Chapter 13
Pituitary Transcription Factor Mutations Leading to Hypopituitarism

Peter Gergics

Abstract Congenital pituitary hormone deficiency is a disabling condition. It is part of a spectrum of disorders including craniofacial midline developmental defects ranging from holoprosencephaly through septo-optic dysplasia to combined and isolated pituitary hormone deficiency. The first genes discovered in the human disease were based on mouse models of dwarfism due to mutations in transcription factor genes. High-throughput DNA sequencing technologies enabled clinicians and researchers to find novel genetic causes of hypopituitarism for the more than three quarters of patients without a known genetic diagnosis to date. Transcription factor (TF) genes are at the forefront of the functional analysis of novel variants of unknown significance due to the relative ease in in vitro testing in a research lab. Genetic testing in hypopituitarism is of high importance to the individual and their family to predict phenotype composition, disease progression and to avoid life-threatening complications such as secondary adrenal insufficiency.

This chapter aims to highlight our current understanding about (1) the contribution of TF genes to pituitary development (2) the diversity of inheritance and phenotype features in combined and select isolated pituitary hormone deficiency and (3) provide an initial assessment on how to approach variants of unknown significance in human hypopituitarism. Our better understanding on how transcription factor gene variants lead to hypopituitarism is a meaningful step to plan advanced therapies to specific genetic changes in the future.

Keywords Pituitary hormone deficiency · Transcription factor · Inheritance · Genetic testing · Variants of unknown significance

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List of Abbreviations

ACTH	Adrenocorticotropic hormone
AL	Anterior lobe of pituitary
Bmp	Bone morphogenetic protein
CGA	Choriogonadotropin alpha subunit
CNS	Central nervous system
CPHD	Combined pituitary hormone deficiency
Fgf	Fibroblast growth factor
FSH	Follicle-stimulating hormone
GH	Growth hormone
GHD	Growth hormone deficiency
GR	Glucocorticoid receptor
HH	Hypogonadotropic hypogonadism
IAD	Isolated ACTH deficiency
IGHD	Isolated growth hormone deficiency
IL	Intermediate lobe of pituitary
LH	Luteinizing hormone
MSH-α	Melanocyte-stimulating hormone, alpha
ONH	Optic nerve hypoplasia
PC(SK)	Proprotein convertase (subtilisin/kexin)
PL	Posterior lobe of pituitary
POMC	Pro-opiomelanocortin
PRL	Prolactin
RAR	Retinoic acid receptor
Shh	Sonic hedgehog
TF	Transcription factor
TR	Thyroid hormone receptor
TSH	Thyroid-stimulating hormone
VUS	Variants of unknown significance
WES	Whole exome sequencing
Wnt	Wingless-type MMTV integration site family/beta-catenin

13.1 Introduction

13.1.1 Incidence and Diagnosis of Human Hypopituitarism

Hypopituitarism affects around 1 in 4000 live births (Castinetti et al. 2012, 2008a; Regal et al. 2001). Combined pituitary hormone deficiency (CPHD) is defined by the deficiency of GH (growth hormone) and at least one more hormone of TSH, ACTH, LH, FSH, PRL (thyroid-stimulating hormone, adrenocorticotropic hormone, luteinizing hormone, follicle-stimulating hormone, and prolactin, respectively). The

incidence of CPHD is estimated to be 1:8000 according to the Genetics Home Reference at the National Institutes of Health (ghr.nlm.nih.gov). The most common pituitary hormone deficient is GH in 1:4000–1:10,000 individuals (Alatzoglou and Dattani 2010), while other isolated pituitary hormone deficiencies are rare. Congenital hypothyroidism has an incidence of 1:3000 (Grosse and Van Vliet 2011), isolated hypogonadotropic hypogonadism (isolated HH) has an incidence under 1:10,000 and is frequently associated with anosmia/hyposmia (Hayes et al. 1998; Seminara et al. 2000). The incidence of congenital isolated ACTH (corticotrope) deficiency (IAD) is largely unknown (Patti et al. 2018). Overall, this places hypopituitarism in the upper end of rare diseases (Richter et al. 2015).

Genetic factors substantially influence height, and short stature is a common cause for referrals to endocrinologists (Pfäffle 2006). The diagnosis of pituitary hormone deficiency is based on guidelines by professional organizations and medical institutes (Ergin et al. 2015). We refer to these for specific details regarding the clinical diagnosis of growth hormone deficiency (GHD) in children (Chinoy and Murray 2016), GHD in adults (Molitch et al. 2011), congenital HH (Boehm et al. 2015) and congenital central hypothyroidism (Leger et al. 2014). Guidelines are not yet established for isolated ACTH deficiency (IAD) (Andrioli et al. 2006) or PRL deficiency in particular. The focus of this chapter is to explore the non-acquired/genetic causes with special attention to transcription factor (TF) genes.

Transcription factors are widely recognized as regulators of pituitary development. Mouse models provided the fundamental evidence for their role in pituitary development; however, not all of the orthologous human genes turned out to be involved in human pituitary disease. An extensive list of TFs involved in vertebrate pituitary development is provided in Table 13.1 [TF classification is based on http://tfclass.bioinf.med.uni-goettingen.de (Wingender et al. 2015)].

Around 2000 TFs are known today. Nearly a third of them are known to have functions during development. They are classified based on protein domains and about 80% of all TFs have C2H2-zinc-finger, homeodomain or helix-loop-helix motifs (Vaquerizas et al. 2009). Most of the genes currently known in the pathogenesis of human isolated growth hormone deficiency (IGHD) or CPHD are TFs discussed in this review. Genes predominantly involved in HH are discussed elsewhere (Maione et al. 2018). Also, those genes that are involved in signaling (*BMP4, CDON, FGF8, FGFR1, GPR161, HHIP, IGSF1, PROKR2, SHH, WDR11*), RNA processing (*EIF2B5, HNRNPU, POLR3A, RBM28, RNPC3*), and other processes (*CHD7, IFT72, 52KCNQ1, PNPLA6, ZSWIM6*) (Di Iorgi et al. 2016; Fang et al. 2016b; Norppa et al. 2018; Tommiska et al. 2017) are not the focus of this review.

Table 13.1 Transcription factors in pituitary development

TF superclass	TF class and family	Human gene	Full name	Human chromosomal localization
Helix-turn-helix	HD-LIM-type	*LHX3* *LHX4* *ISL1*	LIM homeobox 3 LIM homeobox 4 ISL LIM homeobox 1	9q34.3 1q25.2 5q11.1
	HD-NK	*NKX2–1*	NK2 homeobox 1	14q13.3
	HD-paired	*PAX6*	Paired box 6	11p13
	HD-paired-related	*PROP1* *HESX1* *OTX2* *PITX2* *PITX1*	PROP paired-like homeobox 1 Homeobox, ES cell expressed 1 Orthodenticle homeobox 2 Paired-like homeodomain 2 Paired-like homeodomain 1	5q35.3 3p14.3 14q22.3 4q25 5q31.1
	HD-POU	*POU1F1*	POU class 1 homeobox 1	3p11.2
	HD-SINE	*SIX3* *SIX6*	Sine oculis homeobox homolog 3 Sine oculis homeobox homolog 6	2p21 14q23.1
	HD-TALE type-PKNOX	*TGIF1*	TGFB-induced factor homeobox 1	18p11.31
	HD-VAX	*VAX1*	Ventral anterior homeobox 1	10q25.3
	Forkhead and winged helix	*FOXA2* *FOXL2* *FOXO1*	Forkhead box A2 Forkhead box L2 Forkhead box O1	20p11.21 3q22.3 13q14.11
Helix-loop-helix	Per-Arnt-Sim (PAS)-ARNT	*ARNT2*	Aryl-hydrocarbon receptor nuclear translocator 2	15q25.1
Basic helix-loop-helix (bHLH)	MyoD-ASC-related	*ASCL1*	Achaete-scute family bHLH transcription factor 1	12q23.2
	Tal-related-Neurogenin-ATO	*NEUROD1* *NEUROD4*	Neuronal differentiation 1 Neuronal differentiation 4	2q31.3 12q13.2
All alpha helical	HMG-SOX-related-group B	*SOX2* *SOX3*	SRY (sex determining region Y)-box 2 SRY (sex determining region Y)-box 3	3q26.33 Xq27.1
	HMG-TCF-related	*TCF7L1*	Transcription factor 7-like 1	2p11.2
Basic leucine zipper	CEBP related-PAR	*TEF*	Thyrotrophic embryonic factor	22q13.2

