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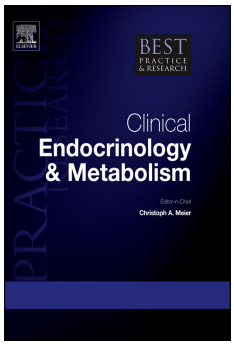

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Mara Giordano, PhD

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GENETIC CAUSES OF ISOLATED AND COMBINED PITUITARY HORMONE DEFICIENCY

Mara Giordano, PhD

Department of Health Sciences, Laboratory of Human Genetics, University of Eastern Piedmont, Novara, Italy

e-mail: mara.giordano@med.uniupo.it

telephone: +39 0321 660637

Fax: +39 0321 320421

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ABSTRACT

Research over the last 20 years has led to the elucidation of the genetic aetiologies of Isolated Growth Hormone Deficiency (IGHD) and Combined Pituitary Hormone Deficiency (CPHD).

The pituitary plays a central role in growth regulation, coordinating the multitude of central and peripheral signals to maintain the body's internal balance. Naturally occurring mutation in humans and in mice have demonstrated a role for several factors in the aetiology of IGHD/CPHD.

Mutations in the *GH1* and *GHRHR* genes shed light on the phenotype and pathogenesis of IGHD whereas mutations in transcription factors such as *HESX1, PROP1, POU1F1, LHX3, LHX4, GLI2* and *SOX3* contributed to the understanding of CPHD. Depending upon the expression patterns of these molecules, the phenotype may consist of isolated hypopituitarism, or more complex disorders such as septo-optic dysplasia (SOD) and holoprosencephaly.

Although the exponentially increasing advances in our understanding in the genetic of growth led to the identification of numerous monogenic causes of growth disorders most of the patients with IGHD/CPHD remain with an explained aetiology as shown by the mutation detection rate.

The introduction of novel diagnostic approaches is now leading to the disclosure of novel genetic causes in disorders characterized by pituitary hormone defects.

Key words:

IGHD, CPHD, *GH1*, *GHRHR*, *PUO1F1*, *PROP1*, *LHX3*, *LHX4*

INTRODUCTION

Height is a highly heritable and easily measurable complex continuous trait determined by a multitude of genetic, hormonal, nutritional and other environmental determinants. It has been estimated that about 80% is determined by genetic factors (1).

The anterior pituitary is the end-product of a carefully orchestrated pattern of expression of signalling molecules and transcription factors that leads to the development of different cell types specialized to produce and secrete specific hormones, including growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and adrenocorticotropic hormone (ACTH).

Studies on mutant mice have led to the understanding of mechanisms underlying pituitary organogenesis defects. Resequencing studies in patients presenting with hypopituitarism have confirmed the participation of some of the respective human orthologs in human pituitary function.

Among them there are many transcription factors that orchestrate the ontogeny of the pituitary, maintain the differentiated state of the developed gland and mediate the coordinated cell specific expression of the pituitary hormones (2).

Factors involved in pituitary organogenesis, particularly early-acting factors, are not pituitary specific, but are also required for the development of other organs and structures. Lesions in these genes can cause also defects in development of craniofacial or other structures. Instead factors acting later in the specifications of pituitary cell lineages are rather specific for each cell type but not

necessarily restricted to the developing or mature pituitary gland.

Impaired expression or functioning of these factors causes disorders of the pituitary gland and eventually leads to the diminished or entirely missing secretion of pituitary hormones.

This review summarizes the genetic causes of IGHD and CPHD and the prevalence of mutations identified in known genes as well as the new advances in the comprehension of novel genetic mechanisms of these disorders provided by novel molecular diagnostic approaches.

ISOLATED GROWTH HORMONE DEFICIENCY

GH1 and GHRHR

The first genetic factors that were recognized as monogenic causes of IGHD were the genes encoding the growth hormone (*GH1)* (3,4,5*)* and the receptor of growth hormone releasing hormone (*GHRHR) (6*). It has been estimated that mutations in these genes can be detected in up to 34% of familial cases of IGHD (7). In table 1 are reported the genes in which mutations have been identified to date in IGHD patients.

The *GH1* gene is located on chromosome 17q23 within a cluster of five highly homologous genes including the placentally expressed growth hormone gene *GH2*, two chorionic somatomammotropin genes *CSH1* and *CSH2* and a pseudogene *CSHP1* (Fig1a). When correctly spliced, *GH1* produces the 22kDa isoform which includes all the five exons with the complete biological activity of GH. The presence of an in-frame cryptic splice site within exon 3 gives rise to alternative spliced transcripts lacking the first 45 bp of exon 3 and produces a 20-kDa peptide missing aa 32-46 [8,9]. Complete skipping of exon 3, accounting for 1-5% of the total GH1 transcripts results in a 17.5 kDa form lacking aa 32-71. Two isoforms lacking exons 3-4 and exons 2-4, encoding a 11.3- and a 7.4-kDa peptides have also been detected.

The most severe form of IGHD, namely IGHD type IA, is characterized by short lengths at birth and hypoglycemia in infancy and severe dwarfism by six months of age [reviewed in 10] . In

response to replacement therapy with exogenous GH, these subjects develop anti-GH antibodies blocking the response to GH replacement.

The molecular defects in most cases are deletions of different size (Fig1a) encompassing the whole *GH1* gene (11,12). It is generally reported that about 10–15% of subjects with severe IGHD and height <−4.5 SD carry GH1 gene deletions. However, this is an average estimate and geographic differences as well as differences in patient selection criteria between studies. Frameshift and nonsense *GH1* mutations have also been also found in subjects with the IGHD IA phenotype (see Tables 1 and Fig 1b) [11-13].

Bialleic mutations in the *GH1* and GHRH receptor (*GHRHR*) gene give rise to the recessive IGHD type IB (Tab1) characterized by low but detectable levels of GH, mild to severe short stature (<-2SDS) height velocity less then 25th percentile, significantly delayed bone age and a good response to GH replacement therapy.

