and Institute of Child Health
Research Ethics Committee

Consent Form for parents or guardians of Children Participating in Research Studies

Title: A genetic analysis of children with forebrain, eye and / or pituitary defects

NOTES FOR PARENTS OR GUARDIANS

1. Your child has been asked to take part in a research study. The person organising that study is responsible for explaining the project to you before you give consent.

2. Please ask the researcher any questions you may have about this project, before you decide whether you wish to participate.

3. If you decide, now or at any other stage, that you do not wish your child to participate in the research project, that is entirely your right, and if your child is a patient it will not in any way prejudice any present or future treatment.

4. You will be given an information sheet, which describes the research project. This information sheet is for you to keep and refer to. *Please read it carefully.*

5. If you have any complaints about the way in which this research project has been or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee, by post via The Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH or if urgent, by telephone on 020 7905 2620 and the committee administration will put you in contact with him.

I/We _____ being the parent(s)/guardian(s) of _____ agree that the Research Project named above has been explained to me to my/our satisfaction, and I/We give permission for our child to take part in this study. I/We have read both the notes written above and the Information Sheet provided, and understand what the research study involves.

SIGNED (Parent (s)/Guardian (s)) PRINTED DATE

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SIGNED (Researcher) PRINTED DATE

IV. PROTOCOLS USED FOR EVALUATING PITUITARY FUNCTION

(A) Short (Standard) Synacthen test

Test procedure: A cannula should be inserted at least 1 hour prior to the test being performed. The test should ideally be performed at 0900, and fasting is not required.

Synacthen dose: Synacthen (Novartis Pharma AG, Stein, Switzerland) Doses are administered IM or IV (dilute in 2 ml normal saline and give slowly over 2 minutes). Anaphylaxis has been reported, but it is extremely rare.

Age	Dose
< 6 months	62.5 micrograms
6 – 24 months	125 micrograms
> 2 years	250 micrograms

Sampling: Serum cortisol should be measured at 0, 30 and 60 minutes.

(B) Spontaneous Cortisol Day Curve

Prior arrangements: Inform the laboratory

Test Procedure and Sampling: Patients to be admitted early in the morning and an indwelling intravenous catheter to be inserted 2 hours prior to blood sampling. Blood samples to measure spontaneous cortisol secretion to be drawn at 2-hour (h) intervals from 1000h until 0800h the following day.

The 0800h sample was used in analysis of the role of this timed sample in the assessment of the H-P-A axis. The mean spontaneous serum cortisol concentration is calculated as the average concentration from the 12 samples obtained.

(C) LHRH test

Test procedure: Collect basal (time 0) samples. Then administer LHRH [Gonadarelin (HRS, Intrapharm, UK)] 2.5 micrograms/kg by IV bolus (maximum of 100 micrograms).

Sampling: Basal (time 0) plasma LH, FSH, oestradiol or testosterone. Repeat plasma LH and FSH at 20 and 60 minutes.

(D) TRH Test

Test procedure: TRH 7 micrograms/kg (max of 200 micrograms) slow IV (over 3 minutes).

Sampling: Basal free T4, TSH as well as free T3. Repeat TSH at 20 and 60 minutes. If clinically indicated, prolactin or growth hormone may need to be measured as well.

(E) Intramuscular Glucagon stimulation test for GH secretion

Glucagon Dose: 100 micrograms/kg IM (maximum 1mg if <90kg; 1.5mg if >90kg).

Timing of test samples:

Time (minutes)	BM stix	Glucose	GH	Cortisol
-30	☐	☐	☐	☐
0	☐	☐	☐	☐
30	☐	☐	☐	
60	☐	☐	☐	
90	☐	☐	☐	
120	☐	☐	☐	☐
150	☐	☐	☐	☐
180	☐	☐	☐	☐

(F) **Insulin tolerance test (ITT) for GH & cortisol secretion**

Test Procedure:

- If the BM stix reading at 0 minutes is under 2.6 mmol/l, then no dose of insulin should be administered. Sampling for hormone levels should proceed as per protocol, but the study should be continued for 60 minutes only. Glucose should be administered orally as described below.
- If the BM stix level is between 2.6 – 3.5 mmol/l then 0.1 IU/kg of soluble insulin should be administered intravenously as a bolus.
- If the BM stix level is > 3.5 mmol/L, then the dose of insulin should be increased to 0.15 IU/kg of soluble insulin, UNLESS the child is suspected of suffering from panhypopituitarism or in children post-cranial surgery or post-cranial radiotherapy (which includes total body irradiation), when the child should only receive 0.1 IU/kg of soluble insulin. Consider administering IV hydrocortisone (< 3 years: 50 mg; > 3 years: 100 mg) at the end of the test.
- The dose may need to be increased in patients with diabetes mellitus, insulin resistance, pre-treatment acromegaly, pre-treatment Cushing syndrome, obesity, or when the test is being repeated due to a failure to achieve hypoglycaemia at the first attempt.
- **NB:** The dose of insulin should NEVER exceed 10 units

Timing of test samples:

Time (minutes)	BM stix	Glucose	GH	Cortisol
-30 (before insulin)	☐	☐	☐	☐
0 (before insulin)	☐	☐	☐	☐
20	☐	☐		
30	☐	☐	☐	☐
60	☐	☐	☐	☐
90	☐	☐	☐	☐
120	☐	☐	☐	☐

Failure to reach a low blood glucose at 30 minutes: If the BM stix has not gone ≤ 2.6 mmol/L, or < 50% basal blood glucose, and the child is asymptomatic, wait for 30 minutes but do NOT repeat insulin under any circumstances. Continue the test as per protocol.

(G) Combined pituitary function test

Give the following successively:

- Insulin (0.1 – 0.15 IU/kg IV) ***or*** glucagon (100 micrograms/kg IM, maximum 1 mg).
- GnRH (LHRH) 2.5 micrograms/kg IV (maximum 100 micrograms).
- TRH 7 micrograms/kg IV slowly over 3 minutes (maximum 200 micrograms).

Timing of test samples:

Time (minutes)	BM stix	Glucose	Cortisol	GH	LH	FSH	TSH	Prolactin
-30	☐	☐	☐	☐				
0	☐	☐	☐	☐	☐	☐	☐	☐
20	☐	☐			☐	☐	☐	☐
30	☐	☐	☐	☐				
60	☐	☐	☐	☐	☐	☐	☐	☐
90	☐	☐	☐	☐				
120	☐	☐	☐	☐				
And continuing if glucagon has been used:								
150	☐	☐	☐	☐				
180	☐	☐	☐	☐				

(H) Water deprivation test

Fluids must not be limited before the test, but tea and coffee must be excluded overnight. A light breakfast with minimal fluid (in the form of water) may be taken early in the morning before the test. Weigh the child, and calculate 5% of the body weight.