(continued)

Table 13.1 (continued)

TF superclass	TF class and family	Human gene	Full name	Human chromosomal localization
Zn-finger (ZnF)	C2H2-ZnF-three-ZnF-Kruppel-related-EGR	*EGR1*	Early growth response 1	5q31.2
	C2H2-ZnF-more than three adjacent ZnF	*GLI2* *GLI3* *ZIC2*	GLI family zinc finger 2 GLI family zinc finger 3 ZIC family member 2	2q14.2 7p14.1 13q32.3
	C2H2-ZnF-multiple dispersed ZnF	*INSM1*	Insulinoma-associated 1	20p11.23
	C4-ZnF-FTZF1-related	*NR5A1*	Nuclear receptor subfamily 5, group A, member 1	9q33.3
	C4-ZnF-GATA-double	*GATA2*	GATA binding protein 2	3q21.3
Immunoglobulin fold	RHR-NFKB-related	*NFKB2*	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	10q24.32
	T-box-Brachyury-related	*TBX19*	T-box 19	1q24.2

CEBP CCAAT/enhancer-binding protein beta, *HD* homeodomain, *LIM* Lin-11, Isl-1, Mec-3, *HMG* high mobility group, *FTZF1* Fushi tarazu transcription factor-1, *GATA* ability to bind to GATA nucleotide sequence, *NK* homologous to the naked cuticle or 93D/E gene cluster in Drosophila, *PAR* proline and acidic amino acid-rich, *PKNOX* Pre-B-Cell Leukemia Homeobox (PBX)/Knotted 1 Homeobox 1, *POU* Pit1, OCT1/2 unc-86, *RHR* Rel homology region, *SINE* sine oculis, *TALE* Three Amino acid Loop Extension, *TGFB* Transforming growth factor beta, *ZIC* zinc finger protein of the cerebellum

13.1.2 Why Does Genetic Diagnosis Matter in Hypopituitarism?

About 15% of CPHD cases have mutations in *PROP1, POU1F1, LHX3, LHX4,* or *HESX1* but systematic screens have not been done for all genes implicated in the disorder (De Rienzo et al. 2015; Fang et al. 2016b). Genetic diagnosis in hypopituitarism has consequences for disease progression and family screening. The international GENHYPOPIT network—with more than 1200 patients (Brue 2018)—reported that only ~25% of their GHD patients were diagnosed neonatally, 32% during puberty, and about 10% well into adulthood (Brue et al. 2017). Pituitary hormone deficiency can evolve over the course of time; therefore, intermittent screening for new hormone deficiency is warranted. For example, IGHD diagnosed in childhood can evolve to

CPHD with TSH and LH deficiency in young adulthood, and with ACTH deficiency later in adulthood (>30 year) (Brue et al. 2017; Coya et al. 2007; Halasz et al. 2006). While some gene deficiencies present with a consistent phenotype (*PROP1*, *POU1F1*), incomplete penetrance and variable expressivity pose a challenge in predicting pituitary disease progression and extra-pituitary manifestations (i.e., *LHX4*, *GLI2*). The size of the pituitary is often smaller than normal in patients with hypopituitarism; however, patients with *PROP1* variants may exhibit pituitary hyperplasia and apparent dynamic changes in the organ size (waxing and waning) (Obermannova et al. 2011; Turton et al. 2005a). The diagnosis of *PROP1* variants in these cases can prevent invasive procedures and spontaneous regression can be anticipated (Dattani 2005). Additionally, the rationale for genetic testing of close family members is quintessential to prevent serious/life-threatening conditions such as secondary adrenal insufficiency (Pekic et al. 2011).

13.1.3 Genetic Diagnostics in Hypopituitarism

Endocrinologists and medical geneticists typically share the responsibility of establishing the genetic diagnosis in hypopituitarism. There is no "state of the art" hypopituitarism-specific genetic diagnostics guideline published by a medical society to date. Family history is the most essential component in the analysis. To identify the genetic origin for hypopituitarism it is important to consider several genetic models: (1) large families with multiple affected individuals suggesting a dominant inheritance; (2) consanguineous families where the odds for recessive disorders is increased; or (3) trios with an affected child with at least one unaffected parent, suggesting incompletely penetrant dominant, recessive, or de novo variants in the proband. In addition, people from the Iberian Peninsula or Lithuania have a higher probability of carrying one of the two founder mutations of *PROP1* (Dusatkova et al. 2016).

The technology used to detect genetic changes includes single gene Sanger sequencing, panel sequencing of the most well-established genes, or next-generation sequencing technologies to assess coding regions genome-wide (Whole Exome Sequencing—WES). Single gene sequencing revealed that around 11% of CPHD patients had variants in *PROP1*, whereas *POU1F1*, *LHX4*, *LHX3*, and *HESX1* were around 1% each, respectively (Fang et al. 2016b). In most diseases the overall genetic diagnosis "solve rate" of WES is ~30% and that would be an excellent progress from the current 15% at best with traditional methods (Trujillano et al. 2017). Papers reporting on results with whole genome sequencing are scarce in hypopituitarism. Only a few publications provide insight into the incidence of larger, chromosomal changes. Copy number variations account for ~8% of the congenital hypopituitarism cases (Correa et al. 2018; Dateki et al. 2010a; Takagi et al. 2015). Recently, a targeted version of WES using the principle of molecular inversion probes was reported to screen 51 patients for 30 known and 37 candidate genes, which has excellent perspectives in screening and identifying more novel variants (Perez Millan et al. 2018).

13.2 Pituitary Gland Structure, Function, and Development

13.2.1 The Structure of the Pituitary: "One Gland Above All"

The pituitary is the major neuroendocrine gland serving as a key hub between the central nervous system (CNS) and the majority of endocrine organs. The mammalian pituitary can be divided into three lobes: anterior (AL), intermediate (IL), and posterior (PL). The AL and IL are derived from the evaginating oral ectoderm (Rathke's cleft) and ensphere both the stalk and the anterolateral aspect of the PL. The IL is rudimentary in humans and a common site for cystic lesions (Rathke's cleft cysts). A fine mesh of a portal vessel system in the anterior lobe allows direct communication from the hypothalamus to the pituitary through blood flow. The axon terminals are surrounded by glial-like cell types (pituicytes) and form the posterior lobe (PL) (Goto et al. 2015).