Whereas mutations in *GH1* (splice site, nonsense and frameshift, Fig 1) for only less than 2% of the causes IGHD-IB (14) , the mutation frequency is higher in *GHRHR*, reaching about 10% in these patients. GHRHR is a seven transmembrane G protein-coupled receptor mostly expressed in the anterior pituitary the binds the GHRH activating a stimulatory G-protein. The increased intracellular cAMP leads to the opening of a voltage-gated calcium channel releasing the GH stored in the secretory granules.

The first *GHRHR* mutation, identified at the homozygous state in a consanguineous pedigree, was a premature stop codon (E72X) within the extracellular domain, thus abolishing the receptor function (6). Most of the patients carrying *GHRHR* mutations belong to consanguineous families and only in a minority of the families the affected members are compound heterozygous for two distinct *GHRHR* mutations. Detected mutations are missense splice site and frameshift (Fig 2) (15-23).

In addition to these biallelic mutations we previously identified a missense substitution (Val10Gly) in the signal peptide at the heterozygous state in 3 unrelated patients with a drastic effect on the processing and translocation of the GHRHR to the cell surface (24). This represents an exception to the inheritance of the *GHRHR* mutations as it was transmitted as a dominant character with incomplete penetrance.

The autosomal dominantly inherited IGHD form, namely IGHD type-II, is mainly caused by heterozygous mutations leading to an aberrant splicing around the exon 3/IVS3 boundary region of the *GH1* gene resulting in exon 3 skipping of transcripts (Fig1b).

Since *GH1* has weak canonical splice sites, multiple cis acting splicing regulatory elements (exon and intron splicing enhancers) are essential to maintain the correct exon 3 definition through the activation of the canonical intron 2 and 3 splice sites and silencing of the cryptic sites. Several mutations leading to aberrant splicing have been reported in IGHD patients within these enhancer motifs (25-33) as well as within the canonical splicing consensus sequences (34-36) leading to an increased amount of the 17.5 kDa isoform. Functional experiments showed that this isoform exhibits a dominant negative effect both in tissue culture and in transgenic mice by disrupting the secretory pathway and trafficking of normal GH and other hormones, including adrenocorticotropic hormone (ACTH) (37-38).

X-linked IGHD (Type III)

IGHD has been reported in some families segregating as an X-linked disorder in association with X-linked agammaglobulinemia (XLA). Duriez et al (1994) (39) described for the first time a splicing mutation within the BTK (Bruton's tyrosine kinase) gene in a patients with XLA and IGHD. Subsequently only another study confirmed the presence of a frame-shift mutation in the BTK gene in a patient with XLA and IGHD (40). BTK is a cytoplasmic tyrosine kinase expressed in B lymphocyte and myeloid cells whose mutations have been identified also in several XLA

patients without IGHD. Thus it it cannot be escluded that the co-occurrence of IGHD in the two reported XLA patients is the consequence of an independent cause.

Subsequently alterations in another X-linked gene, namely SOX3 on Xq27.1 were detected in IGHD families. SOX3 is a member of the SOX (SRY-related high mobility group box) family of transcription factors expressed in neuroepithelial progenitor and stem cells beginning in the earliest stages of embryogenesis (41) involved in in different developmental processes, such as gastrulation, neural induction, specification, and the differentiation of many cell types. Both the over- and under-expression of SOX3 can cause different phenotypes ranging from IGHD to CPHD with ectopic neurohypophysis, anomalies of the corpus callosum with or without intellectual disability (42). Micro-duplications and deletions of genomic regions containing *SOX3* as well as expansion of a poly-adenine tract (in one case a deletion) have been reported (43-45).

Nevertheless other X-linked causes for IGHD must occur to justify the distorted sex-ratio frequently observed in IGDH patients (and also in CPHD). For example duplications involving the proximal Xq chromosome have been reported in patients with complex phenotypes including short stature (46-47). Most of these patients were not tested for pituitary hormone levels, but in those that were evaluated, GH deficiency was observed in the majority of the cases suggesting that other genes on this portion of the X-chromosome might be related to GHD.

Mutations in pituitary transcription factors

IGHD may be the first manifestation of an alteration within a gene encoding a transcription factor usually associated with panhypopituitarism such as POU1F1, PROP1, HEXS1 and OTX2. Mutations in these genes (in particular *HESX1*) give rise to different conditions, observed in patients with a broad spectrum of phenotypes ranging from multiple pituitary hormone deficiency

in combination or not with septo-optic dysplasia (as discussed below) to milder form such as IGHD.

Recently, for the first time a heterozygous missense mutation (p.Pro76Leu) has been identified in the *POU1F1* gene co-segregating with IGHD in a large pedigree (48) without any other hormone deficiency in the all the affected members. *POU1F1* mutations are usually found in patients with a well defined pituitary phenotype characterized by GH, PRL and TSH deficiency. In vitro and in vivo functional analysis showed that the mutant protein interferes with the wild-type POU1F1 in the binding to its cognate sites within the GH Locus Control Region, located upstream the *GH1* gene and within the *GH1* proximal promoter, but not to sites in the *PRL* promoter, thus affecting only the *GH1* expression.

Novel causes of IGHD

To date *GH1* and *GHRHR*, represent the best-known genetic causes of IGHD in both familial and sporadic cases albeit efforts have been made to identified other genes mainly through the candidate gene approach. The advent of next-generation sequencing technologies has enabled to move beyond single gene analysis to the simultaneous investigation of hundreds of genes and to the entire genome without *a priory* hypothesis providing new insight into the pathogenic mechanisms of IGHD.

Biallelic mutations have been identified in a IGHD family in the *RNPC3* gene coding for the minor splisosome U11/U12-65K protein trough whole exome sequencing (49) The three affected sisters were compound heterozygous for p.P474T/p.R502X. RNPC3 is a 65Kd protein that binds to the 3' stem loop of U12 snRNA and it essential for the U12 intron type recognition. The RNAseq data reveled aberrant U12 type intron processing in pituitary specific transcripts, providing novel insights into a novel molecular mechanism associated to IGHD and pituitary development. The

patients showed complete GH deficiency, but in contrast to IGHD type-IA showed a good response to GH replacement therapy.

