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PERSPECTIVE

Genetic Causes of Human Reproductive Disease

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Introduction

Disorders of reproduction represent a significant social, medical, and economic burden for individuals and society. Approximately 1 in 10 couples in the United States are infertile, and each partner is equally likely to be affected (1). Although many causes of infertility can now be determined in both men and women, most couples still receive a diagnosis of idiopathic infertility. A subset of these patients is likely to have an underlying genetic disorder that is either inherited (germline) or acquired (somatic). Although the most severe genetic reproductive disorders cause dysgenetic gonads or abnormal hormonal profiles, milder phenotypes are being recognized with increasing frequency.

Over the past decade, many genes have been identified that influence the development and function of the hypothalamic-pituitary-gonadal (HPG) axis. These genes encode an array of transcription factors, matrix proteins, hormones, receptors, and enzymes that are expressed at multiple levels of the HPG axis, and regulate the complex developmental, paracrine, and endocrine interactions that are necessary for spermatogenesis and ovulation.

Identifying naturally occurring genetic mutations provides unique insight into the role that these factors play in the human HPG axis. In addition, defining the genetic basis of disease has significant benefits for the patients, as appropriate and educated counseling can be provided and treatment tailored to the individual. In this review, we focus on single gene mutations that affect the HPG axis in humans. Space limitations preclude discussion of the many chromosomal anomalies (*e.g.* XO, XXY), metabolic alterations (*e.g. GALT*), steroidogenic defects (*e.g. CYP21*), or activating mutations (*e.g.* Gsα, LH-receptor) that also can disrupt human reproduction. Progress resulting from the human genome project, along with advances in genomics and proteomics, will undoubtedly enhance the rate at which human reproductive mutations are found. Capitalizing on these scientific advances to improve patient care will be a major opportunity of the next decade.

GnRH release and action

Abnormalities in GnRH function can result from aberrant neuronal migration, defective synthesis and release of GnRH, or mutations in the GnRH receptor. The clinical response to pulsatile GnRH therapy is often useful to discriminate defects in hormone synthesis *vs.* action (Fig. 1 and Table 1).

KAL and Kallmann syndrome (KS). During development, GnRH-releasing neurons originate in the olfactory placode and migrate with olfactory neurons through the cribiform plate to the olfactory bulb and into the fetal hypothalamus. Abnormalities in these migratory processes explain the association of hypogonadotropic hypogonadism (HH) with anosmia (absent sense of smell) in patients with KS (2).

Mutations or deletions in the gene *KAL* cause the X-linked form of KS (2, 3). *KAL* encodes an extracellular matrix glycoprotein, anosmin-1, which facilitates neuronal growth and migration. Most *KAL* mutations affect the putative fibronectin repeat region of the anosmin-1 and interfere with the migration of olfactory and GnRH neurons into the olfactory bulb. The migrational arrest of GnRH neurons within the meninges has been reported in a study of a 19-wk human fetus with X-linked KS, and olfactory bulb agenesis or hypoplasia has been detected by MRI in some patients with this condition.

Most patients with X-linked KS have a micropenis and bilaterally undescended testes at birth, reflecting congenital GnRH and gonadotropin insufficiency. HH becomes apparent in adolescence as a failure of pubertal development. Consistent with the hypothalamic defect, patients with X-linked KS typically respond to pulsatile GnRH priming with an increase in gonadotropin release over several days, and pulsatile GnRH has been used successfully to induce fertility in patients with *KAL* mutations (4). However, recombinant gonadotropins are also effective and are easier for most physicians to administer.

KAL/anosmin-1 is also expressed in the developing Purkinje cells of the cerebellum, meso-, and meta-nephros, oc-

Abbreviations: AHC, Adrenal hypoplasia congenita; AMH, anti-Mullerian hormone; CPHD, combined pituitary hormone deficiency; DAX1, dosage sensitive sex reversal-AHC critical region on the X chromosome, gene 1; DHT, dihydrotestosterone; hCG, human CG; *HESX1*, homeobox gene expressed in ES cells; HH, hypogonadotropic hypogonadism; HPG, hypothalamic-pituitary-gonadal; KS, Kallmann syndrome; LHX3, Lim homeobox gene 3; PROP-1, Prophet of Pit-1; SF1, steroidogenic factor-1; SOX, SRY-related HMG-box gene; *Sry*, sex-determining region Y.