13.2.2 The Basic Function of the Pituitary

Pituitary function is essential in growth, fertility, lactation, stress response, and general homeostasis. The anterior lobe has five major cell types producing six major hormones: somatotrophs (producing GH); lactotrophs (PRL); melanocorticotropes (POMC) and its cleavage products: ACTH, α-MSH; thyrotrophs (TSH); and gonadotrophs (LH, FSH). TSH, FSH, and LH are heterodimers of the choriogonadotropin alpha subunit (CGA) and specific beta subunits TSHB, FSHB, and LHB, respectively. The proportion of these cell types is unequal in the adult pituitary such as ~40% are somatotrophs, ~40% lactotrophs, ~10% gonadotrophs, 10% corticotropes, and only 5% are thyrotropes (Kulig et al. 1998). While these make up the majority of resident cells in the AL, there is a fraction that is hormone negative and includes non-differentiated stem cells, progenitor cells, folliculostellate cells, endothelial cells, pericytes, and mesenchymal cells. Defects leading to the loss of predominant cell types can frequently result in a hypoplastic AL (Gangat and Radovick 2017). The PL contains the axon terminals of hypothalamic neurons in the supraoptic and paraventricular nuclei producing arginine-vasopressin (AVP) and oxytocin (OXT). While AVP and OXT are stored in the terminals they are surrounded by a subset of glial-like cells (Goto et al. 2015). Single cell sequencing technologies did not reveal a new major physiological cell type so far but a better resolution of known cell types important in critical stages of development, adaptation to stimuli and neoplasia are highly anticipated (Cheung et al. 2018).

13.3 Lessons from Mouse Pituitary Development to Human Hypopituitarism

13.3.1 Early Patterning of the Pituitary Primordium

Spatiotemporal expression of TFs in the ventral diencephalon and the oral ectoderm results in the formation of the AL/IL/PL between mouse E9 and E12 days. In addition to a severe pituitary abnormality, a wide spectrum of features is present when specific TFs are disrupted. Defects in *Pitx2*, *Isl1, Nkx2.1* result in complex anomalies involving the CNS, the eyes, and multiple non-ectodermal organs such as the heart and the thyroid. *Hesx1, Vax1, Pax6, Otx2, Six3, Six6* deficient mice present with CNS, eye, and other malformations predominantly in the head region (McCabe and Dattani 2014). Others show CNS abnormalities and disorders affecting the ventral motor neurons [*Lhx3/Lhx4*, (Gergics et al. 2015)], segmental bone formation [*Gli2*, (Haddad-Tovolli et al. 2015)] or the gonads [*Sox3*, (Rizzoti et al. 2004)]. *Sox2* is essential in the specification of all pituitary hormone producing and folliculostellate cells and is considered as a signature pituitary stem cell marker (Fauquier et al. 2008). *Sox2/Sox9* co-expressing cells are regarded as committed progenitor cells in the pituitary (Rizzoti et al. 2013).

Pituitary organogenesis and hormone cell specification are outlined in Figs. 13.1 and 13.2.

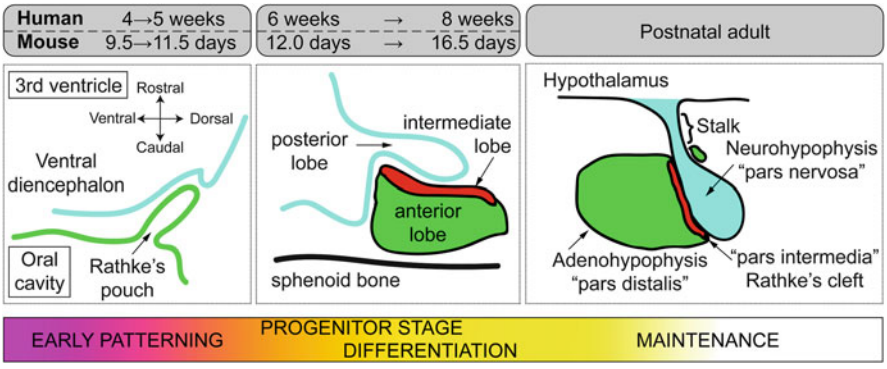

Fig. 13.1 Schematic development of the pituitary in the mouse and human in the midsagittal plane. By mouse embryonic day E16.5, the organ reaches its final shape. Several signaling pathways regulate pituitary development. A continuous Shh expression gets interrupted by Wnt signaling from the diencephalon at E9.5 and the Rathke's pouch protrudes from the rooftop of the oral cavity. These events result in altered expressions of *Bmps* and *Fgfs* and define the pituitary organizer domain of the ventral diencephalon [references within Osmundsen et al. (2017)]. Fgf and Notch signaling orchestrate the evagination of the hypothalamic floor plate of the third ventricle to form the infundibulum and the subsequent PL (Goto et al. 2015)

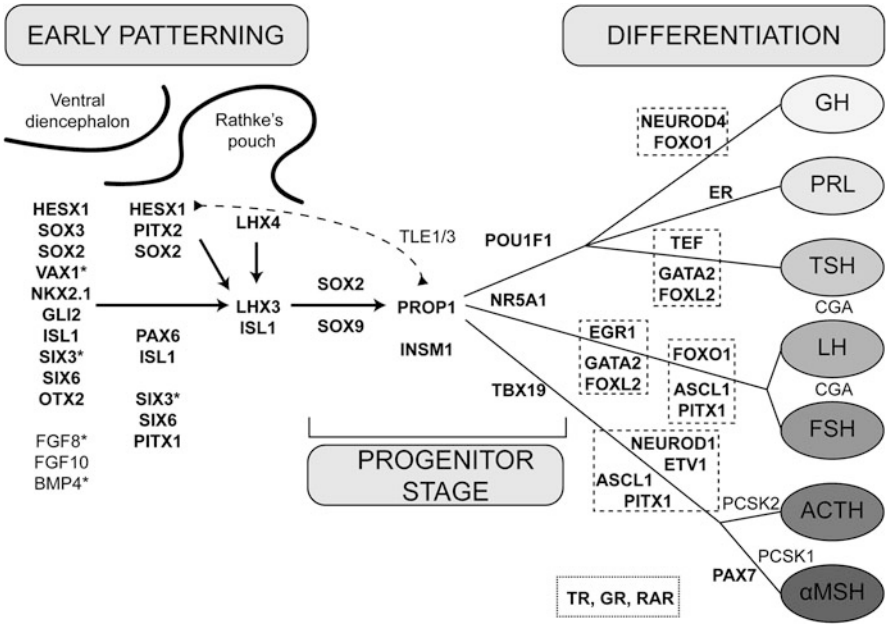

Fig. 13.2 Involvement of transcription factors in the development hormone producing cells in the pituitary anterior lobe. Multiple transcription factors (TFs) participate in the specification of pituitary hormone producing cells. Details of the three main phases of pituitary development are described in the main text. Asterisk: Supernumerary pituitary gland formation is noted in the—Bmp inhibitor—*Noggin*$^{-/-}$ (Davis and Camper 2007), *Tg(Cga-Fgf8)Rsd* (Treier et al. 2001), *Six3*$^{+/-}$ *Hesx1*$^{cre/+}$ (Gaston-Massuet et al. 2008), and *Vax1*$^{-/-}$ mice (Bharti et al. 2011). In the differentiation phase certain TFs are needed for multiple lineages. For example, *Gata2* is a major factor in the transcriptional regulation of *Cga*, *Tshb*, *Lhb* (Dasen et al. 1999). An array of steroid/retinoid/thyroid hormone receptor stimulation is also needed for physiological pituitary hormone expression. Solid arrows mark upstream/downstream relation between factors but do not necessarily mark direct regulation. Dotted curve represents repressive relationship. Acronyms in bold are TFs and correspond to the Table 13.1

13.3.2 Progenitor Cell Determination

Prop1 (Prophet of Pit1) is a key pituitary-specific TF (Sornson et al. 1996). All pituitary hormone producing cell types go through a *Prop1* expressing progenitor stage (Davis et al. 2016). Its main downstream target *Pou1f1* is a lineage determining factor for somatolactotrophs and most thyrotrophs (Li et al. 1990). *Insm1* is key in the differentiation of multiple neuroendocrine cell types. In the absence of *Insm1*, the *Sox2/Sox9*+ pituitary stem/progenitor cell pool is maintained, lineage-specific transcription factors (*Pou1f1, Tbx19, NeuroD1, Nr5a1*) are moderately expressed, but all GH, TSH, LH/FSH cells are missing, and PRL, ACTH, and αMSH cell numbers are drastically reduced (Welcker et al. 2013).