In some cases of IGHD associated with other clinical features a single gene has been identified as the likely monogenic cause of the disorder. Exome sequencing revealed biallelic mutations in *IARS2* a nuclear gene encoding the Mitocondrial Isoleucyò-tRNA Synthetase in patients with a novel recessive disorders, namely CAGSSS (50). The disorder is characterized by cataract (CA), short stature secondary to isolated growth hormone deficiency (G), sensory neuropathy (S), sensorneural hearing loss (S) and skeletal dysplasia. This represents the first report of a mitochondrial protein involved in GH deficiency might shed light on new pathogenetic mechanisms involved in IGHD.

COMBINED PITUITARY HORMONE DEFICIENCY

Combined pituitary hormone deficiency (CPHD) is characterized by the impaired production of GH and one or more other pituitary hormones. The most common recognized genetic defects involved in CPHD include mutations within *PROP1, POU1F1*, *HESX1, LHX3, LHX4, OTX2, GLI2,* and *SOX3* (Table 2).

Mutations within *POU1F1* and *PROP1* , that are "later-acting transcription factors" in the pituitary organogenesis, are responsible for a pituitary specific phenotype characterized by multiple hormone deficiencies without relevant extra-pituitary findings.

Conversely the phenotype of subjects carrying mutations within "early transcription factors", such as HESX1 and GLI2, may present extra-pituitary manifestations, including syndromic hypopituitarism with craniofacial defects, such as septo-optic dysplasia (SOD) or holoprosencephaly (HPE).

The main genetic causes of CPHD are resumed in Table 2.

POU1F1

POU1F1 was the first pituitary-specific transcription factor to be identified in humans and mice (51-52) It belongs to the POU family of transcription factors and it is expressed in the anterior pituitary lobe. It binds to multiple site on the promoter and enhancers of target genes, namely *GH1*, *GHRHR* , *PRL* and *POU1F1* itself (53). It is essential not only for cell-specific gene expression but also for the development of somatotrophs, lactotrophs, and thyrotrophs.

The phenotype associated to *POU1F1* mutations is characterized by profound GH and PRL deficiencies, variable degrees of TSH deficit, severe proportional short stature, atypical facies and feeding difficulties in infancy. Neuroimaging usually shows a normal or hypoplastic anterior gland, pituitary stalk and posterior pituitary.

Both dominant and recessive mutations have been detected (Fig 3) (54-62) with a predominance of one dominant mutation, R271W present in about the 30% of the patients that carry a mutated *POU1F1* gene

To date large genetic screening performed on sporadic patients failed to detect a relevant frequency of mutations, with 1.6% of the sporadic cases of CPHD and no significant differences among populations (reviewed in 63). A higher and considerable frequency of *POU1F1* mutations has been detected in familial cases (21.6%) (63).

PROP1

PROP1 (prophet of PIT1) precedes and induces the expression of POU1F1 and in mice is expressed exclusively in the embryonic pituitary were it is involved in the differentiation of POU1F1 dependent somatotrope, lactotrope, gonadothrope and thyrotrope lineages. In Humans PROP1 expression persists throughout the life suggesting its function in the maintenance of cell lineages

and hormone secretion. Patients harboring mutations within the *PROP1* present GH, PRL TSH deficiency in addition to a variable defect in LH/FSH and ACTH secretion (2). Less severely affected patients may show only IGHD, that might then evolve in CPHD.

To date PROP1 mutations (Fig.4) represent the most common known genetic cause of CPHD both in sporadic (6.7%) and familial cases (48.5%) (63) with a global mutation frequency of 11 % considering all the patients (Tab 2). The mutation rate varies considerably among the different geographical areas: while Western-European, US, Australian and Japanese cohorts presented a mutation prevalence lower than 1% (64-68) the Eastern-European and Russian cohorts showed a much higher frequency reaching 64.8% (69-70) in the Lithuanian population (71). Two variants, namely c. 301_302delAG and c. 150delA are the most common mutations found in *PROP1* and represent more the 90% of the mutated alleles in the Eastern European cohorts. Recently Dusatkova et al (72) genotyped 21 SNPs flanking a 9.6-Mb region around PROP1 and demonstrated an ancestral origin for both variants, that are carried on haplotypes spanning 0.2-0.3 Mb showing that the most frequent *PROP1* variants are not mutation hot spots as previously assumed, but are founder variants.

HESX1

The paired-like homeobox gene, Hesx1 (homeobox expressed in embryonic stem cells) is one of the earliest markers of the mouse pituitary development (2) It is mainly expressed in the developing forebrain and the Rathke's pouch at the embryonic stage (e) 9.5 and is maintained until (e)12.5, after which it is rapidly extinguished. Extinction of Hesx1 is important for activation of downstream genes such as Prop1, suggesting that they act as opposing transcription factors. Null mutant mice for Hesx1 display a phenotype characterized by anophthalmia or microphthalmia and midline neurological defects reminiscent of a rare human syndrome, the septo-optic dysplasia (SOD). This

condition is characterized by hypopituitarism, pituitary hypoplasia, optic nerve hypoplasia, and abnormalities of midline brain structures.

The first homozygous missense mutation (N53C) has been detected within the homeobox domain of HESX1 in two siblings born to consanguineous parents with SOD (73). To date, several mutations both at the homozygous and at the heterozygous states in the HESX1 gene have been reported in a broad spectrum of phenotypes ranging from IGHD to CPHD associated in some cases with anomalies such as SOD, pituitary malformations, like ectopy of the neurohypophysis or pituitary aplasia (74-80). Most patients carry mutations at the heterozygous state and generally show a milder phenotype than the rare homozygous patients.