ulomotor nucleus, and facial mesenchyme, explaining the association of X-linked KS with synkinesia (mirror image movements), renal agenesis, visual abnormalities and midline facial defects (5). Unilateral renal agenesis is common, and may be present in family members in the absence of anosmia or HH. This variable penetrance of features is common in families with *KAL* mutations, suggesting that modifier genes or epigenetic phenomena influence phenotypic expression (4). Furthermore, the association of anosmia with HH due to an apparently autosomal dominant or recessive mode of inheritance in some families indicates that additional genes are involved in GnRH neuronal migration (6).

Obesity, metabolism, and reproduction. Although associations between obesity, metabolism, and reproduction have been proposed for many years, these complex interactions are now beginning to be unraveled. For example, direct evidence for the role of leptin in reproductive function is provided by the HH seen in patients with obesity due to mutations in leptin (7) or the leptin receptor (8), as well as the *ob/ob* mouse. It appears likely that leptin facilitates HPG activity through its central action on GnRH release. Recombinant leptin therapy has been used successfully to induce pulsatile gonadotropin activity and puberty, as well as weight loss, in a girl with congenital leptin deficiency (9). Preliminary data suggest that leptin treatment does not induce premature puberty in younger children. Therefore, leptin appears to be necessary but not sufficient for pubertal development.

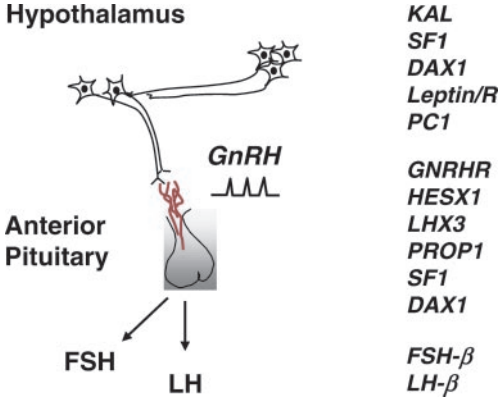

FIG. 1. Overview of the hypothalamic-pituitary (gonadotrope) axis. Mutations in the genes listed in *italics* have been shown to cause HH in humans.

Mutations in the endopeptidase, prohormone convertase-1, have been described in association with obesity, HH and hypocortisolemia (10). Prohormone convertase-1 regulates posttranslational modification of prohormones and neuropeptides, but it is unclear whether the reproductive defects reported result from impaired GnRH processing, abnormalities in neuropeptides related to GnRH secretion, or an alternative mechanism.

Recently, hypothalamic-gonadotrope dysfunction has been reported in female mice with targeted deletion of insulin-related substrate-2, -4, or tissue-specific (neuronal) deletion of the insulin receptor. Although HH has not been reported in patients with insulin receptor mutations, these signaling systems are likely to play an important role in human reproductive function. The relationship of insulin resistance, increased LH secretion, and hyperandrogenemia in polycystic ovary syndrome remain enigmatic but also suggests an interplay between insulin action and reproduction. It is notable that patients with monogenic obesity due to mutations in POMC or the melanocortin-4 receptor do not have reproductive abnormalities, suggesting that the melanocortin system regulates appetite and body weight without influencing the HPG axis.

GnRH and the GnRH receptor. No human *GnRH* mutations have been reported, although some rare cases of hypogonadism have been described in patients with 8p deletions and the *hpg/hpg* mouse is hypogonadal due to a GnRH gene deletion.

An increasing number of GnRH receptor mutations have been described. It currently appears that up to 20% of patients with idiopathic HH may have mutations in this receptor (11–13). Most of these GnRH receptor mutations are compound heterozygous changes that reduce GnRH binding and/or activation of IP3 or PLC signaling pathways. At present, R262Q mutations within the third intracellular loop and Q106R mutations in the first extracellular loop of the GnRH receptor occur most commonly.