Test Procedure:

	Time	Wt (kg)	HR	BP	Urine volume	Specific gravity	Notes
T = 0	0830						*, **
T = 1hr	0930						**
T = 2hr	1030						*, **
T = 3hr	1130						**
T = 4hr	1230						*, **
T = 5hr	1330						*, **
T = 6hr	1430						**
T = 7hr	1530						*, **

*Blood and **urine specimens to be sent for Na^+ and osmolality. Stop the test and proceed to the second part of the test (i.e. the DDAVP test) if one of the following occurs:

1. The patient's weight falls by 5% from the starting weight.
2. Serum osmolality rises (> 295 mOsm/kg), in the face of an inappropriately dilute urine (< 300 mOsm/kg).
3. If the patient becomes clinically dehydrated.

Stop the test and do not proceed to the DDAVP test if:

1. Any urine osmolality exceeds 750 mOsm/kg.
2. At 1530 hours, even if the above criteria have not been met.

(I) DDAVP TEST

The DDAVP test follows the 7-hour water deprivation test. Administer either IM or intranasal DDAVP at a dose of:

Age	DDAVP dose	
	Nasal	Intramuscular
< 2 years	5 micrograms each nostril	0.05 microgram
2 – 8 years	7.5 micrograms each nostril	0.1 microgram
>8 years	10 micrograms each nostril	0.2 microgram

A light meal may be taken and the patient is allowed to drink fluids in volumes strictly equal to the previous hour's urine output. Over the next 4 hours, record urine volume and specific gravity, body weight, BP, HR, and the patient's thirst.

The test ends when of the following occurs: After 4 hours; Urine specific gravity > 1.014.

Sampling at the end of the test: Blood and urine samples taken for sodium and osmolality. fluid restricted to the previous hour's urine output. Serum U&Es need to be checked on the following morning.

(J) Protocol for investigation of children with suspected SOD

The following investigations are required for the evaluation of a child with SOD:

1. Assessment of cortisol secretion in the form of a cortisol day-curve and/or a physiological/standard Synacthen test is required.
2. Random serum prolactin – if low, then a formal TRH provocation test
3. Thyroid function tests – if both the plasma thyroxine (free or total) and TSH are low, then formal provocation with TRH may be indicated.
4. Plasma LH and FSH may be helpful in the first 18 months of life and then again at the time when puberty normally commences. Some of these children may have precocious puberty and a GnRH (LHRH) test may then be indicated. An HCG test may be indicated in boys with undescended testes to assess testicular function.
5. Paired plasma and urine osmolalities, preferably first thing in the morning, if a diagnosis of diabetes insipidus is a possibility.
6. Other tests should include a full blood count, U&E and a karyotype.
7. Growth should be carefully monitored. If the growth velocity is reduced, assessment of GH and cortisol secretion should be considered using one of a number of GH provocation tests e.g. insulin-induced hypoglycaemia or a glucagon test.
8. Neuroradiological imaging in the form of an MRI scan with pituitary views.
9. Ophthalmologic assessment with visual evoked responses (VER) and electrophysiology (e.g. ERG).
10. Referral to an appropriate Developmental Paediatrician.
11. DNA analysis. To be sent to *Dr Mehul Dattani* (Professor in Endocrinology, Biochemistry, Endocrinology and Metabolism Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH. Email address: mdattani@ich.ucl.ac.uk)

V. EXPANDED PATIENT DATA

Abbreviations used in this section:

A	absent
Ab	abnormal
ACTH	corticotrophin
AP	anterior pituitary
AVP	arginine vasopressin
C	cavum
CC	corpus callosum
CH	central hypothyroidism
E	enlarged
F	female
FSH	follicle stimulating hormone
GH	growth hormone
Gn	gonadotrophins
H	hypoplastic
HPE	holoprosencephaly
LH	luteinising hormone
M	male
MR	magnetic resonance
N	normal
N/A	not available
N/T	not tested
ONH	optic nerve hypoplasia
PP	posterior pituitary
Pt	patient number
PRL	prolactin
TSH	thyrotrophin
SP	septum pellucidum
SST	short Synacthen test
U	undescended
Δ	delta TSH

V-1 MR imaging and endocrinology in patients with hypopituitarism.

Pt	sex	Presentation			Deficiencies of					Prolactin (mU/L)	ONH	MR imaging				
		Age (years)	Height (SDS)	Weight (SDS)	GH	TSH	ACTH	Gn	AVP			AP	PP	SP	CC	Stalk
1	M	8.3	-4.8	-2.1	+	-	-	-	-	520	-	N	N	N	N	N
2	M	8.0	-5.9	-1.5	+	-	-	-	-	210	-	N	N	N	N	N
3	M	N/A	N/A	N/A	+	-	-	-	-	230	-	N	N	N	N	N
4	M	5.6	-5.6	-0.8	+	-	-	-	-	240	-	N	N	N	N	N
5	M	5.0	-6.9	-4.7	+	-	-	-	-	540	-	N	N	N	N	N
6	F	16.2	-4.4	0.1	+	-	-	+	-	440	-	N	N	N	N	N
7	M	0.2	-6.0	0.1	N/T	+	+	-	-	280	-	N	N	N	N	N
8	F	2.0	-7.5	-2.7	+	+	-	N/T	-	150	-	N	N	N	N	N
9	M	0.0	N/A	N/A	N/T	+	-	N/T	+	480	-	N	N	N	N	N
10	F	6.0	-2.5	3.7	+	-	-	+	+	270	-	N	N	N	N	N
11	M	1.3	-9.1	-4.8	N/T	+	+	N/T	-	1664	-	N	N	N	N	N
12	M	0.0	N/A	N/A	+	+	+	+	-	412	-	N	N	N	N	N
13	F	1.2	-9.3	-3.8	+	+	-	N/T	-	67	-	N	N	N	N	N
14	M	N/A	N/A	N/A	+	-	-	-	-	N/A	-	H	N	A	N	N
15	M	2.3	-5.9	-1.7	+	-	-	-	-	142	-	H	N	N	N	N
16	F	3.8	-8.0	3.4	+	-	-	-	-	150	-	H	U	N	N	N
17	M	2.0	-7.1	-4.3	+	-	-	-	-	N/A	-	H	N	N	N	N
18	M	3.6	-6.4	-3.7	+	-	-	-	-	110	-	H	N	N	H	N
19	F	5.8	-8.9	-4.6	+	-	-	-	-	N/A	-	H	N	N	N	N
20	M	10.5	-7.7	-2.1	+	-	-	-	-	178	-	H	U	N	N	N
21	F	4.0	-5.7	-1.2	+	-	-	-	-	345	-	H	N	N	N	N
22	F	6.1	-7.4	-3.1	+	-	-	-	-	129	-	H	N	N	N	N
23	M	8.6	-8.1	-5.8	+	-	-	-	-	95	-	H	U	N	N	N
24	M	4.0	N/A	N/A	+	-	-	-	-	N/A	-	H	U	N	N	A