Whereas the condition in homozygous subjects displays full penetrance, the heterozygous *HESX1* mutations are invariably associated with reduced penetrance. Consequently, most of the patients are sporadic as their carrier relatives are frequently unaffected. Most of the HESX1 screenings performed in large CPHD cohorts (63) failed to detect mutations. The only mutations identified were detected in sporadic subjects with an estimated global frequency of 0.45%.

LHX3

LHX3 is a member of the LIM-homeodomain (LIM-HD) subfamily of transcription factor proteins that are characterized by a DNA-binding homeodomain in the middle of the protein and two amino terminal LIM domains (cysteine-rich structures important for protein-protein interactions) (2). It is expressed in the embryonic brain, spinal cord and Rathke's pouch, an early structure in pituitary development. LHX3 represents one of the most important “early transcription factors” being involved in the determination and differentiation of all five pituitary cell types.

Patients carrying biallelic *LHX3* mutations (Fig.5) show deficiencies of GH, PRL, TSH, LH, and FSH. Other associated symptoms can include normal, hypoplastic, or enlarged anterior pituitaries, a rigid spine with limited neck rotation, hearing loss, and ACTH deficiency [81-85].

LHX3 mutations showed very low mutation frequency in genetic screening (0.3% in sporadic and 11.1% in familial cases) and the unexpectedly high prevalence of LHX3 mutations in apparently unrelated in the two most northern countries of Sweden has been explained by a common ancestor dating back to the 17th century. The patients, presenting the characteristic LHX3 features, were homozygous for the same mutation whose carrier frequency was estimated 1/50 (86).

LHX4

LHX4, is another member of the LIM homeodomain that acts as a of transcription factor during pituitary gland and nervous system development. It contains two LIM domains in its N-terminus and a DNA-binding homeodomain (2). In murine studies, Lhx4 is expressed in the developing neural tube, hindbrain, Rathke's pouch, and pituitary gland. However the proper expression of Lhx4 is also crucial for the normal development of other organs such as lungs. Mice homozygous for Lhx4 die shortly after birth due to pulmonary failure, whereas heterozygous appear to be normal. In humans LHX4 mutations are usually transmitted as an autosomal dominant trait with variable penetrance. Patients present CPHD with variable GH, TSH, ACTH and gonadotropin deficiency deficiencies; MRI analyses shown in most cases hypoplasia of the pituitary and inabout half of the reported cases, the sella turcica is poorly developed. However there's a large inter- and intra-familial variability and mutations have been rarely detected with only 9 described in literature (fig 6) (87-90). Recently a novel recessive mutation have been reported (pT126M) associated with a lethal form of congenital hypopituitarism (91). The pathogenicity of this mutation remains however to be demonstrated as the functional assay failed to demonstrated any difference from the wild type LHX4.

GLI2 and the Sonic hedgehog (SHH) pathway

SHH and to lower extent *GLI2* mutations were initially reported in patients with Holoprocensephaly (HPE), a severe neurological characterized by incomplete or failed forebrain separation, or HPE-like phenotypes with pituitary anomalies and postaxial polydactyly. As the SHH pathway is also involved in pituitary development, mutations in SHH and GLI2 have been subsequently searched in CPHD patients. Franca et al (93) reported novel heterozygous frame-shift or nonsense GLI2 mutations and high frequency of non-synonymous GLI2 variants in patients with congenital hypopituitarism without HPE and most of these patients presented with CPHD and an ectopic posterior pituitary lobe. More recently, several individuals with truncation mutations in GLI2 were reported with the presence of typical pituitary anomalies, polydactyly and subtle facial features rather than HPE. In all the patients so far identified with GLI2 mutations, the pattern of inheritance was dominant with incomplete penetrance and variable phenotype (93,94).

It has to be considered that *GLI2* is a large and highly polymorphic gene with several rare variations reported in the exome server database (http://evs.gs.washington.edu/EVS/). Thus it is quite difficult to assess the pathogenicity of in the absence of functional studies. In a previous review of the literature we estimated that the most realistic *GLI2* mutation frequency in CPHD is about 1.5% (63).

Novel causes of CPHD

Mutations within *KAL1, FGFR1, FGF8* and *PROKR2* cause Kallmann syndrome characterized idiopathic hypogonadotropic hypogonadism and anosomia/hyposomia. Some of these genes are expressed in the Rathke's pounch. *PROKR2* mutations have been identified in patients with CPHD , including septo-optic dysplasia (SOD) suggesting a potential role for the PROK2 pathway in

pituitary development (98). The implication of this gene in panhypopituitarism has benn strengthened by the identification by next-generation targeted sequencing of a novel heterozygous *PROKR2* substitution (c.742C>T; p.R248W) in a patient with CPHD with marked pituitary dysplasia

Webb et al (2013) identified in a consanguineous family with 6 affected members with CPHD, microcephalia loss of vision and other congenital anomalies, a homozygous frameshift mutation (c1373_1374dupTC) in *ARNT2* (encoding the Aryl-Hydrocarbon Receptor Nuclear Translocator 2) through exome sequencing.

Recently the novel resequencing approach lead to the identification of a homozygous mutation in *GRP161* encoding the orphan G protein coupled receptor 161, a signal transducing molecule, in a family with short stature, CPHD and pituitary stalk interruption (101).

CONCLUSIONS AND PERSPECTIVES

In the last two decades, the exponentially increasing advances in the understanding of the genetic of pituitary hormone deficiencies led to the identification of numerous monogenic causes of growth disorders. However, most of the patients remain with an explained aetiology and without a correctly addressed therapeutic programme. Fully penetrant mutations within known genes have been detected in approximately 20-30% of familial cases, whereas the frequency mutations in sporadic cases is much lower. These suggest that other yet unidentified genes are responsible for the diseases both in familial and sporadic case.

