

Inherited disorders of the gonadotropin hormones

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Abstract

Pulsatile GnRH acts at the GnRH receptor on gonadotropes to stimulate gonadotropin gene expression, hormone synthesis and secretion. The pituitary gonadotropins, LH and FSH, stimulate steroid production and gametogenesis in males and in females. Gonadotropin production thus requires the normal development and function of hypothalamic GnRH-producing neurons and pituitary gonadotrope cells. Genes involved in gonadotrope development and/or gene expression include *SF1*, *DAX1*, *KAL*, *GNRHR*, *PC1*, *HESX1*, *LHX3*, *PROP1*, *LH β* , and *FSH β* . Given the complex control of gonadotropin biosynthesis and secretion, it is not surprising that genetic abnormalities have been identified at several of these steps. Some of the mutations that will be reviewed include: (1) *SF1* and *DAX1*-orphan nuclear receptors that are expressed at multiple levels throughout the reproductive axis; (2) *KAL*-X-linked Kallmann syndrome, where there is abnormal development of hypothalamic GnRH-producing neurons; (3) *PC1*-causing abnormal processing of GnRH and *GNRHR* mutations that impair action at the GnRH receptor; (4) *HESX1*, *LHX3*, *PROP1*-abnormal development/function of the gonadotrope cell lineage; (5) *LH β* and *FSH β* -mutations in the gonadotropin genes that cause structural abnormalities in the hormones. Although all of these gene defects lead to gonadotropin deficiency, each disorder is associated with unique phenotypic or hormonal features. Characterization of the molecular basis of gonadotropin deficiency is useful for directing therapy and for genetic counseling. Identification of these mutations also provides insight into the pathways that govern reproduction. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Over the past decade, a number of genes have been identified that regulate the development and function of the hypothalamic–pituitary gonadal (HPG) axis in humans (Fig. 1). Mutations in several of these genes have been found in patients with sporadic or familial forms of hypogonadotropic hypogonadism (*KAL*, *DAX1*, *GnRHR*, *HESX1*, *LHX3*, *PROP1*) or abnormal sexual maturation due to impaired gonadotropin function (*FSH β* , *LH β*) (Table 1). Although mutations in these genes are relatively rare, it is important to consider the genetic basis of disorders of go-

nadotropin hormones. First, appropriate treatment can be provided to an affected individual if the pathological basis of the underlying disorder is known. For example, pulsatile GnRH therapy may be effective in a patient with a hypothalamic defect such as Kallmann syndrome, but is less beneficial when pituitary gonadotropes or the gonadotropin hormones themselves are abnormal. Second, defining the genetic basis of the condition allows appropriate genetic counseling for the patient and family. Finally, identifying naturally occurring mutations has provided crucial information about the roles that these genes play in human sexual development and fertility, and may allow the development of better infertility treatments or approaches to contraception in the future. This review will provide an update of the range of single gene disorders that are associated with impaired gonadotropin release or function in humans.

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2. Orphan nuclear receptors

Steroidogenic factor-1 (SF-1/FTZF1/NR5A1) and DAX-1 (NR0B1) are two orphan nuclear receptors that are expressed widely throughout the reproductive axis (hypothalamus, gonadotropes, gonads) and adrenal gland. Mutations in these genes in humans and targeted mutagenesis studies in mice have confirmed that these transcription factors play a crucial role in the development and function of the HPG axis at multiple levels, including gonadotropin production.

2.1. Steroidogenic factor-1 (SF-1)

SF-1 is a key regulator of the transcription of many genes involved in sexual differentiation, steroidogenesis and reproduction. Accordingly, targeted mutagenesis of *Sf1* ($-/-$) in mice results in complete adrenal and gonadal agenesis and XY sex-reversal with persistence of Müllerian structures in males. Target genes in the hypothalamus and pituitary include the GnRHR, α -GSU, and LH β (Parker and Schimmer, 1997). *Sf1* knock-out mice also show marked abnormalities in the development of the ventromedial hypothalamus and impaired development of pituitary gonadotropes (Ikeda et al., 1995). The reduced gonadotropin expression seen in animals kept alive by corticosteroid treatment can be partially restored by GnRH treatment, suggesting that abnormalities in GnRH production may be more important than defective gonadotropin synthesis and release, as the basis for gonadotropin deficiency.