V-1 [continued]

Pt	sex	Presentation			Deficiencies of					Prolactin (mU/L)	ONH	MR imaging				
		Age (years)	Height (SDS)	Weight (SDS)	GH	TSH	ACTH	Gn	AVP			AP	PP	SP	CC	Stalk
25	M	4.9	-6.7	-3.1	+	-	-	-	-	160	-	H	N	N	N	N
26	M	5.7	-6.9	-4.2	+	-	-	-	-	614	-	H	U	N	N	A
27	F	2.7	-7.4	-3.3	+	-	-	-	-	147	-	H	N	N	N	N
28	M	6.0	-6.5	-0.7	+	-	-	-	-	857	-	H	N	N	N	N
29	M	3.4	-5.7	-3.8	+	-	-	-	-	124	-	H	U	N	N	N
30	M	5.1	-5.5	-2.2	+	-	-	-	-	112	-	H	N	N	N	N
31	M	10.3	-6.4	-1.3	+	-	-	-	-	N/A	-	H	U	N	N	A
32	M	5.0	-7.7	N/A	+	-	-	-	-	228	-	H	U	N	N	A
33	F	3.8	-8.0	-4.0	+	-	-	-	-	648	-	H	U	N	N	A
34	M	11.0	-6.8	-2.7	+	-	-	-	-	N/A	-	H	N	N	N	N
35	M	3.0	N/A	N/A	+	-	-	-	-	216	-	H	U	N	N	N
36	M	1.6	-5.4	-5.0	+	-	-	-	-	260	-	A	N	N	N	N
37	M	4.5	N/A	N/A	+	-	-	-	-	192	-	H	N	N	N	N
38	M	17.3	-5.8	0.5	+	-	-	-	-	222	-	H	N	N	N	A
39	M	N/A	N/A	N/A	+	-	-	-	-	N/A	-	H	N	N	N	N
40	F	1.3	-6.1	-4.6	+	-	-	-	-	N/A	-	H	N	N	N	N
41	F	11.0	-4.9	0.2	+	-	-	-	-	N/A	-	H	N	N	N	N
42	M	6.9	-7.5	-4.0	+	-	-	-	-	N/A	-	H	N	N	N	N
43	M	6.0	-2.8	N/A	+	-	-	-	-	N/A	-	H	U	N	N	H
44	M	3.0	-6.0	-1.5	+	-	-	-	-	34	-	H	U	N	N	N
45	M	N/A	N/A	N/A	+	-	-	-	-	1400	-	H	N	N	N	N
46	F	3.5	-5.6	-1.8	+	-	-	-	-	410	+BL	H	U	A	N	A
47	M	0.5	-6.9	-4.2	+	-	-	-	-	296	+BL	H	N	A	H	H
48	F	0.6	-3.9	-0.2	+	-	-	-	-	485	+BL	H	U	N	N	A

V-1 [continued]

Pt	Sex	Presentation			Deficiencies of					Prolactin (mU/L)	ONH	MR imaging				
		Age (years)	Height (SDS)	Weight (SDS)	GH	TSH	ACTH	Gn	AVP			AP	PP	SP	CC	stalk
49	F	8.5	-5.8	N/A	+	-	-	-	-	300	+BL	H	N	A	N	H
50	M	7.6	-6.6	-2.9	+	-	-	-	-	N/A	+BL	H	N	N	N	H
51	F	2.0	N/A	N/A	+	-	-	-	-	192	+UL	H	U	N	N	A
52	M	3.1	-6.7	-3.6	+	-	-	-	-	278	+BL	H	N	N	N	A
53	F	1.5	-8.7	-3.3	+	+	+	+	-	98	+BL	H	N	N	A	A
54	M	0.5	-5.1	-1.2	+	-	+	+	+	644	+BL	H	N	A	A	A
55	F	0.3	-5.1	-2.0	+	+	+	+	-	567	+BL	H	U	A	N	N
56	M	0.6	-3.1	0.3	+	+	+	+	-	N/A	+BL	N	U	C	H	H
57	F	0.9	-0.3	4.2	+	-	+	N/T	-	270	+BL	H	A	N	H	A
58	F	0.1	-6.1	-0.8	+	+	+	+	+	1360	+BL	H	U	A	N	H
59	M	3.6	-5.3	-1.9	N/T	-	+	N/T	+	N/A	+BL	H	U	N	A	N
60	M	2.9	-8.3	-3.6	+	+	+	N/T	-	1223	+BL	H	N	N	A	N
61	M	0.5	-5.0	-1.5	+	+	+	+	-	206	+BL	H	U	C	N	H
62	F	1.9	-4.4	0.4	+	+	+	N/T	-	248	+UL	H	U	N	N	A
63	M	2.0	N/A	N/A	+	+	+	+	-	1336	+BL	H	N	A	N	N
64	M	0.2	-9.0	-4.1	+	+	+	-	+	355	+BL	H	N	A	A	N
65	F	10.9	-4.2	-0.7	+	+	+	+	-	106	+BL	N	U	A	N	N
66	M	0.8	-4.8	N/A	+	+	+	+	-	1875	+BL	H	N	N	A	N
67	F	1.0	-6.2	-2.9	+	+	+	N/T	-	665	+UL	H	U	N	N	A
68	M	0.4	-3.9	-0.7	N/T	+	+	N/T	-	N/A	+UL	A	U	N	H	N
69	M	0.6	-8.0	-6.7	+	+	+	+	-	1260	+BL	H	N	N	N	A
70	M	0.4	-5.6	-0.9	+	+	+	N/T	-	661	+BL	H	A	N	H	H
71	F	0.0	-4.9	-1.4	+	+	+	+	-	2160	+BL	H	A	A	H	H
72	M	3.5	-6.4	-2.8	+	+	-	N/T	-	N/A	+BL	N	N	N	N	H

V-1 [continued]