The introduction of next generation sequencing technology in the diagnostic workflow will lead to the identification of novel genetic determinants in patients coming to the paediatrics' consultation for pituitary defects. This will allow to decrease the cost and the response time for the clinical and

molecular diagnostic providing an important contribution to the improvement of the therapeutic choices.

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LEGEND TO FIGURES:

FIGURE 1:

a) Scheme of the GH1 gene cluster. The bars indicate the deletions most frequently detected in IGHD-type 1A patients.

b) Scheme of the GH1 gene with the point mutations detected IGHD patients. In the upper part are reported mutations causing splicing defects. In the lower part frame-shift and missense mutations.

FIGURE 2:

Scheme of the GRHRH gene with the mutations detected in IGHD patients. In the upper part are reported mutations causing splicing defects. In the lower part frame-shift, missense and nonsense mutations.

FIGURE 3:

Scheme of the POU1F1 gene with the mutations detected in CPHD patients. In the upper part are reported mutations causing splicing defects. In the lower part frame-shift, missense and nonsense mutations.

FIGURE 4

Scheme of the PROP1 gene with the mutations detected in CPHD patients. In the upper part are reported mutations causing splicing defects and frame-shift mutations in exon 2 (in red). In the lower part frame-shift, missense and nonsense mutations.

FIGURE 5

Scheme of the POU1F1 gene with the mutations detected in CPHD patients. In the upper part are reported mutations causing splicing defects. In the lower part frame-shift, missense and nonsense mutations.

FIGURE 6

Scheme of the LHX3 gene with the mutations detected in CPHD patients. In the upper part are reported mutations causing splicing defects. In the lower part frame-shift, missense and nonsense mutations.

FIGURE 7

Scheme of the LHX4 gene with the mutations detected in CPHD patients. In the upper part are reported mutations causing splicing defects. In the lower part frame-shift, missense and nonsense mutations.

Table 1. Genetic causes of IGHD

gene	inheritance	Mutation type	Phenotypes	Mutation frequency
GH1	AR (IGHD 1A)	Deletions (6.0, 7.0,7.6,45 Kb)	Severe short stature No serum GH Anti GH-ab	15%
	AR (IGHD 1B)	Nonsense frameshift	Mild to severe short stature Low serum GH	2%
	AD (IGHD II)	Splicing defect	Mild to severe short stature Low serum GH	5-10%
GHRHR	AR (IGHD 1B)	Missense Nonsense	Mild to severe short stature Low serum GH	10%
HESX1	AD	Missense splicing	Low serum GH (SOD)	rare
SOX3	X-linked	Deletion/Duplication polyA expansion	Lo serum GH (Intellectual disability)	rare
*RNPC3**	AR	Nonsense Missense	Severe Short stature No serum GH	mutations detected in only one family
*PIT-1**	AD	Missense	Severe short stature Low serum GH	mutations detected in only one family
*IARS2**	AR	missense	Low serum GH Skeletal dysplasia Hearing loss	mutations detected in only one family

AR=Autosoma Recessive AD=Autosomal Dominant

*mutations were detected in only one family

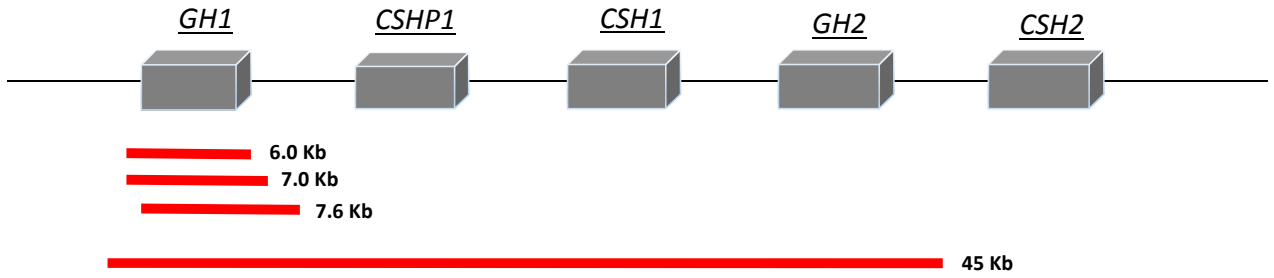
Tab 2 Genetic causes of CPHD

Gene	inheritance	Mutation type	Pituitary hormone deficiency	Associated extrapituitary phenotypes	Mutation frequency*
POU1F1	AR, AD	Missense, nonsense, frameshift, splicing	GH, PRL, TSH	none	2.8%
PROP1	AR	Missense, nonsense, frameshift, splicing	GH, PRL, TSH, LH, FSH, evolving ACTH	none	11.2 %
HESX1	AD,AR	Missense	from IGHD to variable CPHD	SOD	0.4%
LHX3	AR	Missense, nonsense, frameshift, splicing	GH, PRL, TSH, LH, FSH, ACTH	Sensoneural hearing loss, limited neck rotation, short cervical spine	0.5%
LHX4	AR,AD	GH, PRL, TSH, LH, FSH, evolving ACTH	GH, PRL, TSH, LH, FSH, ACTH	Cerebellum anomalies	0.9%
OTX2	AD	Missense, nonsense	from IGHD to variable CPHD	Anofthalmia/microfth almia, developmental delay	rare
SOX3	X-linked	Variation poly-A length duplication/deletion	from IGHD to variable CPHD	Intellectual disability Midline defects Infudibular hypoplasia	rare
GLI2	AD	Frame-shift, missense	GH, PRL, TSH, LH, FSH, ACTH	Polydactily, holoprosencephaly Craniofacial abnormalities	7.1%

*This is a global mutation frequency, considering both familial and sporadic cases, as reported in De Rienzo et al. 2015 (63)