The role of SF-1 in humans has been clarified by the description of a heterozygous missense mutation in the DNA-binding domain of SF-1 in a patient with complete XY sex-reversal, testicular dysgenesis, and adrenal failure (Achermann et al., 1999b). Gonadotropins were elevated after gonadectomy (FSH > LH), and declined after estrogen treatment, suggesting relative preservation of gonadotrope function in this heterozygous patient compared to homozygous *Sf1* knock-out mice. No *SF1* mutations have been reported yet in genotypic females, where sex-reversal would not be expected. Identifying such patients could provide important insight into the role of SF-1 in ovarian development and function, as well as providing additional information about the role of SF-1 in gonadotrope function in humans.

2.2. DAX-1

Mutations in the *AHC* (Xp21) gene, which encodes DAX-1, cause X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism (HHG) (Muscatelli et al., 1994; Zanaria et al., 1994). Affected (hemizygous) boys develop primary adrenal failure in infancy or childhood. Approximately 10% of boys with

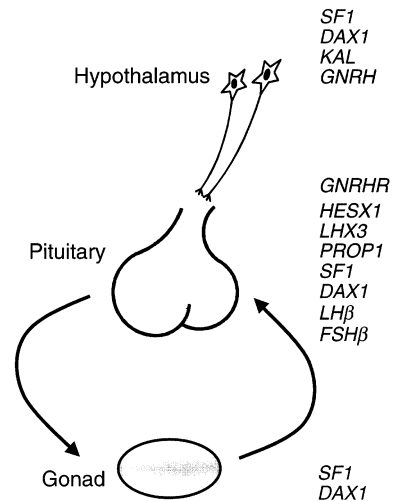


Fig. 1. Overview of the hypothalamic–pituitary gonadal axis. Mutations in several genes have been shown to cause impaired gonadotropin release or function in humans.

DAX1 mutations have evidence of congenital gonadotropin deficiency, such as cryptorchidism or micropenis. Conversely, a number of reports have documented normal HPG axis activity in infancy in patients with *DAX1* mutations. By the time of puberty, HHG appears to be a universal feature of this condition, although partial pubertal development has been reported in rare cases.

Pulsatile GnRH has been used in an attempt to induce puberty in several patients with *DAX1* mutations. These efforts have generally been unsuccessful, probably reflecting a combined hypothalamic and pituitary defect (Habiby et al., 1996). Gonadotropins have also been used in a limited number of patients to try to induce spermatogenesis directly. Treatment with human chorionic gonadotropin stimulates testosterone produc-

Table 1
Single gene disorders causing impaired gonadotropin release or function in humans^a

Gene	Locus	Product	Reported mutations
<i>SF1</i>	9p33	Orphan nuclear receptor	1
<i>DAX1</i>	Xp21	Orphan nuclear receptor	70
<i>KAL</i>	Xp22	Extracellular matrix protein	34
<i>GNRHR</i>	4q21	G-protein coupled receptor	6
<i>HESX1</i>	3p21	Paired-like homeodomain TF	1
<i>LHX3</i>	9q34	Paired-like homeodomain TF	2
<i>PROP1</i>	5q35	Paired-like homeodomain TF	45
<i>FSHβ</i>	11p13	Glycoprotein hormone	5
<i>LHβ</i>	19q13	Glycoprotein hormone	1

^a TF, transcription factor. Contiguous gene deletion syndromes (*DAX1*, *KAL*) and polymorphic variants (*LH β*) not included.

tion in most patients, but the induction of spermatogenesis after treatment with gonadotropins has been less successful (Seminara et al., 1999; Tabarin et al., 2000). This observation may reflect a direct role for DAX-1 in Sertoli cell function and spermatogenesis, as seen following targeted disruption of the *Ahch* gene in mice (Yu et al., 1998). Additional human data are required before the extent of this defect is known.