Pt	Sex	Presentation			Deficiencies of					Prolactin (mU/L)	ONH	MR imaging				
		Age (years)	Height (SDS)	Weight (SDS)	GH	TSH	ACTH	Gn	AVP			AP	PP	SP	CC	stalk
73	M	1.3	-2.9	2.8	+	+	-	-	+	114	+BL	H	U	N	N	A
74	M	0.5	-2.2	1.0	+	+	+	-	+	900	+BL	H	N	A	N	N
75	F	0.6	-3.9	0.0	+	+	+	-	-	448	+BL	H	U	A	N	A
76	M	9.2	-3.0	2.4	+	-	+	N/T	-	766	+BL	H	N	C	N	H
77	F	4.5	-3.5	2.7	+	+	+	-	+	343	+BL	H	U	C	A	A
78	F	0.3	-5.7	-3.2	-	+	+	+	-	N/A	+BL	H	N	N	A	N
79	M	0.1	-4.5	-1.0	+	+	+	+	-	N/A	+BL	H	U	N	N	A
80	M	10.7	-5.6	-1.2	+	-	+	-	+	N/A	+UL	H	U	A	N	A
81	F	0.5	-1.4	-4.1	+	+	+	N/T	+	160	+BL	H	U	A	N	A
82	F	0.8	-6.7	-4.0	+	+	+	-	-	12	+BL	H	U	N	N	A
83	M	0.9	-3.2	-0.3	+	+	+	N/T	-	555	+BL	H	N	A	N	N
84	F	5.0	N/A	N/A	+	+	+	-	-	489	+BL	H	N	N	N	A
85	M	1.0	N/A	N/A	+	-	+	N/T	+	N/A	+BL	H	N	A	N	N
86	F	2.3	-7.3	-1.3	+	+	+	N/T	-	407	+BL	H	U	A	N	A
87	F	0.0	N/A	N/A	+	+	+	-	+	812	+BL	H	N	N	A	N
88	F	3.6	-6.9	-4.0	+	+	+	N/T	-	2947	+BL	N	U	A	A	A
89	M	0.1	-5.2	-0.6	+	+	+	-	-	N/A	+BL	H	N	A	N	N
90	M	0.5	N/A	N/A	+	+	+	+	+	N/A	+BL	H	N	A	N	N
91	F	0.2	-6.2	-1.3	N/T	+	+	N/T	-	N/A	+UL	H	N	A	H	N
92	M	11.3	N/A	N/A	+	+	+	N/T	+	202	+BL	H	E	A	N	A
93	F	2.2	-5.1	N/A	+	-	+	N/T	+	432	+BL	A	U	N	A	N
94	M	3.3	-5.7	-1.6	+	-	+	N/T	-	N/A	+BL	H	U	N	N	N
95	F	2.8	-5.7	0.9	+	+	+	N/T	-	N/A	+BL	A	N	A	N	A
96	M	0.2	-6.0	-1.3	+	+	+	N/T	-	669	+BL	H	U	N	N	A

V-1 [continued]

Pt	Sex	Presentation			Deficiencies of					Prolactin (mU/L)	ONH	MR imaging				
		Age (years)	Height (SDS)	Weight (SDS)	GH	TSH	ACTH	Gn	AVP			AP	PP	SP	CC	stalk
97	M	1.0	-4.8	-1.6	+	+	+	+	-	316	-	H	U	N	N	A
98	M	0.6	N/A	N/A	+	+	+	+	-	N/A	-	H	U	N	N	A
99	M	3.0	-4.8	0.3	+	+	+	+	-	N/A	-	H	U	N	N	A
100	M	3.4	-4.9	0.2	+	+	-	N/T	-	441	-	N	U	N	N	A
101	M	4.9	-6.7	-2.1	+	+	-	N/T	-	264	-	H	U	N	N	A
102	M	2.0	-7.9	-4.9	+	+	-	N/T	-	196	-	H	U	N	N	A
103	M	1.8	-2.5	1.7	N/T	+	+	N/T	-	558	-	H	U	N	H	A
104	M	1.6	-4.3	-0.1	+	-	+	N/T	-	70	-	H	N	N	N	A
105	M	10.8	-7.7	-4.5	+	+	+	+	-	N/A	-	H	U	N	N	A
106	F	3.9	-9.5	-5.4	+	+	+	+	-	158	-	H	U	N	N	A
107	M	0.0	N/A	N/A	+	+	+	+	+	N/A	-	H	N	N	N	A
108	F	2.5	-7.5	-3.9	+	+	-	N/T	-	537	-	H	N	N	N	N
109	M	4.5	N/A	N/A	+	+	-	-	-	298	-	H	N	N	N	N
110	M	0.1	-7.9	-2.1	+	+	+	+	-	132	-	H	U	N	A	A
111	M	3.0	-9.9	-6.8	+	-	-	+	-	153	-	H	N	N	N	A
112	M	0.5	-7.8	-4.9	+	+	-	-	-	44	-	H	N	N	N	N
113	M	0.6	-4.7	-1.8	+	-	-	+	+	151	-	H	N	A	N	N
114	M	2.9	-6.8	-4.1	+	+	+	N/T	-	446	-	H	U	N	N	A
115	M	0.4	-6.8	-1.6	+	+	+	+	-	N/A	-	H	U	N	N	A
116	M	3.2	-5.3	0.9	+	-	-	+	-	281	-	H	U	N	N	N
117	M	9.0	-4.5	-3.8	+	+	-	+	-	323	-	E	N	N	N	N
118	M	4.5	-6.2	-1.7	+	+	+	+	-	735	-	H	N	N	N	A
119	M	6.8	-6.1	-2.9	+	+	+	+	-	475	-	N	U	N	N	A
120	M	N/A	N/A	N/A	+	+	+	+	-	11	-	H	U	N	N	N

V-1 [continued]

Pt	Sex	Presentation			Deficiencies of					Prolactin (mU/L)	ONH	MR imaging				
		Age (years)	Height (SDS)	Weight (SDS)	GH	TSH	ACTH	Gn	AVP			AP	PP	SP	CC	stalk
121	F	0.1	-4.9	-1.0	+	+	+	+	-	3280	-	H	U	N	N	N
122	F	0.0	-3.1	1.5	+	+	+	+	-	667	-	N	U	N	A	A
123	M	N/A	N/A	N/A	+	-	+	-	-	N/A	-	H	U	N	N	A
124	F	0.5	-3.7	0.6	+	+	+	N/T	-	810	-	H	U	N	N	N
125	M	10.0	N/A	N/A	+	+	+	-	-	336	-	H	U	N	N	N
126	M	7.0	-6.0	-2.2	+	+	+	+	-	1051	-	H	U	N	N	A
127	F	N/A	N/A	N/A	+	+	+	+	-	N/A	-	H	U	N	N	A
128	M	3.0	-7.6	3.7	+	+	+	N/T	-	N/A	-	N	U	N	N	A
129	M	N/A	N/A	N/A	+	+	-	-	-	N/A	-	H	N	N	N	A
130	F	0.7	-4.2	-0.8	+	+	+	N/T	-	242	-	H	U	N	N	N
131	M	4.8	-7.0	-3.1	+	-	+	-	-	591	-	N	U	N	N	A
132	F	N/A	N/A	N/A	+	+	+	+	-	N/A	-	H	N	N	A	N
133	M	5.3	-5.1	1.1	+	+	+	N/T	-	477	-	N	U	N	N	A
134	M	1.9	-7.1	-2.7	+	-	-	+	-	105	-	H	U	N	N	A
135	M	0.5	-6.8	-4.2	+	+	+	+	-	N/A	-	H	N	N	N	A
136	M	0.3	-5.7	-0.9	+	+	+	+	-	19	-	H	N	N	N	N
137	M	11.3	-6.5	-2.6	+	+	-	-	-	732	-	H	N	N	N	N