FIG.1

a) ***GH1*** **gene cluster (17q23)**



b) ***GH1***

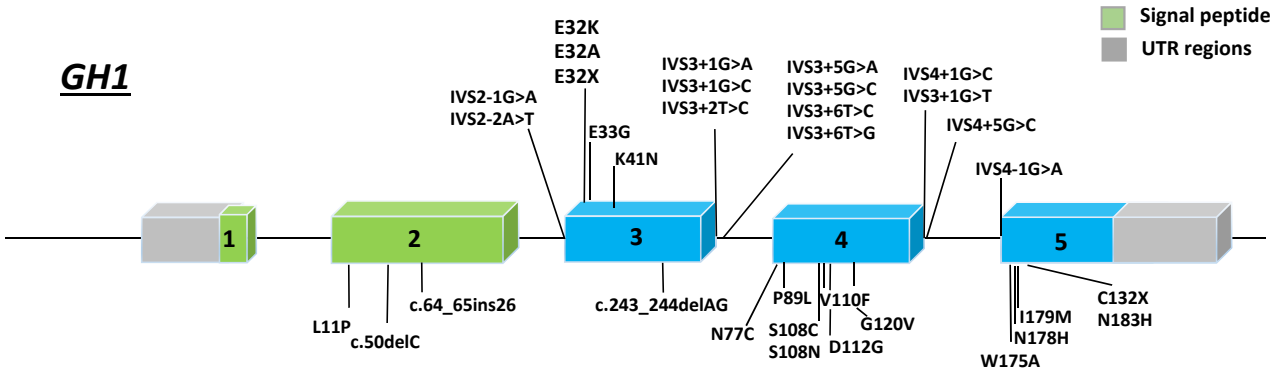


FIG.2

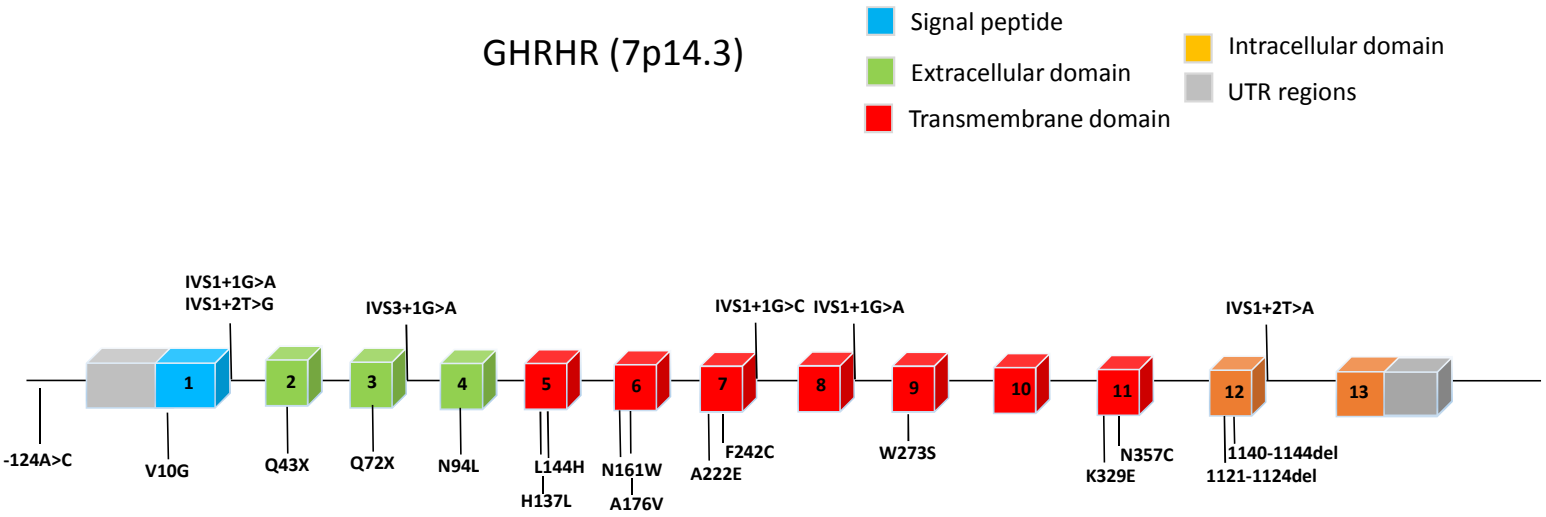

GHRHR (7p14.3)
Signal peptide
Extracellular domain
Transmembrane domain
Intracellular domain
UTR regions
-124A>C
V10G
IVS1+1G>A
IVS1+2T>G
Q43X
IVS3+1G>A
Q72X
N94L
L144H
H137L
N161W
A176V
IVS1+1G>C
A222E
F242C
IVS1+1G>A
W273S
K329E
N357C
IVS1+2T>A
1121-1124del
1140-1144del
1
2
3
4
5
6
7
8
9
10
11
12
13