More than 60 different *DAX1* mutations have been identified in over 70 families with X-linked AHC. Most of these mutations are frameshift or nonsense mutations that cause premature truncation of the carboxy-terminus of the DAX-1 protein, impairing its function as a repressor of SF-1 mediated transcription. Recently, the phenotypic spectrum associated with *DAX1* mutations has been extended to include HHG in a woman homozygous for a *DAX1* mutation through gene conversion (Merke et al., 1999), delayed puberty in female carriers of *DAX1* mutations (Seminara et al., 1999), and adult-onset adrenal failure and partial HHG in a man with an I439S missense mutation (Tabarin et al., 2000). The I439S mutant was shown to have only partial loss of repression in transient gene expression assays, consistent with the milder phenotype in this patient. With the exception of this case, however, genotype–phenotype correlations have been elusive, suggesting a potentially important role for modifier genes. In addition, *DAX1* mutations were not detected in more than 100 patients with HHG or delayed puberty, suggesting that abnormalities in this gene are rare in the absence of a personal or family history of adrenal failure (Achermann et al., 1999a).

3. Abnormalities in gonadotropin-releasing hormone secretion and action

Abnormalities in GnRH secretion and action can result from impaired migration of GnRH secreting neurons during development (*KAL*), defective GnRH synthesis, release, and processing (leptin, *PC1*), or mutations in the GnRH receptor (*GnRHR*).

3.1. *KAL* and Kallmann syndrome (see review by Hardelin in this issue)

During early fetal development, GnRH-releasing neurons and olfactory neurons arise in the olfactory placode and migrate through the cribiform plate to reach their final positions in the hypothalamus and olfactory bulb, respectively. These ontological phenomena explain the association of HHG with anosmia (absent sense of smell) in Kallmann syndrome. Many patients with the X-linked form of this condition have mutations in the *KAL* gene (Franco et al., 1991; Legouis et al., 1991), which encodes an extracellular

matrix glycoprotein, anosmin-1, that facilitates neuronal growth and migration. Associated features, which include unilateral renal agenesis and synkinesia (mirror-image movements), show variable penetrance in families with this condition, suggesting that modifier genes or epigenetic phenomena may influence phenotypic expression (Seminara et al., 1998). Further, the genetic causes of the more frequent autosomal dominant and recessive forms of Kallmann syndrome remain unknown (Georgopoulos et al., 1997).

3.2. *GNRH* gene

The *GNRH* gene is an obvious candidate for mutation in patients with HHG, as GnRH plays such a pivotal role in sexual development and function, and *hpg/hpg* mice have recessively inherited HHG caused by a *GnRH* gene deletion. Although hypogonadism has been described in some patients with 8p deletions involving the *GNRH* locus, no specific mutations or deletions within the human *GNRH* gene have been reported (Weiss et al., 1991).

There is increasing evidence that leptin plays an important role in reproductive function, probably through its central effects on the release of neurotransmitters, such as neuropeptide Y, that ultimately regulate GnRH release. HHG has been described in patients with obesity due to mutations in leptin (Strobel et al., 1998) or the leptin receptor (Clement et al., 1998). Leptin treatment appears to restore puberty in leptin-deficient patients, although experience is limited at present.

The endopeptidase prohormone convertase-1 (*PC1*) plays a crucial role in the post-translational modification of prohormones and neuropeptides, and a compound heterozygous mutation in *PC1* has been described in a woman with obesity, hypocortisolemia and HHG (Jackson et al., 1997). The reproductive defect in this case may be the result of impaired GnRH processing and/or abnormalities in neuropeptides related to GnRH secretion.