V-2 "Control" group B: Patients with optic nerve hypoplasia and normal endocrinology

Pt	sex	Age at presentation	Deficiencies of					Prolactin (mU/L)	ONH	MR imaging				
			GH	TSH	ACTH	Gn	AVP			AP	PP	SP	CC	Stalk
138	M	3.6	N/T	-	-	-	-	152	+UL	H	N	N	N	N
139	M	2.4	N/T	-	-	-	-	60	+BL	H	N	A	N	H
140	F	4.0	-	-	-	-	-	117	+BL	H	N	N	N	N
141	M	1.5	N/T	-	-	-	-	213	+BL	N	A	A	N	H
142	M	1.4	N/T	-	-	-	-	191	+BL	N	N	A	H	H
143	M	5.2	N/T	-	-	-	-	189	+BL	H	N	N	N	N
144	M	6.0	N/T	-	-	-	-	174	+UL	H	N	N	N	H
145	F	0.8	N/T	-	-	-	-	N/A	+BL	H	N	N	N	H
146	F	0.9	N/T	-	-	-	-	N/A	+BL	H	N	A	H	N
147	F	0.6	N/T	-	-	-	-	401	+BL	N	N	N	H	N
148	M	0.3	N/T	-	-	-	-	423	+BL	N	N	A	A	N
149	F	0.3	N/T	-	-	-	-	338	+BL	N	N	N	H	N
150	F	0.2	N/T	-	-	-	-	277	+BL	H	N	A	H	N
151	M	5.7	-	-	-	-	-	55	+BL	H	N	N	N	N
152	M	1.1	-	-	-	-	-	86	+BL	H	N	C	A	N
153	F	1.6	N/T	-	-	-	-	322	+BL	N	N	N	N	H
154	M	0.4	N/T	-	-	-	-	N/A	+BL	H	A	N	N	H
155	F	0.9	N/T	-	-	-	-	196	+BL	N	N	A	H	N
156	F	1.6	N/T	-	-	-	-	300	+UL	H	N	N	N	H
157	F	0.6	N/T	-	-	-	-	261	+BL	H	A	N	N	N
158	M	6.5	N/T	-	-	-	-	487	+BL	N	N	N	N	H
159	F	0.5	N/T	-	-	-	-	573	+BL	N	N	A	H	N
160	M	0.6	N/T	-	-	-	-	220	+BL	H	N	A	H	N
161	F	1.1	N/T	-	-	-	-	322	+BL	N	N	C	H	H
162	F	0.2	N/T	-	-	-	-	2082	+BL	N	N	A	N	N

V-2 [continued]

Pt	Sex	Age at presentation	Deficiencies of					Prolatin (mU/L)	ONH	MR imaging				
			GH	TSH	ACTH	Gn	AVP			AP	PP	SP	CC	stalk
163	M	0.7	N/T	-	-	-	-	344	+BL	H	A	A	H	H
164	M	0.2	N/T	-	-	-	-	244	+BL	H	A	A	N	H
165	F	1.6	N/T	-	-	-	-	241	+BL	H	A	C	N	H
166	M	0.7	-	-	-	-	-	254	+BL	N	N	A	N	N
167	M	1.1	N/T	-	-	-	-	208	+BL	H	U	N	H	H
168	M	3.4	-	-	-	-	-	170	+UL	N	N	N	A	N
169	F	0.9	N/T	-	-	-	-	1812	+UL	H	N	N	H	H
170	M	2.0	-	-	-	-	-	572	+UL	H	N	N	N	H

V-3 Patients with isolated GH deficiency and no midline defects on MR scanning [Group A].

Pt	sex	Birth (SDS)			MPH SDS	age at GH treatment (years)	Peak GH (mU/L)	Deficiencies of					MR imaging					
		weight	length	BMI				TSH	ACTH	Gn	AVP	PRL (mU/L)	AP	PP	SP/CC	Stalk	ONH	HPE
1	F	-1.6	0.7	-2.7	-1.7	3.8	7.1	-	-	N/T	-	150	N	U	N	N	-	-
2	M	-0.3	-1.0	0.7	-2.0	3.0	1.0	-	-	N/T	-	N/A	H	N	N	N	-	-
3	M	0.1	1.0	-0.4	-0.2	2.9	19.8	-	-	N/T	-	306	N	N	N	N	-	-
4	F	-2.4	-1.7	-2.3	-2.3	6.1	2.6	-	-	N/T	-	N/A	H	N	N	N	-	-
5	F	-0.7	1.9	-2.1	-1.1	2.0	7.9	-	-	N/T	-	974	N	N	N	N	-	-
6	M	-2.1	-1.9	-1.6	-1.9	4.9	12.0	-	-	N/T	-	171	H	N	N	N	-	-
7	F	0.0	0.0	0.3	-1.4	4.2	7.0	-	-	N/T	-	648	H	N	N	Ab	-	-
8	M	-1.8	-1.5	-1.4	-0.7	3.8	6.9	-	-	N/T	-	215	H	U	N	N	-	-
9	M	-1.3	-0.8	-1.0	-0.7	4.9	17.6	-	-	N/T	-	144	N	N	N	N	-	-
10	M	-0.7	-1.0	0.0	0.9	2.2	1.3	-	-	N/T	-	259	H	N	N	N	-	-
11	F	-0.5	N/A	N/A	-2.4	2.8	0.5	-	-	-	-	199	H	N	N	N	-	-
12	M	-1.6	-1.7	-0.9	-1.1	5.6	11.0	-	-	N/T	-	540	N	N	N	N	-	-

V-4 Patients with CPHD but no midline defects on MR scanning [Group B].