FIG.3

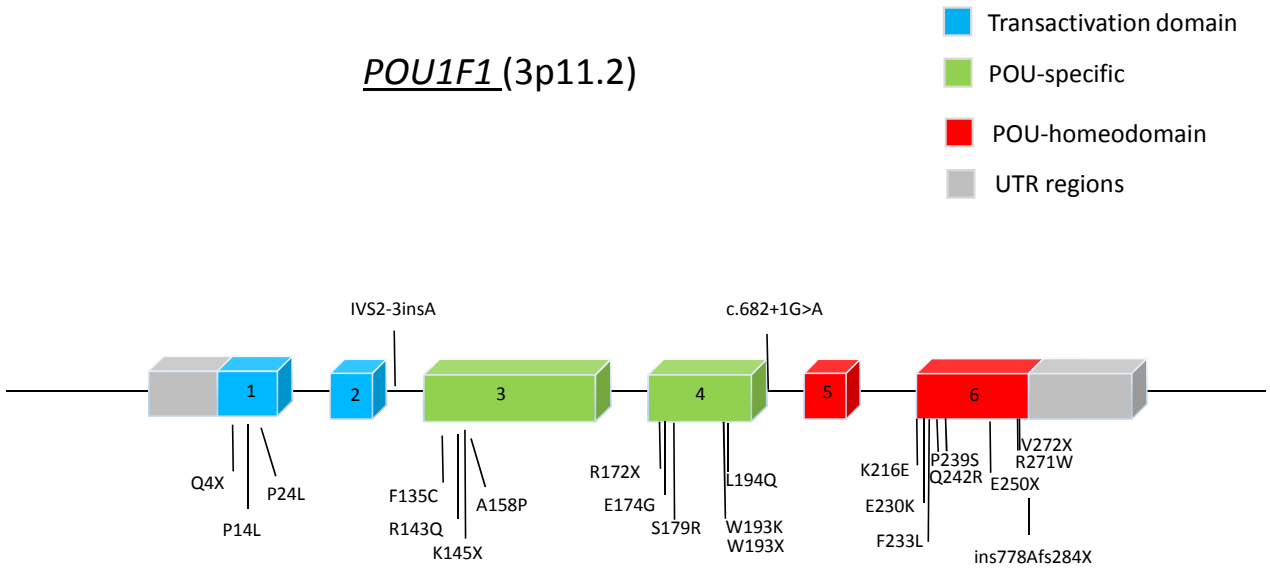

POU1F1 (3p11.2)
Transactivation domain
POU-specific
POU-homeodomain
UTR regions
IVS2-3insA
c.682+1G>A
1
2
3
4
5
6
Q4X
P24L
P14L
F135C
R143Q
A158P
K145X
R172X
E174G
S179R
L194Q
W193K
W193X
K216E
E230K
F233L
P239S
Q242R
E250X
V272X
R271W
ins778Afs284X

Figure 4

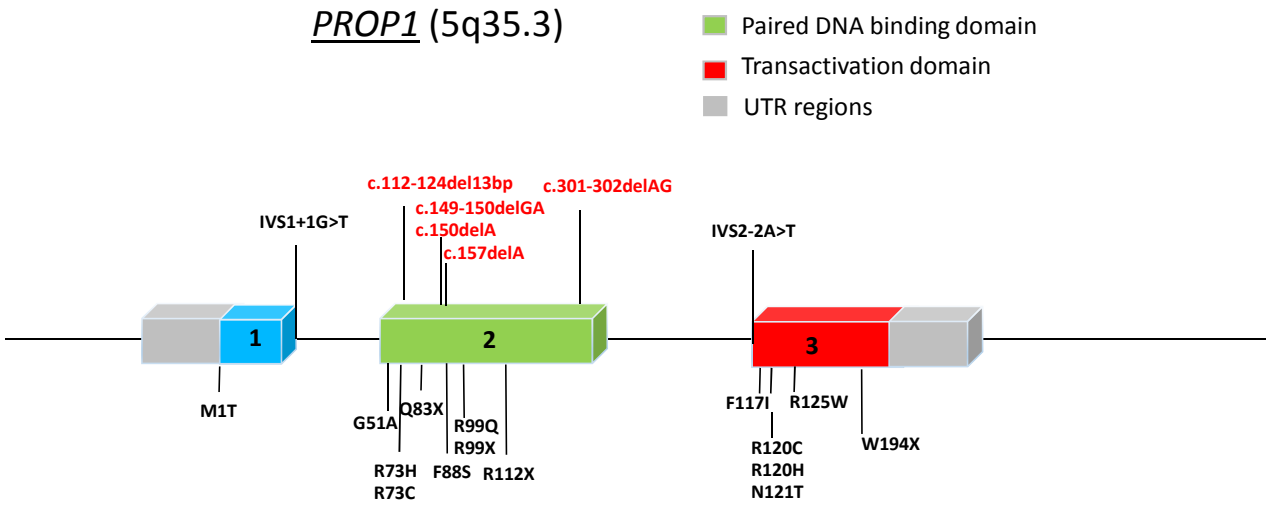

PROP1 (5q35.3)
Paired DNA binding domain
Transactivation domain
UTR regions
c.112-124del13bp
c.301-302delAG
c.149-150delGA
c.150delA
c.157delA
IVS1+1G>T
IVS2-2A>T
1
2
3
M1T
G51A
Q83X
R99Q
R99X
R73H
R73C
F88S
R112X
F117I
R125W
R120C
R120H
N121T
W194X