3.3. *GnRH* receptor (see review by Milgrom in this issue)

An increasing number of families with compound heterozygous mutations in the GnRH receptor have been described within the past 3 years (de Roux et al., 1997; Layman et al., 1998). These *GnRHR* mutations reduce GnRH binding and/or activation of phospholipase C and inositol triphosphate production. Variable clinical features may be seen, even within the same kindred, and range from complete to partial HHG. Equally variable basal and GnRH-stimulated gonadotropin concentrations have been reported, although spontaneous gonadotropin release has reduced

pulse amplitude in most patients. High-dose pulsatile GnRH treatment may therefore be effective in inducing fertility in some patients who appear to have partial GnRH resistance (Seminara et al., 2000).

4. Anterior pituitary development

Development of the anterior pituitary gland, and differentiation of the sub-types of cells found within it, is regulated by a number of transcription factors. Many of these are homeobox genes that are expressed in complex spatial and temporal patterns. Mutations in genes involved in the early stages of pituitary development cause marked defects in all pituitary hormones (e.g. *HESX1*), whereas mutations in genes that regulate the later stages of development affect a more limited number of hormones (e.g. *LHX3*, *PROPI*). No mutations in gonadotrope specific genes (e.g. *Egr1*) have been described yet.

4.1. *HESX-1*

Hesx1 is a homeobox gene that is expressed in early forebrain and pituitary development. Targeted mutagenesis of *Hesx1* in mice causes variable anterior central nervous system defects and pituitary dysplasia. A homozygous R53C missense mutation within the DNA-binding homodomain of *HESX1* was reported in two children with familial septo-optic dysplasia and panhypopituitarism (Dattani et al., 1998). Although these children are prepubertal, investigations suggest that gonadotropin release is impaired.

4.2. *LHX3*

Targeted mutagenesis of *Lhx3/Lim3* in mice produces panhypopituitarism, suggesting that *LHX3* is a candidate gene for disorders of human pituitary development. Recently, this has been confirmed by Netchine et al. (2000), who described *LHX3* mutations in four patients from two families with combined pituitary hormone deficiency, but preserved corticotrope function. In addition, limited head rotation due to cervical spine rigidity was reported. Evaluation of HPG function showed impaired gonadotropin release. One boy presented with cryptorchidism and micropenis at birth, and the three oldest patients failed to show any signs of pubertal development by 15 years of age.

4.3. *PROP-1*

The Ames dwarf (*df*) mouse has GH, prolactin, and TSH deficiency due a homozygous missense mutation (S83P) in the *Prop1* (*prophet of Pit-1*) gene. Gonadotrope differentiation is also impaired, and homozygous females and most males are infertile. *PROPI*

was therefore considered a candidate gene in patients with combined pituitary hormone deficiencies, and *PROPI* mutations have now been identified in more than 20 families (Wu et al., 1998). Most of these mutations are clustered within the PROP-1 homeodomain, and reduce binding affinity and transcriptional activation of target genes. The delA301G302 mutation is found frequently, even in unrelated kindreds, and may represent slipped strand mispairing in this AG repeat region. Most patients with *PROPI* mutations develop GH and TSH deficiency in childhood. The reproductive phenotype is more variable; some patients demonstrate HHG and pubertal failure in adolescence, whereas others progress through puberty spontaneously, but develop hypogonadism or amenorrhea later in adult life. This variability in reproductive phenotype can be seen within families with the same mutation (Fluck et al., 1998). In rare cases, impaired corticotrope function has been reported in older patients with *PROPI* mutations.

5. Gonadotropins

The gonadotropins are heterodimers consisting of specific β -subunits that are non-covalently bound to a common α -subunit. No human α -subunit mutations have been reported. Based on targeted disruption of the α -subunit gene in mice, a marked dysfunction of all glycoprotein hormones (including TSH and hCG) would be expected. In fact, because hCG plays an essential role in the maintenance of pregnancy in humans but not in rodents, human α -subunit mutations might be non-viable. In contrast, a limited number of mutations in the *FSH β* and *LH β* genes have been described. These mutations have provided important information about the relative roles of the gonadotropins in men and women.