Pt	sex	Birth (SDS)			MPH SDS	age at GH treatment (years)	Peak GH (mU/L)	Deficiencies of					MR imaging					
		weight	length	BMI				TSH	ACTH	Gn	AVP	PRL (mU/L)	AP	PP	SP/CC	Stalk	ONH	HPE
13	M	-4.0	-2.0	-6.0	-0.4	2.5	15.8	+	-	N/T	-	196	H	U	N	Ab	-	-
14	M	-0.8	-1.4	0.3	-1.2	1.1	0.9	-	+	+	-	70	H	N	N	Ab	-	-
15	M	-1.0	0.8	-1.8	-1.8	2.0	1.0	+	-	-	-	44	H	N	N	N	-	-
16	M	0.4	-1.3	2.0	0.3	3.0	2.8	+	+	+	-	446	H	U	N	Ab	-	-
17	M	-0.4	2.1	-1.8	-0.4	2.0	1.7	+	+	+	-	1887	H	U	N	Ab	-	-
18	F	0.2	-1.6	1.9	0.2	0.7	N/T	+	+	N/T	-	3280	H	U	N	N	-	-
19	F	1.3	N/A	N/A	0.0	2.0	N/T	+	+	+	-	810	H	U	N	N	-	-
20	F	0.5	-0.5	1.5	0.3	1.7	3.4	+	-	N/T	-	67	H	N	N	N	-	-
21	F	0.0	0.0	0.4	-0.2	2.2	1.2	+	+	+	-	242	H	U	N	N	-	-
22	M	-1.1	-1.0	-0.5	0.6	1.0	N/T	+	+	+	-	19	H	N	N	N	-	-

V-5 Patients with CPHD and midline defects on MR scanning [Group C]

Pt	sex	Birth (SDS)			MPH SDS	age at GH treatment (years)	Peak GH (mU/L)	Deficiencies of						MR imaging			
		weight	length	BMI				TSH	ACTH	Gn	AVP	PRL (mU/L)	AP	PP	SP/CC	Stalk	ONH	HPE
23	F	1.7	1.5	1.5	0.2	2.6	1.7	+	+	+	-	98	H	N	Ab	Ab	+	-
24	F	-1.0	N/A	N/A	-1.2	1.2	6.4	-	+	N/T	+	644	H	N	Ab	Ab	+	-
25	M	0.6	-2.2	3.1	0.7	3.1	2.9	+	+	N/T	-	343	H	N	Ab	N	-	+
26	F	-0.2	-1.3	1.1	-1.5	2.0	20.0	-	-	-	+	419	N	N	Ab	N	-	+
27	M	-1.2	0.0	-1.6	-0.5	2.5	N/T	+	+	N/T	-	N/A	N	N	Ab	N	+	-
28	F	0.3	-1.5	2.0	0.6	1.2	5.4	+	+	+	+	1360	H	U	Ab	Ab	+	-
29	M	-0.1	-1.2	1.2	-0.5	2.0	5.7	+	+	N/T	-	266	N	N	Ab	N	+	-
30	M	-0.3	-1.6	1.2	0.7	1.8	5.2	+	+	-	+	355	N	N	Ab	N	-	-
31	M	-1.0	-0.8	-0.5	0.9	1.6	10.5	+	+	+	-	665	H	U	N	Ab	+	-
32	M	-0.7	-4.6	3.5	-1.6	1.0	6.5	+	+	+	-	1260	H	N	N	Ab	+	-
33	M	-0.6	N/A	N/A	-2.0	1.5	8.4	+	+	N/T	-	661	H	U	Ab	N	+	-
34	F	-1.3	-1.6	-0.3	-0.3	1.2	11.5	+	+	-	-	2160	H	N	Ab	N	+	-
35	M	0.4	-2.1	2.8	-0.2	0.6	N/T	+	+	N/T	-	132	H	U	Ab	Ab	-	-
36	M	-0.7	-1.0	0.0	-0.7	2.0	2.2	+	+	N/T	+	900	N	N	Ab	N	+	-
37	F	0.6	N/A	N/A	1.4	1.5	6.9	+	+	-	-	448	H	U	Ab	N	+	-
38	M	0.2	-0.1	0.5	0.1	2.3	9.3	+	+	+	-	N/A	H	U	N	Ab	+	-
39	F	1.9	N/A	N/A	0.0	4.0	18.6	+	+	N/T	+	166	H	U	Ab	Ab	+	-
40	M	1.6	0.5	1.9	-0.6	1.5	9.0	+	-	N/T	-	555	H	N	Ab	N	+	-
41	F	0.1	-1.1	1.3	0.5	1.0	6.7	+	+	+	-	667	N	N	Ab	N	-	-
42	F	-0.8	-3.1	1.7	0.0	3.3	N/T	+	+	N/T	-	392	H	U	Ab	Ab	+	-
43	M	0.2	-1.0	1.3	-0.6	1.1	20.0	+	+	-	-	N/A	H	N	Ab	N	+	-
44	M	-1.0	N/A	N/A	0.1	1.9	10.3	+	+	-	-	669	H	U	N	N	+	-

V-6 Patients with central hypothyroidism without midline defects (Group A)

Pt	Sex	Age at (yrs)		FT4	TT4	TSH (mU/L)				Deficiencies of				PRL	MR imaging				
		diagnosis	CH	(pmol/L)	(nmol/L)	Unstimulated	Peak	Delayed	Δ	GH	ACTH	Gn	AVP	(mU/L)	AP	PP	SP/CC	stalk	ONH
1	M	5.0	5.0	6.6	-	2.2	N/T			+	+	+	-	316	H	U	N	Ab	-
2	M	0.6	0.6	-	62.0	3.6	N/T			+	+	N/T	-	N/A	H	U	N	Ab	-
3	M	2.0	2.0	8.7	-	2.3	N/T			+	-	N/T	-	196	H	U	N	Ab	-
4	M	11.0	11.0	7.3	-	1.5	15.0	-	13.5	+	+	N/T	-	366	H	U	N	Ab	-
5	F	6.1	6.1	5.5	-	1.0	N/T			+	+	N/T	-	158	H	U	N	Ab	-
6	M	0.5	0.5	9.9	-	3.1	13.1	-	10.0	N/T	+	+	-	222	H	N	N	N	-
7	F	1.2	1.2	11.5	-	2.1	25.9	+	23.8	+	-	-	-	537	H	N	N	N	-
8	M	1.5	1.5	-	38.0	1.0	N/T			+	+	-	-	44	H	N	N	Ab	-
9	M	2.9	2.9	6.9	-	3.2	31.2	+	28.0	+	+	-	-	446	H	U	N	Ab	-
10	M	0.0	0.0	7.7	-	0.3	N/T			N/T	+	-	-	1664	N	N	N	N	-
11	M	0.4	0.4	9.0	-	3.0	41.2	+	38.2	+	+	+	-	1887	H	U	N	Ab	-
12	M	9.0	9.0	6.9	-	2.2	4.8	-	2.6	+	-	+	-	323	H	N	N	N	-
13	M	0.3	3.7	-	60.0	1.0	1.0	-	0.0	+	+	+	-	735	H	N	N	Ab	-
14	M	6.3	6.3	-	58.0	2.8	20.7	+	17.9	+	+	+	-	475	N	U	N	Ab	-
15	F	0.0	0.0	6.4	-	3.6	49.3	-	45.7	+	+	+	-	87	N	N	N	N	-
16	M	2.5	2.5	5.1	-	1.4	1.4	-	0.0	-	-	-	-	N/A	N	N	N	N	-
17	F	0.0	0.0	5.9	-	0.0	N/T			N/T	+	+	-	1163	H	U	N	N	-
18	M	10.0	16.0	-	50.0	2.2	4.0	-	1.8	+	+	-	-	336	H	U	N	N	-
19	M	7.0	7.0	8.4	-	2.6	14.0	-	11.4	+	+	+	-	1051	H	U	N	Ab	-
20	M	3.0	3.0	8.8	-	3.3	6.5	-	3.2	+	+	-	-	N/A	N	U	N	Ab	-
21	F	1.2	1.2	5.0	-	2.1	3.7	-	1.6	+	-	N/T	-	67	H	N	N	N	-
22	F	0.7	0.7	6.9	-	7.7	N/T			+	+	-	-	242	H	U	N	N	-
23	M	0.1	0.1	9.6	-	4.1	N/T			+	+	N/T	-	N/A	H	N	N	Ab	-
24	M	0.3	0.3	10.2	-	3.3	6.6	-	3.3	+	+	+	-	14	H	N	N	N	-