13.3.3 Differentiation Phase

The *POU1F1* Lineage (Somatolactotrophs and Thyrotrophs)
Pou1f1 (formerly *Pit-1*) is a signature pituitary transcription factor that directly regulates the transcription of *Gh*, *Prl*, *Tshb*, and *Cga* (Gordon et al. 1993; Li et al. 1990). A cluster of thyrotrophs in the rostral tip develops independently of *Pou1f1* (Lin et al. 1994). Notable significant other factors for this lineage are: *Neurod4* (Ando et al. 2018), *Foxo1* (Kapali et al. 2016) for somatotrophs, the estrogen receptor for lactotrophs (Day et al. 1990), and thyrotroph embryonic factor (TEF) for thyrotrophs (Drolet et al. 1991).

Gonadotroph and Melanocorticotroph Lineages
Nr5a1 (previously known as *Sf1*) is a hallmark TF for gonadotroph commitment (Zhao et al. 2001). *Egr1* is expressed predominantly in gonadotrophs (Man et al. 2014). *Tbx19* (previously known as *Tpit*) is a signature TF of melanocorticotrope commitment and in the transcriptional regulation of POMC (Budry et al. 2011). *Pax7* is a pioneer transcription factor acting as a selector to melanotrope over corticotrope faith through chromatin remodeling (Budry et al. 2012). The expression of specific proprotein convertases (PC or PCSK) is key in the differential cleavage of POMC (Marcinkiewicz et al. 1993).

13.4 Human Gene Variants in Pituitary Hormone Deficiency

13.4.1 Interpretation of Novel Genes/Variants in the Era of Whole Exome/Genome Sequencing

The discovery of specific genes in hypopituitarism started in the 1990s with *Pou1f1* about 60 years after the discovery of the Snell dwarf (*Pou1f1*$^{dw/dw}$) (Li et al. 1990). A dozen other genes such as *PROP1, HESX1, LHX3, LHX4* were described in human hypopituitarism in the next two decades. Thanks to the Human Genome Project and the availability of Sanger sequencing, genetic testing improved for patients with hypopituitarism. Single gene sequencing was amenable as long as a limited number of candidate genes needed to be screened. As the number of candidate genes increased, automated panel sequencing took over (Klee et al. 2011).

The rise of next-generation sequencing technologies from around 2007 changed the landscape dramatically and dozens of novel candidate genes and variants were identified in a decade (Warr et al. 2015). This flipped the order such that the candidate genes and variants were found in the human first and then functional studies in cell lines and vertebrate model organisms were implemented to discern the pathogenicity and disease mechanism. In this new era, the most difficult task is to evaluate the many novel genes and variants with unknown significance (VUS).

Analyzing VUS in patients with hypopituitarism is of utmost importance since less than 15% of hypopituitarism patients have a genetic diagnosis (De Rienzo et al. 2015; Fang et al. 2016b). Professional organizations such as the American College of Medical Genetics (ACMG), the Association for Molecular Pathology (AMP) (Richards et al. 2015), ClinGen Sequence Variant Interpretation (SVI) Working Group (ClinGen SVI WG) (Strande et al. 2017) in the USA, or the Association for Clinical Genomic Science in the UK developed recommendations for variant interpretation. This effort is ongoing and expanding to develop some disease-specific guidelines as well. These recommendations classify VUS based on evidence of (1) known physiological expression and function, (2) changes in these when the variant is present, as well as on (3) animal and in vitro model systems and rescue experiments corresponding to the human disease.

Initial evaluation of VUS includes the assessment of the (1) probability for loss of intolerance (pLI) of the gene; (2) frequency of the VUS in a matched population [e.g., Genome Aggregation Database (gnomAD), gnomad.broadinstitute.org (Lek et al. 2016)]; (3) protein structure and function prediction combined with evolutionary conservation [e.g. Combined Annotation Dependent Depletion (CADD), cadd.gs.washington.edu, (Rentzsch et al. 2019)].

A more detailed analysis includes investigation of (4) spatial and temporal expression of the mRNA/protein especially in the disease-affected tissues (postnatally, e.g., Genotype-Tissue Expression (GTEx) project, gtexportal.org/home/, Tabula Muris, tabula-muris.ds.czbiohub.org (Schaum et al. 2018), The Human Protein Atlas www.proteinatlas.org/humanproteome/tissue) or embryonic ages (Brinkmeier et al. 2009; Ma et al. 2009) (5) knockout vertebrate models (e.g., Mouse Genome Informatics: www.informatics.jax.org/phenotypes.shtml, The Zebrafish Information Network zfin.org).

These surveys can support the role of a VUS but further (6) in vitro and (7) in vivo vertebrate studies are necessary to elevate the level of proof for pathogenicity. The in vitro studies need to demonstrate the biological difference between the wild type and the variant protein. The more elegant approach uses cultured native cells from healthy and affected individuals, or immortalized or engineered cells such as induced pluripotent stem cells (iPS) or CRISPR-edited cells (Strande et al. 2017). Established in vitro assays are excellent to use if they are available, but many times there is no available assay and the validation of a new one can be tedious. In vivo studies still pose the greatest bottleneck in the analysis as time to generate a mouse carrying the orthologous VUS can be at least 6 months. Other model systems like the Zebrafish are excellent for knock-down and rescue experiments in a shorter time frame (Davis et al. 2014).

13.4.2 TF Gene Variants in Patients with Hypopituitarism

13.4.2.1 TF Gene Variants in Patients with Combined Pituitary Hormone Deficiency and Isolated Growth Hormone Deficiency

This chapter has two comprehensive goals for clinicians and researchers who encounter patients with hypopituitarism: (1) to describe the landscape of genetic and phenotypic heterogeneity in hypopituitarism and (2) to create a resource for the first steps of in vitro testing for VUS in select hypopituitarism genes based on published scenarios.

Phenotypic Heterogeneity in Hypopituitarism
The majority of human genes tested in patients with CPHD, IGHD, IAD to date are TFs, which are illustrated in Tables 13.2, 13.3, and 13.4. We aimed to collect information on the genetics and common phenotypic features of around 300 probands and families. Due to space limitations of this chapter not all original references could be cited.

An Approach to Perform In Vitro Testing of VUS in TF Genes
One can achieve a detailed analysis on a computer for a small set of novel genes/variants in a shorter time; however, the next step is frequently to start in vitro testing. A generalized view of active TFs is that they localize to the nucleus, bind to specific promoter/enhancer DNA sequences with partner proteins, and change the mRNA expression of target genes (Vaquerizas et al. 2009). Loss of function (nonsense and select frame shift) variants may require little to no testing if the gene is sensitive to haploinsufficiency indicated by dominant inheritance and a high pLI score (Lek et al. 2016) (Tables 13.2 and 13.4).

Depending on the affected functional domain in the TF, the assessment can include the following by overexpressing the TF from a plasmid DNA in cell culture: (1) quantitative assessment of protein expression by Western blot; (2) subcellular localization of the green fluorescence protein tagged TF, (3) protein-DNA binding assays such as electrophoretic mobility shift assay (EMSA) where the TF binds to specific DNA sequences, (4) transactivation reporter assays, and (5) protein–protein binding by co-immunoprecipitation.