5.1. *FSH β*

Homozygous or compound heterozygous mutations in the *FSH β* gene have been reported in three women who presented with delayed puberty, absent breast development, and primary amenorrhea (Val61X; C51G/Val61X; Val61X) (Layman et al., 1997; Matthews and Chatterjee, 1997; Matthews et al., 1993). In these cases, serum FSH was undetectable, and serum LH was elevated. Normal primordial follicles were detected in the ovary, and in two cases, follicular maturation, ovulation and fertility were achieved following treatment with exogenous FSH. These findings confirm the role of FSH in antral development and granulosa cell estrogen production, and reflect the phenotype seen following targeted disruption of *FSH β* in female mice (Kumar et al., 1997). A similar block in folliculogenesis is seen in

female FSH receptor knock-out mice (Dierich et al., 1998) and women with FSH receptor mutations (Aitomaki et al., 1995).

Using the crystal structure of hCG as a guide (neither the FSH β nor the LH β structures have been solved), the mutations reported in these patients can be seen to affect different regions of the molecule (Lapthorn et al., 1994). Val61X causes a deletion of the C-terminus, including the so-called “seat-belt” region (residues 90–110) that is essential for heterodimer formation (Fig. 2). C51 is involved in the “cysteine-knot” motif that organizes the core of the protein subunit and determines the remaining folds. The functional effects of the Val61X and C51G mutations were tested by co-expressing wild-type and mutant FSH β subunits in vectors containing the normal α -subunit gene (Layman et al., 1997). Consistent with the structural predictions, immunoradiometric assay and bioassay of culture media showed normal expression of wild-type FSH, but undetectable levels of mutant FSH.

The effect of FSH mutations on spermatogenesis is less clear. Male FSH β and FSH receptor knockout mice are fertile despite having a reduced testicular size and partial spermatogenic failure (Kumar et al., 1997). Men harboring an FSH receptor mutation (A189V) have a variable reduction in testicular volume and a range of spermatogenic failure (Tapanainen et al., 1997). It was predicted, therefore, that FSH is not absolutely required for spermatogenesis, and that

testosterone may play a compensatory role in supporting spermatogenesis in the absence of FSH in these cases.

The recent description of homozygous mutations in the FSH β gene in two azoospermic men has challenged this concept. In the first report, the finding of delayed puberty and low testosterone may have contributed to the lack of spermatogenesis (Val61X) (Phillip et al., 1998). In the other patient, however, puberty occurred normally, and testosterone was within the normal range, yet no spermatozoa were detected, and a testicular biopsy showed spermatogenic arrest (Lindstedt et al., 1998). This patient was homozygous for a C82R mutation that participates in the cysteine knot and is predicted to interfere with dimer formation. Additional cases of naturally occurring human FSH β and FSH receptor mutations are needed, therefore, to clarify the role of FSH in spermatogenesis.

5.2. LH β

To date, only one human LH β gene mutation has been reported (Weiss et al., 1992). The affected male had delayed puberty, low testosterone, and arrested spermatogenesis. Upon presentation, his serum immunoreactive LH was elevated and FSH normal. Long-term treatment with hCG resulted in testicular enlargement, virilization, and increased sperm count (11 million per milliliter, 50% motility), but fertility was not