V-7 Patients with central hypothyroidism and midline defects (Group B)

Pt	Sex	Age at (yrs)		T4		TSH (mU/L)				Deficiencies of				PRL (mU/L)	MR imaging				
		Initial diagnosis	CH	FT4 (pmol/L)	TT4 (nmol/L)	Unstimulated	Peak	Delayed peak	Δ	GH	ACTH	Gn	AVP		AP	PP	SP/CC	stalk	ONH
25	F	1.5	6.0	-	48.0	3.5	4.5	-	1.0	+	-	+	-	98	N	N	Ab	N	+
26	M	0.0	0.0	7.0	-	1.2	N/T			+	-	+	+	N/A	H	N	N	Ab	+
27	M	0.0	0.0	8.8	-	2.1	N/T			+	+	-	-	343	H	N	Ab	N	-
28	F	0.0	0.0	10.4	-	3.1	25.3	-	22.2	+	+	+	-	567	H	U	Ab	N	+
29	F	0.4	1.0	-	54.0	4.6	N/T			+	+	+	+	N/A	H	N	N	N	+
30	M	0.6	0.6	8.4	-	4.9	N/T			+	+	N/T	-	N/A	N	N	Ab	N	+
31	F	0.0	0.0	10.2	-	4.1	48.9	+	44.8	+	+	+	+	1360	H	U	Ab	Ab	+
32	M	3.8	3.8	10.4	-	9.1	N/T			+	-	N/T	-	1223	H	N	Ab	N	+
33	F	0.0	0.0	7.6	-	0.3	N/T			N/T	+	-	-	N/A	N	U	Ab	N	-
34	M	0.0	0.0	8.8	-	0.9	12.6	-	11.7	N/T	-	-	-	410	N	N	Ab	N	-
35	M	0.0	0.0	10.8	-	3.7	N/T			+	+	N/T	-	266	H	N	Ab	N	+
36	M	2.0	2.0	7.0	-	1.3	8.8	-	7.5	+	+	+	-	1336	H	N	Ab	N	+
37	M	0.2	0.2	7.0	-	2.2	26.3	+	24.1	+	+	-	+	355	H	N	Ab	N	+
38	F	7.6	12.0	11.4	-	0.9	N/T			+	+	+	-	106	N	U	Ab	N	+
39	M	0.0	0.0	7.8	-	2.0	N/T			+	+	+	-	1875	H	N	Ab	N	+
40	F	0.0	0.0	6.9	-	11.0	N/T			+	+	N/T	-	665	H	U	N	Ab	+
41	M	4.3	4.3	8.6	-	1.2	N/T			+	-	+	-	N/A	N	N	N	N	+
42	M	0.6	0.6	-	45.0	0.2	0.2	-	0.0	+	+	+	-	1260	H	N	N	Ab	+
43	F	0.0	0.0	6.4	-	1.7	27.9	+	26.2	+	+	+	-	2160	H	N	Ab	N	+

V-7 [continued]

Pt	Sex	Age at (yrs)		T4		TSH (mU/L)				Deficiencies of				PRL (mU/L)	MR imaging				
		Initial diagnosis	CH	FT4 (pmol/L)	TT4 (nmol/L)	Unstimulated	Peak	Delayed peak	Δ	GH	ACTH	Gn	AVP		AP	PP	SP/CC	Stalk	ONH
44	M	0.1	0.1	-	42.0	5.0	N/T			+	+	N/T	-	N/A	H	U	Ab	Ab	-
45	M	0.3	0.3	9.9	-	2.8	N/T			+	+	-	+	900	N	U	N	N	+
46	F	0.1	11.9	6.5	-	1.8	20.5	-	18.7	+	-	-	+	343	H	U	Ab	Ab	+
47	M	4.5	4.5	9.8	-	1.4	9.9	-	8.5	+	+	+	-	N/A	H	U	Ab	Ab	+
48	M	4.0	4.0	7.0	-	2.3	23.6	-	21.3	+	+	-	-	N/A	H	N	N	N	+
49	F	0.0	1.0	9.0	-	2.2	N/T			+	+	+	-	667	N	N	Ab	N	-
50	F	3.6	3.6	8.0	-	1.3	11.5	+	10.2	+	+	N/T	-	2947	N	U	Ab	Ab	-
51	M	0.0	0.0	-	65.0	4.1	22.4	-	18.3	+	+	-	-	N/A	H	N	Ab	N	+
52	M	0.5	0.5	9.1	-	6.3	13.1	+	6.8	+	+	-	+	N/A	N	N	Ab	N	+
53	F	2.0	12.0	9.7	-	1.6	10.2	-	8.6	+	-	+	-	N/A	H	N	Ab	N	-
54	M	0.0	0.0	9.4	-	5.5	N/T			+	+	+	-	669	H	U	N	N	+

V-8 Clinical, MR imaging and endocrine data in patients analysed to assess the efficacy of the short Synacthen test