PROP1

Patients with *PROP1* variants present with a highly consistent phenotype: GH, TSH, and more than two-thirds of the cases have ACTH, FSH/LH, PRL deficiency. Typically, they present with AL hypoplasia, 1:10 patients have AL hyperplasia, and PL is intact. Waxing and waning of the pituitary size over time is common (Obermannova et al. 2011; Turton et al. 2005a). Patients carry homozygous or compound heterozygous variants. About three-quarters of variants are missense/nonsense while one-quarter are splicing or large deletions (Fang et al. 2016b; Madeira et al. 2017). Two founder mutations are well known: c. 301-302delAG from the Iberian Peninsula

Table 13.2 Heredity and pituitary hormone deficiency in patients with CPHD/IGHD and pathogenic variants in prevalent pituitary transcription factor genes by 2019[a]

			Inheritance				Deficient hormone other than GH in CPHD			
Gene	pLI	Number of index cases[a]	Typical	Rare	IGHD	CPHD	TSH	ACTH	FSH LH	PRL
PROP1	0.094	>60	R	–	None	+++++	+++++	++++	+++++	++++
POU1F1	0.036	~40	R	D (5)	+	+++++	+++++	None	None	+++++
LHX3	0.007	~30	R	DIP (2)	None	+++++	+++++	++	+++	++++
LHX4	0.018	~30	DIP	R (1)	+	+++++	+++++	++++	+++	+
HESX1	0.001	>20	D	R (6)	++	++++	+++++	+++++	+++	++
OTX2	0.939	~10	D	R (1)[b]	+++	+++	+++++	+++	+++	+
GLI2	0.990	>50	DIP	–	++	++++	+++++	+++++	++++	Rare
GLI3	1.000	~20	D	–	+++++	Rare	Rare	Rare	Rare	Rare

pLI probability for loss of function intolerance (Source: https://gnomad.broadinstitute.org). A pLI $\geq$ 0.9 suggests extreme intolerance to loss of function variants. pLI $\leq$ 0.1 are tolerant. Those genes with a low pLI have a propensity to tolerate damages to one allele and both alleles need to be disrupted for dysfunction. This can be seen with cases of recessive inheritance when carrying homozygous or compound heterozygous variants

Proportion of published cases/families: $\geq$80%: +++++; 79–60%: ++++; 59–40%: +++; 39–20%: ++; 19–10%: +; <10%: rare; CPHD is defined as GHD plus one more pituitary hormone deficient. *R* recessive, *D* dominant, *DIP* dominant with incomplete penetrance, *(N)* of published probands with nontypical inheritance

[a]Assessment of CPHD/IGHD patients is based on literature review (1990-early 2019), Human Gene Mutation Database Professional (www.biobase-international.com) (Qiagen, Germantown, MD) and Online Mendelian Inheritance in Man (www.omim.org McKusick-Nathans Institute of Genetic Medicine at Johns Hopkins University School of Medicine, Baltimore MD, & National Center for Biotechnology Information, Bethesda, MD)

[b]Evidence for causality is low in Catania et al. (2019)

Table 13.3 Pituitary and extra-pituitary morphological features in patients with CPHD/IGHD and pituitary transcription factor gene variants

	Incidence					
	Pituitary AL					
Gene	Hypoplasia	Hyperplasia	EPP	PSIS	CNS	Other
PROP1[a]	+++++[b]	+	ND	ND	ND	Various minor features
POU1F1[a]	+++++	Rare	ND	ND	ND	Various minor features
LHX3	+++	+	ND	ND	Rare	Limited neck rotation, enlarged fontanel, hearing impairment, frontal bossing (all +++++)
LHX4	++++	Rare	+++ +	ND	Chiari I. (+)	Underdeveloped sella (++), thin stalk (+), micropenis (+)
HESX1	+++	ND	+++	Rare	SOD (+), ONH (+)	Various minor features
OTX2	+++	ND	+++ +	++	Chiari I. (+)	Micro/anophtalmia (++++), ONH (++), facial and genital defects (+)
GLI2	++++	ND	+++	+	HPE-like (+ +)	Postaxial polydactyly (++), cleft lip and palate (+)
GLI3	++[c]	ND	ND	ND	See other	PHS or subPHS (all)

Assessment and grading are identical to Table 14.2. Note that pituitary size/morphology can be normal

AL anterior lobe, *EPP* ectopic posterior pituitary, *PSIS* pituitary stalk interruption syndrome, *CNS* central nervous system, *ND* not described, *SOD* septo-optic dysplasia, *ONH* optic nerve hypoplasia, *HPE* holoprosencephaly

[a]See details for PSIS/CNS in (Brue et al. 2017). Incidence cannot be established

[b]Waxing and waning of pituitary size was described in Turton et al. (2005b) and Obermannova et al. (2011)

[c]"Pituitary agenesis" was described

(Cogan et al. 1998; Dusatkova et al. 2016) and c. 296-297delGA from Lithuania (Navardauskaite et al. 2014). Functional testing includes binding to/transactivation on the PRDQ9 sequence (Kelberman et al. 2009). While the pituitary phenotype is extremely consistent there are two reports of stating extra-pituitary features: (1) two patients with pituitary stalk interruption syndrome (PSIS) and four with heart/kidney malformations and deafness were described from the GENHYPOPIT network in consanguineous patients with *PROP1* and *POU1F1* variants (Brue et al. 2017); (2) one CPHD patient was described with ectopic posterior pituitary (EPP) who carried a heterozygous c.301_302delAG, which is inconsistent with the recessive inheritance (Avbelj Stefanija et al. 2015). However, it is possible that these individuals had additional genetic or environmental causes of the atypical features.

Table 13.4 Probability for loss of function intolerance in transcription factor genes in CPHD/IGHD patients with few or no published cases

Gene	pLI	Gene	pLI	Gene	pLI
PAX6	1	*ISL1*	0.896	***SOX3***	0.475
NFKB2	1	*PITX1*	0.892	***NKX2–1***	0.378
FOXO1	0.997	*FOXL2*	0.88	***TCF7L1***	0.36
FOXP3	0.994	*VAX1*	0.784	*EGR1*	0.327
ARNT2	0.991	*NEUROD1*	0.772	*INSM1*	0.269
NR5A1	0.991	***FOXA2***	0.742	*NEUROD4*	0.097
GATA2	0.981	***SOX2***	0.735	***TGIF1***	0.01
PITX2	0.979	*ASCL1*	0.697	*TBX19*	0
ZIC2	0.974	*TEF*	0.615		
SIX3	0.951	***SIX6***	0.554		

Genes with few published CPHD/IGHD cases are in bold. Cases with IAD are underlined

POU1F1

Patients with *POU1F1* variants show the most consistent manifestation of all CPHD patients with GH, TSH, PRL deficiency and no other hormones being affected, AL hypoplasia, and PL placed normally. Typical inheritance is recessive (homozygous and compound heterozygous), although there are some examples of dominant inheritance. There are two published families with IGHD carrying a heterozygous p.P76L (Sobrier et al. 2016) or a homozygous p.E230K (Gat-Yablonski et al. 2002). Functional testing is performed by transactivation on the *Gh*, *Prl* and *Tshb* promoters (Hendriks-Stegeman et al. 2001; Turton et al. 2005b), altered promoter/enhancer autoregulation (Vallette-Kasic et al. 2001), exon trapping of splice variants (Inoue et al. 2012; Takagi et al. 2017; Turton et al. 2012).

POU1F1 variants with dominant inheritance have an incomplete penetrance and reveal mechanisms other than roles as a transcriptional regulator on the known promoters. The most common dominant variant p.R271W was shown as a dominant negative transcriptional repressor as well as amino acid R271 binding to MATR3 and SATB1 in the nuclear matrix enabling features in chromatin remodeling (Cohen et al. 2006; Pellegrini et al. 2006; Skowronska-Krawczyk et al. 2014). POU1F1 p.P76L presented with IGHD, and the variant protein exhibited increased interaction with ELK1, PITX1, and LHX3a and with the enhancer region of *GH1* (Sobrier et al. 2015). Splice variants revealed either complete skipping of exon 2 (c.142+3A>G and c.214+1G>T) (Inoue et al. 2012; Turton et al. 2012) or splicing into the longer beta isoform of POU1F1 possessing repressor activities in vitro (c.143-83A>G) (Takagi et al. 2017). The variant p.K216E has an increased (not decreased!) activation of *Gh* and *Prl* and drastically reduced retinoic acid dependent autoactivation of the *Pou1f1* enhancer (Cohen et al. 1999).