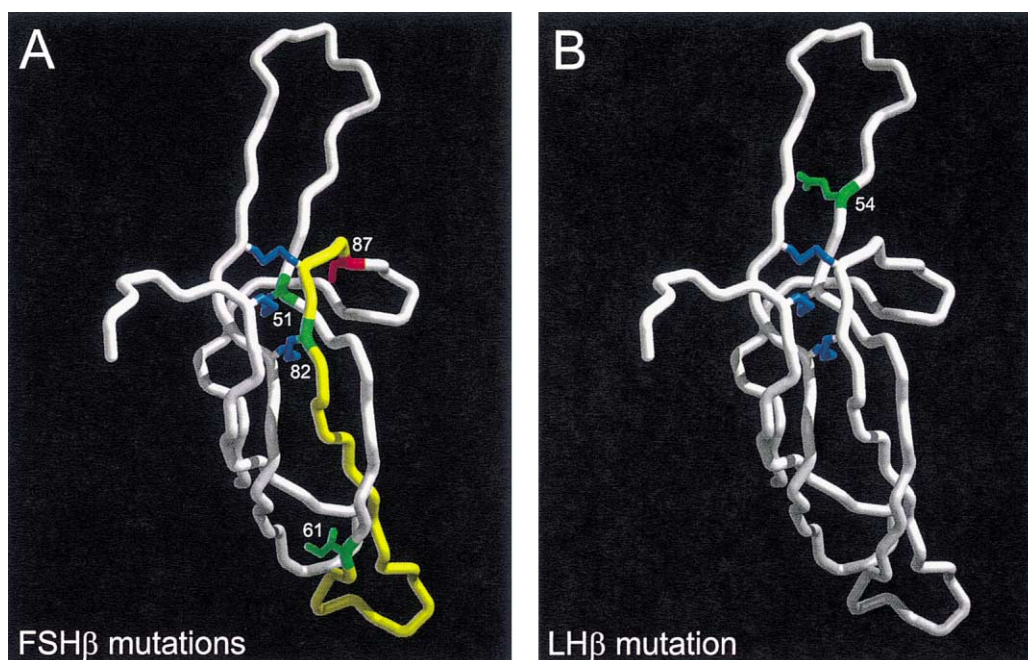


Fig. 2. Locations of reported mutations in the FSH and LH β -subunits. Identified mutations in hFSH β and hLH β were mapped onto the crystal structure of hCG β (Lapthorn et al., 1994) relative to the positions of the cysteine residues, which are fully conserved among the three β -subunits. Mutated residues are shown in green. Residue numbers (in white) refer to the position in the FSH and LH β -subunits rather than the number of the residue in the hCG structure. The three disulfide bridges involved in the ‘cysteine knot’ motif are shown in blue. In panel A, a 2 bp deletion at residue 61 changes the ensuing sequence (colored yellow) up to residue 86 and results in a stop codon at residue 87 (colored red).

Table 2
Phenotypic features of patients with single gene disorders causing impaired gonadotropin release or function^a

Gene	Associated features	Gonadotropins		Reproductive phenotype	
		LH	FSH	Sex	Features
<i>SFI</i>	XY sex reversal Müllerian structures Adrenal failure	N/↓	N/↓	XY	Gonadectomy performed
<i>DAX1</i>	Adrenal failure	N/↓	N/↓	M	Pubertal failure/HHG
<i>KAL</i>	Anosmia Renal agenesis Synkinesia	↓	↓	M	Pubertal failure/HHG
<i>GNRHR</i>	–	N/↓	N/↓	M F	Delayed puberty, hypogonadism, severe oligospermia Delayed puberty, variable thelarche, amenorrhea
<i>HESX1</i>	Septo-optic dysplasia Panhypopituitarism	↓	↓	M/F	Children prepubertal but HHG predicted
<i>LHX3</i>	GH, TSH, PRL Cervical spine rigidity	↓	↓	M/F	Pubertal failure/HHG
<i>PROP1</i>	↓ GH, TSH, PRL ? ACTH	N/↓	N/↓	M/F	Range from pubertal failure to delayed-onset HHG
<i>FSHβ</i>	–	↑	↓	M F	Normal/delayed puberty hypogonadism, azospermia Absent thelarche, primary amenorrhea
<i>LHβ</i>	–	↓	N/↑	M	Delayed puberty, arrested spermatogenesis

^a N, normal; GH, growth hormone; TSH, thyroid-stimulating hormone; PRL, prolactin; ACTH, adrenocorticotropin; M, male; F, female; HHG, hypogonadotropic hypogonadism. Additional associated features may occur as part of contiguous gene deletion syndromes. These features include Duchenne muscular dystrophy and glycerol kinase deficiency for *DAX1* (Xp21) and X-linked ichthyosis and chondrodysplasia punctata for *KAL* (Xp22).

achieved. Gonadotropin measurements at 44 years of age showed elevated LH (64.2 U/L) and FSH (113.6 U/L). Why FSH levels increased with age is unclear, but suggests features of Sertoli cell dysfunction.