Pt.	Sex	Age at test (years)	Cortisol (nmol/L)					Deficiencies of				PRL	MR imaging				
			Mean spontaneous	SST (minutes)			0800 hour	GH	TSH	Gn	AVP	(mU/L)	AP	PP	SP/CC	stalk	ONH
				0	30	60											
1	M	0.6	28	28	98	100	28	N/T	+	N/T	-	1664	N	N	N	N	-
2	F	0.3	28	28	64	69	28	N/T	+	N/T	-	1163	N	U	N	N	-
3	M	0.4	39	75	214	229	52	+	+	-	+	355	H	N	Ab	N	+
4	F	0.1	55	157	268	284	28	+	+	N/T	-	448	H	U	Ab	N	+
5	F	0.1	68	8	259	259	28	+	+	-	-	567	H	U	Ab	N	+
6	M	0.5	73	59	314	409	76	+	-	N/T	+	644	H	N	Ab	Ab	+
7	M	0.3	78	28	28	28	28	+	+	N/T	-	669	N	U	N	N	+
8	F	1.1	83	60	320	492	131	N/T	+	N/T	-	270	H	N	Ab	N	+
9	F	2.4	83	82	358	510	56	N/T	+	N/T	-	407	N	N	Ab	N	+
10	M	0.5	98	56	728	766	61	+	+	N/T	-	266	H	N	Ab	N	+
11	M	1.0	99	274	469	478	162	+	+	+	+	900	N	N	Ab	N	+
12	F	0.2	120	41	752	1141	153	+	+	-	-	11	H	N	N	Ab	+
13	F	1.6	120	101	488	652	221	N/T	-	N/T	-	300	N	N	N	N	+
14	M	0.9	141	88	525	680	157	+	+	N/T	-	555	N	N	Ab	N	+
15	F	0.8	142	145	736	901	122	N/T	-	-	-	277	H	U	Ab	N	+
16	M	5.1	148	90	457	596	233	+	+	N/T	-	1223	H	N	Ab	N	+
17	F	0.1	157	55	823	1133	440	N/T	-	-	-	1811	N	N	N	N	+
18	F	0.5	171	121	871	1048	121	N/T	-	N/T	-	338	N	N	Ab	N	+
19	F	1.0	174	175	896	935	268	N/T	-	N/T	-	503	N	N	N	N	+
20	M	0.7	198	229	842	862	193	N/T	-	-	-	344	N	N	Ab	N	+
21	F	0.8	199	326	722	780	457	N/T	-	N/T	-	401	N	N	Ab	N	+
22	M	1.8	204	191	730	930	315	N/T	-	-	-	213	H	U	Ab	Ab	+
23	M	1.4	205	145	586	779	215	+	+	+	+	114	H	U	N	Ab	+
24	M	0.5	220	353	742	930	311	N/T	-	-	-	220	N	N	Ab	N	+
25	F	0.0	228	116	480	549	193	+	+	+	-	2160	H	N	Ab	N	+
26	F	1.8	238	288	830	1053	375	N/T	-	N/T	-	188	N	N	N	N	+
27	M	2.3	243	273	505	647	403	+	-	+	-	70	N	N	N	Ab	-
28	F	0.9	258	183	800	968	175	N/T	-	-	-	455	H	N	N	N	+

V-9 Clinical, neuroradiological and endocrine data of patients who underwent the LHRH test

Pt.	sex	age at	LH (U/L) at minutes			FSH (U/L) at minutes			genital	Deficiencies of				PRL	MR imaging				
		test (yrs)	0	20	60	0	20	60	abnormality	GH	TSH	ACTH	AVP		AP	PP	SP/CC	stalk	ONH
1	M	4.4	0.7	5.3	3.7	0.9	4.2	4.2	-	N/T	-	-	-	317	N	U	Ab	N	-
2	F	3.9	0.7	2.0	1.9	1.1	8.4	17.6	-	+	+	+	-	28	H	N	Ab	N	+
3	F	0.7	0.7	0.7	0.7	0.2	1.7	2.4	-	+	+	+	+	1360	H	N	Ab	Ab	+
4	F	2.9	0.7	14.2	15.7	4.3	14.9	22.4	-	-	+	+	-	2005	N	N	Ab	N	-
5	M	3.0	5.1	37.5	21.9	6.8	16.4	22.8	-	N/T	+	+	-	247	N	U	N	N	-
6	M	17.4	0.7	2.0	1.8	0.3	1.0	3.6	+	+	-	-	-	659	H	U	Ab	Ab	+
7	M	4.6	1.1	6.9	5.8	1.0	2.0	3.4	+	+	+	+	+	355	H	U	Ab	N	+
8	M	7.6	0.7	2.6	1.9	0.2	1.6	1.6	-	+	-	+	-	423	N	U	Ab	N	-
9	M	5.0	0.7	0.7	0.7	0.2	0.2	0.2	+	N/T	+	-	-	222	H	U	N	N	-
10	F	9.2	0.7	7.6	4.9	5.4	39.7	48.2	-	N/T	-	-	-	277	H	U	Ab	N	+
11	F	0.3	0.7	0.7	0.7	0.3	3.5	3.4	-	+	+	+	-	2160	H	U	Ab	N	+
12	M	16.8	0.7	2.5	1.6	0.6	3.8	4.2	-	+	+	-	+	114	H	N	N	Ab	+
13	F	7.4	0.7	13.0	11.7	15.8	44.0	49.1	-	+	+	+	+	448	H	N	Ab	Ab	+
14	M	14.9	0.7	6.4	6.8	0.8	2.1	3.8	+	-	-	-	-	86	H	U	Ab	N	+
15	M	11.5	0.7	0.7	0.7	0.2	0.9	1.6	-	+	-	-	-	90	H	U	N	N	-
16	M	15.0	0.7	0.7	0.7	0.2	0.2	0.2	-	+	+	+	-	636	H	U	N	N	-
17	M	6.0	0.7	0.7	0.7	0.2	0.2	0.2	+	+	+	+	-	1887	H	N	N	Ab	-
18	M	3.1	0.7	11.8	8.6	1.1	19.6	23.1	+	-	-	-	-	166	N	U	Ab	N	+
19	M	12.7	0.2	1.1	0.8	0.6	2.1	2.5	+	+	+	+	-	N/A	H	N	Ab	Ab	+
20	F	11.1	0.7	8.5	5.2	3.4	48.4	66.1	-	N/T	-	-	-	456	H	U	Ab	Ab	+
21	F	2.3	0.9	5.9	4.9	14.9	28.2	32.5	-	+	+	-	-	12	H	N	N	Ab	+
22	M	6.3	0.8	24.6	27.9	2.8	9.7	16.5	-	N/T	-	-	-	220	H	U	Ab	N	+
23	F	8.8	0.5	0.5	0.5	0.3	0.3	0.3	-	+	+	+	-	667	N	U	Ab	N	-
24	M	8.3	0.7	5.8	4.0	0.4	1.3	1.5	-	N/T	-	-	-	344	H	N	Ab	Ab	+
25	F	2.4	0.7	0.7	0.7	0.6	3.6	4.1	-	+	+	+	-	1319	N	N	N	Ab	-
26	M	9.9	0.7	6.6	4.2	0.6	2.0	2.8	-	-	-	-	-	254	N	U	Ab	N	-
27	F	0.7	0.7	0.7	0.7	0.2	0.2	0.2	-	N/T	+	+	-	1777	N	N	Ab	N	+
28	F	1.2	0.7	0.7	10.9	15.4	56.9	91.4	-	N/T	-	-	-	1812	H	U	Ab	Ab	+
29	F	16.7	0.7	11.4	11.6	9.6	52.9	78.6	-	-	-	-	-	101	N	U	Ab	N	-
30	M	5.9	0.7	2.7	2.4	0.8	1.3	1.6	+	+	+	+	-	14	H	U	N	N	-