LHX3

The typical hypopituitarism patient with homozygous *LHX3* variants presents with GH and TSH deficiency, two-thirds with LH/FSH and PRL deficiency while only a third of them have ACTH deficiency (Bechtold-Dalla Pozza et al. 2012). Less than half of the patients have abnormal pituitary size, with AL hypoplasia and eutopic PL. Additional features are fairly common, such as limited neck rotation (not present in *Lhx3*$^{-/-}$ mice), enlarged fontanels, hearing impairment, frontal bossing or more rarely thinning of the corpus callosum and dolichocephaly (Bonfig et al. 2011; Jullien et al. 2018; Kristrom et al. 2009; Rajab et al. 2008; Ramzan et al. 2017).

One family was published with a generation of two miscarriages, one child with compound heterozygous *LHX3* p.C118Y & c.252-3 C>G variants with CPHD and limited neck rotation while members of this family carrying the c.252-3 C>G splice variant had a high incidence of limited neck rotation (Sobrier et al. 2012). Another heterozygous variant also showed CPHD but no other distinctive features and the phenotype was incompletely penetrant (Jullien et al. 2018).

Variants are tested by using the activator LHX3A isoform for transactivation and DNA binding on *Cga, Gh, Prl, Tshb* promoters (Bechtold-Dalla Pozza et al. 2012; Rajab et al. 2008). Heterozygous variants are typically tested for dominant negative effects on the same promoters together with POU1F1 interaction (Jullien et al. 2018; Sobrier et al. 2012).

LHX4

These patients typically present with dominant inheritance and incomplete penetrance. GH, TSH deficiency is high while more than half of the cases have ACTH and less than half of them have gonadotroph deficiency and PRL deficiency is rare. AP hypoplasia and EPP are typical, underdeveloped sella is common. Rare features include Chiari I malformation and thin stalk (Castinetti et al. 2008b; Cohen et al. 2017; Dateki et al. 2010a; Machinis et al. 2001; Pfaeffle et al. 2008; Rochette et al. 2015). There are two examples of IGHD described (Cohen et al. 2017; Gucev et al. 2016).

Functional testing is typically carried out on the same promoters as with LHX3 or on the *Pou1f1* and *Fshb* promoters. Haploinsufficiency appears to be the typical mode of dominant action (same references as in previous paragraph and Fuxman Bass et al. 2015).

The only patient with a homozygous allele (p.T126 M) described so far presented with the features of a typical heterozygous *LHX4* patient (CPHD, AP aplasia, EPP, sella defect) but also with midfacial hypoplasia, small upturned nose with depressed nasal bridge, low-set crumpled ears and death during the first postnatal week (Gregory et al. 2015b). Homozygosity mapping and high conservation of amino acid 126 suggested pathogenicity. Transactivation ability of p.T126 M alone on the *Prl* promoter was not different; however, the interaction with POU1F1 was significantly reduced on the reporter construct.

HESX1

Less than two dozen *HESX1* families with hypopituitarism were described to date. Thus far, those with recessive inheritance are all CPHD (Fang et al. 2016a; Reynaud et al. 2011; Sobrier et al. 2006), while heterozygous *HESX1* patients can be IGHD (Cohen et al. 2003; McNay et al. 2007; Vivenza et al. 2011) or CPHD (Corneli et al. 2008; Coya et al. 2007; Reynaud et al. 2012; Tajima et al. 2003; Takagi et al. 2016; Thomas et al. 2001). The variants identified so far are predominantly missense. The onset of hormone deficiency is typically early and frequently evolving from IGHD to CPHD (Coya et al. 2007; Reynaud et al. 2011). GH, TSH, ACTH deficiency is very high while FSH/LH and PRL deficiency is gradually fewer. AL hypoplasia and EPP are common and rare features can include variable penetrance of PSIS, thin stalk, septo-optic dysplasia (SOD), and optic nerve hypoplasia (ONH). *HESX1* is a well-characterized gene in SOD (Dattani et al. 1998). Only a few SOD cases are reported to have hypopituitarism (Cohen et al. 2003; Coya et al. 2007; Thomas et al. 2001). Functional testing of variants includes testing HESX1's ability to repress activation caused by PROP1 on a multimerized paired HD binding site (P3E4) reporter, binding to DNA (Cohen et al. 2003; Fang et al. 2016a; McNay et al. 2007; Reynaud et al. 2012; Sobrier et al. 2006; Takagi et al. 2016).

SOX2

Individuals with *SOX2* variants present with anophtalmia, intellectual disability, and growth delay/short stature. Hypopituitarism is frequently not assessed (Schilter et al. 2013) and is reviewed in the works by Bakrania et al. (Bakrania et al. 2007) and Schneider et al. (Schneider et al. 2009). Information is limited to less than 20 cases that present with isolated HH (Bakrania et al. 2007; Errichiello et al. 2018; Kelberman et al. 2006; Sato et al. 2007; Takagi et al. 2014b), IGHD (Kelberman et al. 2006; Schilter et al. 2013; Schneider et al. 2009), and a few with CPHD (Blackburn et al. 2018; Kelberman et al. 2006; Macchiaroli et al. 2014; Schneider et al. 2009). They often have bilateral/unilateral anophtalmia (missing in *Sox2*-null mice), but other features including ONH, EPP, learning disability are occasionally present. Inheritance is dominant and in patients with hypopituitarism most variants are de novo frame shifts. Functional testing of variants involves transactivation of the *Hesx1* promoter and binding to consensus SOX DNA binding sites (Kelberman et al. 2006; Takagi et al. 2014b). The role of *Sox2* in pituitary tumors requires further investigation.

SOX3

The Xq26–27 chromosomal region of *SOX3* was first implicated in a large family with X-linked mental retardation (XLMR) and IGHD in 1996 (Laumonnier et al. 2002). Very few families were described to date and most of them have CPHD: GH

and TSH deficiencies, occasional LH/FSH deficiency and rarely ACTH or PRL (Alatzoglou et al. 2011; Bauters et al. 2014; Izumi et al. 2014; Takagi et al. 2014a; Woods et al. 2005). In these hemizygous males, most genetic changes are small, in-frame deletions and insertions affecting polyalanine tracts (Alatzoglou et al. 2011; Izumi et al. 2014; Laumonnier et al. 2002). There are two examples of CPHD with complete *SOX3* duplications (Bauters et al. 2014; Woods et al. 2005). Larger chromosomal duplications can result in XX sex reversal (Sutton et al. 2011). The single missense variant example (p.R5Q) had CPHD and a central incisor (Alatzoglou et al. 2011). EPP is occasionally reported (Woods et al. 2005).

The polyalanine tract changes result in perinuclear/cytoplasmic aggregates, impair the ability to transactivate via consensus SOX DNA binding sites, and have a reduced propensity to inhibit Wnt/Ctnnb/TCF mediated transcription (Alatzoglou et al. 2011; Takagi et al. 2014a; Woods et al. 2005).