Mutational analysis revealed a homozygous Q54R missense mutation in *LHβ*. This mutation affects an amino acid that is conserved in all β subunits of the glycoprotein hormones, and falls within a long loop in the protein that has been implicated in receptor binding (Fig. 2). Consistent with this, functional studies showed that the mutation does not interfere with hormone synthesis, heterodimerization, or immunoreactivity, but prevents binding of LH to its receptor (Weiss et al., 1992). Of note, three maternal uncles who are heterozygous for the mutation had a history of infertility despite a reportedly normal pubertal development. However, they were clearly not hypogonadal, and the proband's father was presumably a fertile obligate carrier, suggesting that the effects of heterozygosity on male fertility may be variable. Further, the fact that this patient had normal male external genitalia confirms that hCG can stimulate sufficient testosterone during the critical period of genital development in utero. This scenario contrasts with the abnormalities in genital development seen in males with inactivating mutations of the LH receptor.

No *LHβ* gene mutations have been described in females. Given the phenotype found in women with homozygous LH receptor mutations, amenorrhea or oligomenorrhea might be expected to occur following normal pubertal development. (Latronico et al., 1998).

Heterozygous S102G changes in the *LHβ* gene have been described in two women with infertility (Liao et al., 1998), and homozygous W8R and I15T changes have been found as population polymorphisms. The functional significance of these *LHβ* variants remains unclear.

6. Discussion

Although an increasing number of factors are associated with inherited disorders of gonadotropin release and function, relatively few patients have been identified with mutations in these genes. The most likely explanation for this is that genetic abnormalities that impair reproduction are rapidly excluded from the gene pool and do not lend themselves to easy identification based on studies of large pedigrees. However, the patients identified to date likely represent the most severe end of the phenotypic spectrum, and disorders associated with milder phenotypes may be more common. Identifying mutations in these patients should provide further information about the role of these transcription factors, hormones, and receptors in reproduction.

Associated clinical features can be very useful to guide the differential diagnosis and evaluation of patients with possible inherited gonadotropin deficiency disorders (Table 2). For example, anosmia or a family history of renal agenesis might point towards *KAL*. This diagnosis would be supported by other features of Kallmann syndrome or an X-linked pattern of transmission. Coin-

cident primary adrenal failure should suggest the possibility of mutations in *DAX1*, as these are relatively common because of X-linked transmission. Adrenal insufficiency associated with abnormal gonadal development should raise the possibility of *SF1*, *WT1*, *CYP11A1*, or *Star* mutations. If multiple pituitary hormone deficiencies are present, developmental transcription factors such as *PROPI*, *LHX3*, or *HESX1* should be considered. Isolated deficiencies of LH or FSH suggest specific mutations in the β -subunit genes.

Unfortunately GnRH testing, whether given as a bolus or in a pulsatile mode, does not provide a particularly discriminating test because of variable responses even among patients with similar genetic abnormalities. For example, some patients with *GNRHR* gene mutations show little response to exogenous GnRH, whereas others exhibit apparently normal LH and FSH secretion in response to pharmacologic testing. Similar variability is seen among patients with *DAX1* mutations, though most have blunted GnRH responses. Of note, individuals with GnRH deficiency (e.g. Kallmann syndrome) may show poor responses to initial GnRH testing but gradually develop robust responses to pulses of GnRH as the pituitary is primed. None the less, absent responses to repeated GnRH testing or pulses would argue against GnRH deficiency and point to an abnormality in the gonadotrope cell, including developmental defects, GnRH receptor abnormalities, or gonadotropin gene mutations.

With time, additional patients will be described with these and other genetic abnormalities of gonadotropin synthesis. As genetic testing becomes more available, it will be an important tool for the evaluation of these challenging diagnostic problems. Equally important is the need to include careful clinical and hormonal evaluation and family histories to restrict the number of genes that need to be tested.

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