FIG.5

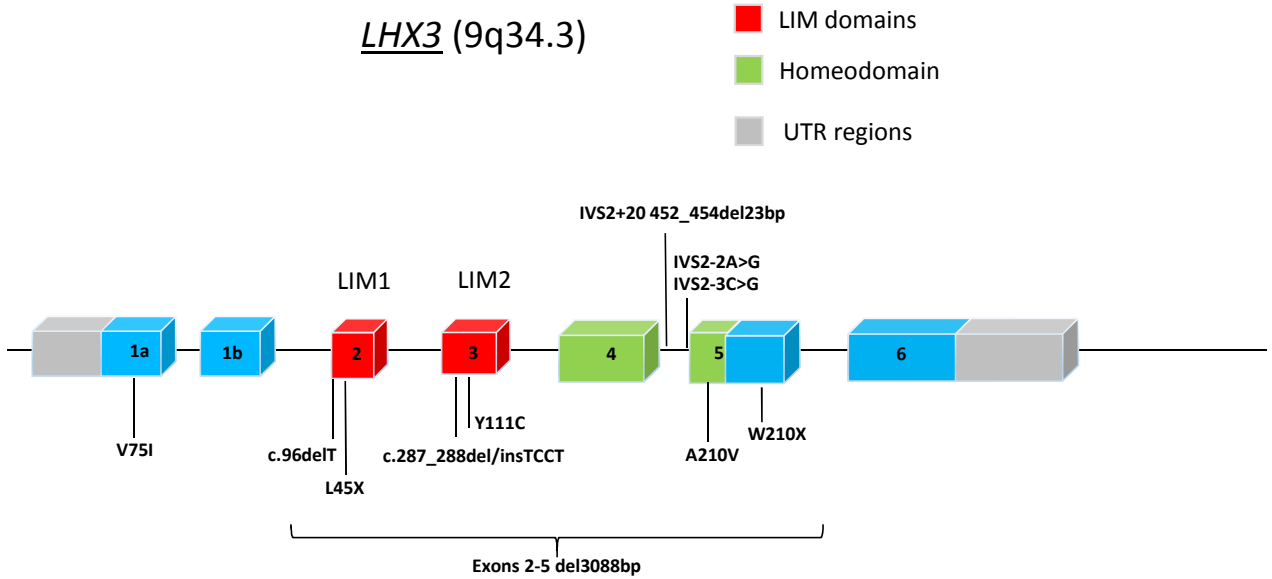

LHX3 (9q34.3)
LIM domains
Homeodomain
UTR regions
IVS2+20 452_454del23bp
IVS2-2A>G
IVS2-3C>G
LIM1
LIM2
1a
1b
2
3
4
5
6
V75I
c.96delT
L45X
Y111C
c.287_288del/insTCCT
A210V
W210X
Exons 2-5 del3088bp

FIG.6

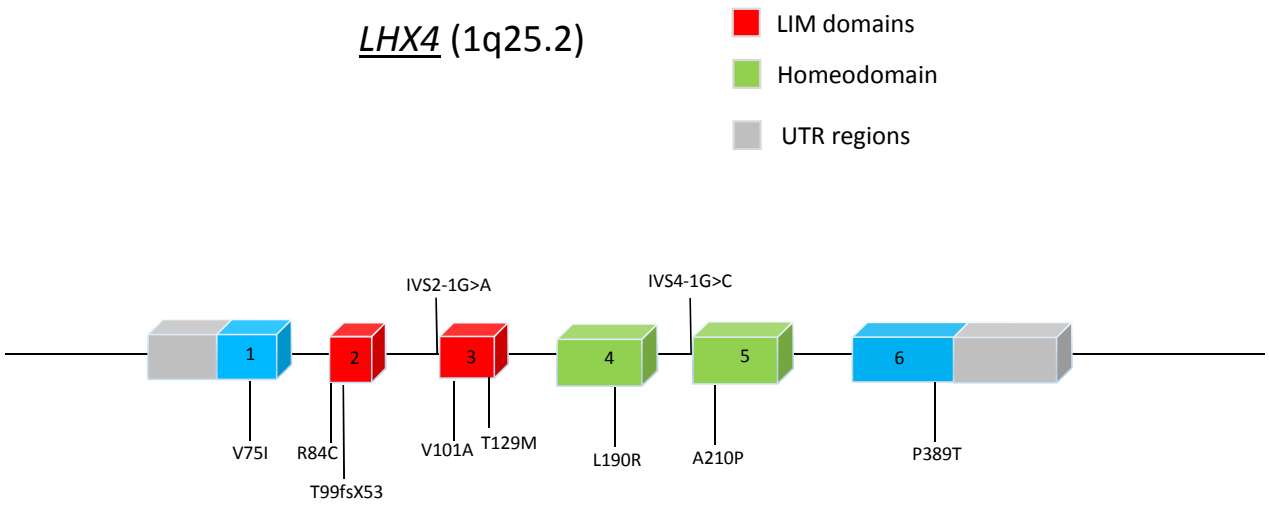
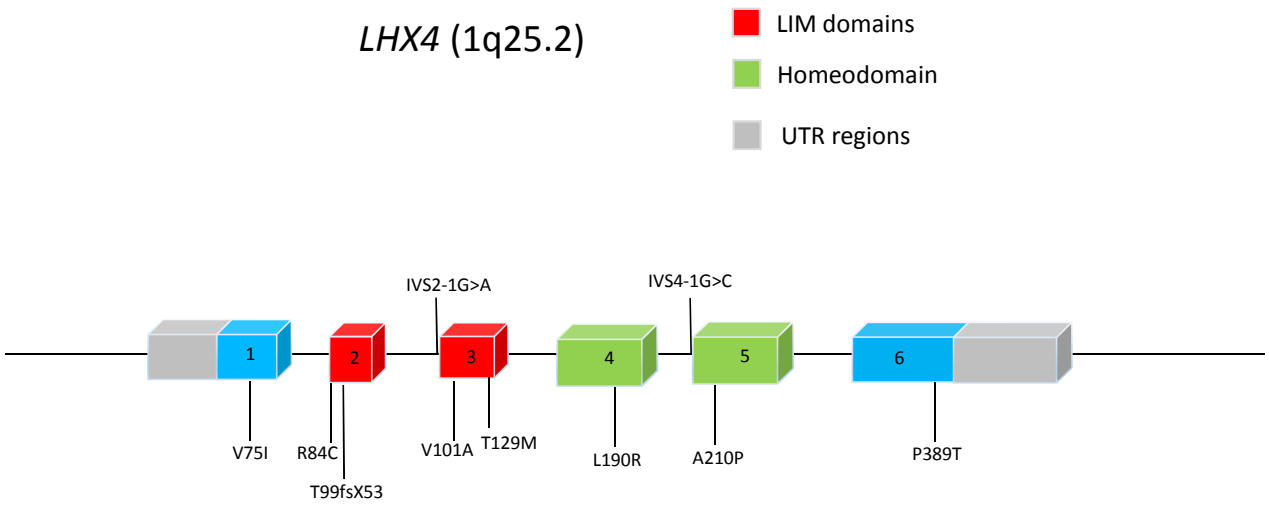

LHX4 (1q25.2)
LIM domains
Homeodomain
UTR regions
IVS2-1G>A
IVS4-1G>C
1
2
3
4
5
6
V75I
R84C
T99fsX53
V101A
T129M
L190R
A210P
P389T

FIG.7


LHX4 (1q25.2)
LIM domains
Homeodomain
UTR regions
IVS2-1G>A
IVS4-1G>C
1
2
3
4
5
6
V75I
R84C
T99fsX53
V101A
T129M
L190R
A210P
P389T