OTX2

Families with *OTX2* variants present with an autosomal dominant inheritance and incomplete penetrance. Incomplete penetrance and variable expressivity are well demonstrated in $Otx2^{-/-}$ mice in a genetic background specific manner (Hide et al. 2002). Patients with heterozygous *OTX2* variants can present with ocular only, or ocular with hypopituitarism phenotypes, while cases of hypopituitarism-only cases are rare (Diaczok et al. 2008). The ocular phenotype is anophtalmia/microphtalmia typically involving both eyes (Gerth-Kahlert et al. 2013 from Ragge and Wyatt). In the cases with ocular and pituitary phenotypes, the same ocular phenotypes were observed as well as optic nerve hypoplasia/dysplasia/aplasia (ONH) (Dateki et al. 2008; Gorbenko Del Blanco et al. 2012; Prasov et al. 2012; Schilter et al. 2011; Tajima et al. 2009). IGHD is almost as common as CPHD (Ashkenazi-Hoffnung et al. 2010; Dateki et al. 2008; Delahaye et al. 2012; Henderson et al. 2009; Lonero et al. 2016). Less than two dozen hypopituitarism cases are described, and they typically present with CPHD (GH, TSH, and fewer ACTH and FSH/LH and rarely PRL deficiency), EPP, and five of them had PSIS (all ONH and IGHD references and Diaczok et al. 2008; Shimada et al. 2016; Takagi et al. 2015; Vincent et al. 2014). The one published recessive CPHD case with an *OTX2* variant is only based on in silico prediction and segregation (Catania et al. 2019). Missense, nonsense, frame shift variants and large deletions are all common with *OTX2*. Although some reviews suggest that variants in the N-terminal region of the protein are associated with ocular features and in the C-terminal with pituitary involvement, we believe there are not enough cases with hypopituitarism to support this idea (Gorbenko Del Blanco et al. 2012; Schilter et al. 2011). In vitro variant testing is performed with single or multimerized consensus bicoid binding sites and transactivation is carried out on native promoters of *Hesx1, Pou1f1* as well (Dateki et al. 2008, 2010b; Diaczok et al. 2008; Gorbenko Del Blanco et al. 2012; Shimada et al. 2016; Tajima et al. 2009). Knock-down of the endogenous zebrafish mRNA in combination with other genes

resulted in a complex eye, head, and mandible phenotype comparable to the human otocephaly-dysgnatia complex (Chassaing et al. 2012).

GLI2 and *ZIC2*

Patients with *GLI2* variants typically show autosomal dominant inheritance with incomplete penetrance (Babu et al. 2019; Bear et al. 2014; Flemming et al. 2013; Franca et al. 2013; Juanes et al. 2016; Roessler et al. 2005; Shirakawa et al. 2018; Simm et al. 2018; Zwaveling-Soonawala et al. 2018). CPHD (GH, TSH, ACTH most of the time, LH/FSH frequently, PRL rarely deficient) is the most common while IGHD is infrequent (Bear et al. 2014; Gregory et al. 2015a; Juanes et al. 2016; Roessler et al. 2005; Shirakawa et al. 2018). Most patients have hypoplastic AL, about half of the patients have EPP, and a few have absent PP/PSIS. About 10% of the patients have postaxial polydactyly and/or HPE-like features. While most variants are unique to the family the allele affected by both p.M1352V and p.D1520N variants was described by multiple authors in CPHD (Flemming et al. 2013; Franca et al. 2013; Zwaveling-Soonawala et al. 2018). Most variants described are missense. Functional assessment includes binding to a consensus GLI-site in the *PTCH1* promoter. Transactivation studies test variants either on the octamerized GLI-binding site from the enhancer of *Hnf3b* (*Foxa2*) or on the single GLI-binding site from the promoter of keratin 17. The variant testing includes two steps on these reporter constructs: (1) Testing the mutated full-length GLI2 alone and (2) testing the mutated full-length GLI2 together with a *GLI2* cDNA construct missing the N-terminal (1–328) repressor domain (ΔN-GLI2). The ΔN-GLI2 acts a potent activator on these reporter constructs and co-transfection of the mutant + ΔN-GLI2 can demonstrate a dominant negative effect. Embryonic sarcoma cell line (C3H10T1/2) was used to demonstrate osteogenic differentiation upon transfection with normal *GLI2*. In vivo assays were demonstrated with frog eggs where injection of normal *GLI2* results in secondary tail formation (Babu et al. 2019; Flemming et al. 2013; Roessler et al. 2005).

ZIC2 is a common HPE gene and a member of the GLI TF subfamily. A heterozygous p.Gln364Leufs∗2 variant was described in a child with alobar HPE, complex facial/dental features, and the involvement of both the AL/PL with subsequent CPHD (GH, TSH) and central diabetes insipidus (Tasdemir et al. 2014).

GLI3

Heterozygous variants in *GLI3* are known to cause Greig cephalopolysyndactyly and Pallister–Hall syndrome (PHS). A clear genotype–phenotype correlation exists where variants affecting the middle-third of the open reading frame (nucleotides 1998–3481) can be found in PHS only. PHS is characterized by the presence of major criteria such as hypothalamic hamartoma and mesaxial polydactyly plus several minor features (bifid epiglottis, IGHD, CPHD, genital hypoplasia,

imperforate anus, and small nails) (Demurger et al. 2015; Johnston et al. 2005; Kang et al. 1997). A sub-PHS is diagnosed when one major criterion is present with at least one minor criterion. Most published cases with hypopituitarism or pituitary agenesis are in patients with PHS and to lesser extent with sub-PHS. Almost all patients have IGHD, several of the minor features and adrenal and renal agenesis (Demurger et al. 2015; Johnston et al. 2005). Very few cases were described with CPHD (Li et al. 2015; Narumi et al. 2010). Detailed imaging of the pituitary region is not available. Functional testing is similar to *GLI2*.

TF Genes with Limited Evidence in Hypopituitarism

FOXA2

A few patients with heterozygous *FOXA2* variants were described so far. They share the features of CPHD (GH, TSH, ACTH), and have a high incidence of hyperinsulinemia, hypoplastic/absent AL, EPP and have a range of minor features such as single central incisor, dysmorphic facial features, biliary tract abnormalities, heart defects, and neurodevelopmental delay (Boda et al. 2018; Giri et al. 2017; Tsai et al. 2015; Vajravelu et al. 2018). These patients have either missense or large deletions affecting 20p11.21. *FOXA2* is expressed in multiple tissues corresponding to the phenotype spectrum (Giri et al. 2017). Transactivation can be tested on the human *GLUT2* (phGT2–294), *ABCC8, KCNJ11, HADH, SHH, GLI2,* and *NKX2–2* promoter reporters (Giri et al. 2017; Vajravelu et al. 2018).

ARNT2

ARNT2 is part of the protein complex that includes the aryl hydrocarbon receptor-interacting protein (AIP), widely studied in specific groups of pituitary adenomas (Raitila et al. 2010; Rostomyan et al. 2017) and cancer (Bogeas et al. 2018). *ARNT2* is highly expressed in the mouse and human CNS, retina, kidney, lung, and the pituitary (Webb et al. 2013). A recessive *ARNT2* c.1373_1374dupTC variant was identified in a large family with CPHD, kidney, urogenital tract, eye, and CNS anomalies (postnatal microcephaly, frontotemporal hypoplasia, seizures) (Webb et al. 2013). This variant resulted in a frame shift and nonsense mediated decay of the transcript.

PAX6

PAX6 patients typically present with aniridia and microphtalmia (Lim et al. 2017). Few patients were described with borderline GHD, HH, central hypothyroidism or low cortisol levels (Hergott-Faure et al. 2012; Shimo et al. 2014; Solomon et al. 2009). *PAX6* heterozygous variants were described in two cases of IGHD (Takagi et al. 2015). One of them presented with cleft palate, optic disc cupping, AL hypoplasia, and EPP and had a 310 kb deletion of the *PAX6* enhancer. The other

case with a missense p.N116S had AL hypoplasia. Functional analysis included showing normal protein expression, subcellular localization, binding to a consensus *PAX6* binding element from the promoter of CD19 (Mishra et al. 2002) as well as transactivation on a hexamer *PAX6* consensus binding element where only the latter showed significant impairment.

TGIF1

TGIF1 is highly expressed in the liver, kidney, gonads, forebrain, and several other tissues including the pituitary during development and postnatally (Hu et al. 2011). TGIF1 acts as a repressor in retinoid X receptor (RXR) mediated transcription in a TGFB/SMAD-dependent manner (Bartholin et al. 2006; Bertolino et al. 1995). Patients with heterozygous variants in *TGIF1* typically present with variable degrees of midline defects ranging from a single central incisor to HPE (Dubourg et al. 2004; El-Jaick et al. 2007). A pool of 30 patients with CPHD were screened, and only one patient had a *TGIF1* variant (p.Q267X). This individual had CPHD (GH, TSH, LH/FSH), AL hypoplasia, and a single central incisor. No functional studies were carried out (Tatsi et al. 2013). The repressor effect of TGIF1 is typically demonstrated in the context of retinoic acid activating on a promoter construct of *RBP2* (DR1-TATA-luc) or TGFB activating a promoter construct of *MMP1* (3-TP-lux). TGIF1 co-immunoprecipitated with RXRA and SMAD3 (El-Jaick et al. 2007). Another patient with a complex CNS phenotype with pituitary hypoplasia and single central incisor had a chromosomal rearrangement affecting *TGIF1* (Kantaputra et al. 2006).

PITX2

Heterozygous *PITX2* variants are one cause of Axenfeld-Rieger syndrome (ARS) characterized by the defects of the eye anterior segment, hypodontia (including single central incisor), maxillary hypoplasia, umbilical protrusion, and heart defects in humans (Franco et al. 2017; Seifi and Walter 2018; Semina et al. 1996). No *PITX2* variants have yet been discovered in patients with hypopituitarism. A few papers studying *PITX2* variants are clear on the lack of hypopituitarism and the presence of ARS with the patient variants studied (Quentien et al. 2011), while others have no more indication of pituitary involvement than a flattened sella turcica (Idrees et al. 2006) and some actually state the lack of *PITX2* variants (Lowry et al. 2007). Mouse mutants heterozygous for *Pitx2* loss of function alleles do not have hypopituitarism.

NKX2–1, TCF7L1, INSM1, SIX3, *and* SIX6

A large chromosomal deletion involving *NKX2–1* (and *MBIP, NKX2.8, PAX9, SLC25A1*) was described in a patient with pituitary stalk duplication and exaggerated response to TRH stimulation (Accornero et al. 2010). So far, one nonsense variant of *NKX2–1* was implicated in one family with IGHD and HH (Balicza et al. 2018). Heterozygous missense variants in the Wnt signaling repressor *TCF7L1* were described in two patients with IGHD and SOD-like features (Gaston-Massuet et al.

2016). There are very few variants reported in *INSM1*; however, it appears to be a very specific marker for neuroendocrine differentiation in primary lung cancer (Mukhopadhyay et al. 2019). HPE and micro/anophtalmia is prevalent in patients with *SIX3* or *SIX6* variants but there is no clear evidence for pathogenicity in human hypopituitarism thus far (Gallardo et al. 1999; Martinez-Frias et al. 2014; Rauchman et al. 2001).

13.4.2.2 Select Genetic Causes of Isolated Pituitary Hormone Deficiency

Isolated ACTH Deficiency (IAD)

Genetic causes for IAD include two established genes: *TBX19* and *POMC*. Pathogenic *TBX19* TF variants result in recessive, neonatal onset ACTH deficiency, and they represent about two-thirds of the patients with IAD (Couture et al. 2012; Metherell et al. 2004; Pulichino et al. 2003). Patients present with severe hypoglycemia and high mortality unless promptly treated with hydrocortisone (Abali et al. 2019; Couture et al. 2012; Vallette-Kasic et al. 2005). Recessive mutations were described in *POMC* resulting in a protein translation defect with red hair pigmentation, severe, early onset obesity, and secondary adrenal insufficiency (Aslan et al. 2014; Krude et al. 1998). The mechanism of corticotrope deficiency remains elusive and likely indirect in patients with heterozygous, de novo *NFKB2* TF mutations who present with IAD, hypogammaglobulinemia similar to common variable immunodeficiency (CVID), alopecia, lymphocyte and NK-cell defects, and trachyonychia (Brue et al. 2014; Chen et al. 2013; Lougaris et al. 2015).

Isolated TSH Deficiency (ITD)

While congenital hypothyroidism has an incidence 1:3000 congenital central hypothyroidism (isolated thyrotroph deficiency) is extremely rare (<1:20,000) (Grosse and Van Vliet 2011; van Tijn et al. 2005). Variants in *TSHB* and *TRHR* are the longest known causes (Collu et al. 1997; Hayashizaki et al. 1989). Variants in *TSHB* typically affect the "seat belt" region where TSHB binds CGA in a tightly regulated process to form biologically active TSH (Matzuk et al. 1988; Nicholas et al. 2017). The mechanism by which heterozygous *TRHR* variants lead to TSH deficiency is not completely understood (Collu et al. 1997). The cause of isolated TSH deficiency can be clarified with TRH stimulation testing. *TSHB* defects preserve the secretory response of CGA and PRL (Bonomi et al. 2001). The response is blunted if *TRHR* is defective (Collu et al. 1997).

Recently, an X-linked cause of TSH deficiency was described in men carrying variants in *IGSF1*. They present with PRL deficiency and macroorchidism, but no GHD (Asakura et al. 2015; Hughes et al. 2016; Joustra et al. 2016; Nakamura et al. 2013; Sun et al. 2012; Tajima et al. 2013; Tenenbaum-Rakover et al. 2016). *TBLX1* is the newest member of genes in isolated congenital central hypothyroidism (Heinen et al. 2016).

Isolated Growth Hormone Deficiency (IGHD)

Typical genetic causes for IGHD remain to be those in *GHRHR*, *GH1* while defects in *SOX3*, *HESX1 GLI3, OTX2* are rare (Alatzoglou and Dattani 2010; Demurger et al. 2015). Overall, *GHRH* and *GH1* defects are recessive (type I GHD) but a non-insignificant pool of patients shows autosomal-dominant or X-linked inheritance (Alatzoglou and Dattani 2012). *SOX3, GLI3*, and *OTX2* were discussed previously.

Isolated LH/FSH Deficiency

Currently more than 30 genes are implicated in congenital HH with or without anosmia. This is a huge increase since 2000, when only four well-established congenital HH genes were known: *KAL1*, *GNRHR*, *DAX1*, and *PCSK1* (Seminara et al. 2000). An extensive review was recently published (Maione et al. 2018).

13.5 Concluding Remarks

Current diagnostic opportunities have enabled physicians to establish the clinical diagnosis of pituitary hormone deficiency with high confidence. Advancements in DNA sequencing technology provided an incredible pool of novel candidate genes and variants to test for the clinician and the researcher. We have just begun to understand the functional consequences of changes in the coding region of the genome. According to the Genetics Home Reference at the NIH, the coding information is only 1% of our genome. Improving of the understanding of large copy number variations as well as the "meaning" of the noncoding genome will be driven by the progression of whole genome sequencing technology and bioinformatics analysis. Currently, the treatment of pituitary hormone deficiency consists of replacement of growth hormone and end organ hormones such as thyroid hormone or steroid hormones. Creating artificial endocrine organs is at its dawn. Gene therapy for specific genetic defects is at its very early stages for non-pituitary diseases. Improving our understanding on how genetic defects in the most common TF genes lead to disease such as hypopituitarism is fundamental in this progress.

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