The clinical features of patients with *GNRHR* mutations are highly variable, even within the same kindred. At the most severe end of the spectrum, complete loss of function

TABLE 1. Single gene disorders in the HPG axis resulting in HH or abnormal gonadotropin release in humans

Gene	Locus	Inheritance	Associated features
KAL	Xp22	X-linked	Anosmia, renal agenesis, synkinesia, cleft lip/palate, oculomotor/visuospatial defects, gut malrotations
Leptin/R	7q31/1p31	AR	Obesity
PC1	5q15–21	AR	Obesity, hypocortisolemia
GNRHR	4q21	AR	
HESX1	3p21	AR	Septo-optic dysplasia, CPHD
		AD	Isolated GH insufficiency
LHX3	9q34	AR	CPHD (ACTH spared), cervical spine rigidity
PROP1	5q35	AR	CPHD (ACTH usually spared)
FSHβ	11p13	AR	↑ LH
LHβ	19q13	AR	↑ LH (bioinactive), (↑ FSH)
SFI (NR5A1)	9p33	AD/AR	Primary adrenal failure, XY sex reversal, uterus
DAX1 (NROB1)	Xp21	X-linked	Primary adrenal failure, impaired spermatogenesis

AD, Autosomal dominant; AR, autosomal recessive. Other features may be seen in patients with contiguous gene deletion syndromes of Xp21 and Xp22 (*e.g.* Xp21: DAX1, glycerol kinase deficiency and Duchenne muscular dystrophy; Xp22: KAL, chondrodysplasia punctata and X-linked ichthyosis).

mutations (*e.g.* A129D, S168R) cause microphallus and cryptorchidism in males, complete failure of pubertal development, and resistance to pulsatile GnRH treatment (14). In contrast, milder loss of function mutations cause partial GnRH resistance. Basal gonadotropins are detectable in these patients and a modest gonadotropin response is seen after bolus GnRH stimulation. Affected females can have spontaneous thelarche and normal breast development, and high-dose pulsatile GnRH has been used to induce ovulation in one woman with partial GnRH resistance (15). Affected males may exhibit incomplete pubertal development. Larger numbers of patients with impaired fertility and detectable gonadotropins need to be evaluated to determine the true prevalence of mild GnRH receptor mutations.

Anterior pituitary development

Mutations in several genes that regulate gonadotrope differentiation and function have been described in patients with combined pituitary hormone deficiency (CPHD) [*e.g.* homeobox gene expressed in ES cells (*HESX1*), Lim homeobox gene 3 (*LHX3*), Prophet of Pit-1 (*PROP1*)], but no mutations in gonadotrope-specific genes have been described yet (*e.g. Egr1*) (Fig. 1, Table 1).

HESX1. Targeted mutagenesis of the homeodomain transcription factor *Hesx1* in mice causes anterior central nervous system defects and pituitary dysplasia, and a homozygous R53C missense mutation in the DNA-binding region of *HESX1* has been reported in two children with septo-optic dysplasia and CPHD (16). Although these children are prepubertal, investigations show impaired gonadotropin release, and spontaneous pubertal development is unlikely. Heterozygous mutations in other regions of HESX1 are associated with the milder phenotype of isolated GH insufficiency (17).

LHX3. Targeted mutagenesis of *Lhx3/Lim3* causes panhypopituitarism and early death in homozygous knockout mice. Homozygous LHX3 mutations have been found in four patients from two families with features of CPHD (but preserved corticotrope function) and limited head rotation due to cervical spine rigidity (18). One boy had cryptorchidism and micropenis at birth, and the three oldest patients failed to show signs of pubertal development by 15 yr of age and there was evidence of impaired gonadotropin release. Notably, patients with mutations in the related transcription factor LHX4 have CPHD with preserved reproductive function.

PROP1. PROP1 was considered a candidate gene for pituitary dysfunction following characterization of the Ames dwarf (*df*) mouse (S83P), and mutations in this transcription factor have now been reported in more than 50 families with CPHD (19). Most patients with *PROP1* mutations develop GH and TSH deficiency in childhood. The onset of gonadotropin insufficiency is variable, even within families with the same mutation; some patients demonstrate pubertal failure in adolescence whereas others develop hypogonadism or secondary amenorrhea later in life (20). Although corticotrope function is usually preserved, progressive ACTH insufficiency and hypocortisolemia have been reported in several kindred.

Gonadotropins and gonadotropin receptors

The gonadotropins are heterodimers consisting of specific β-subunits that are noncovalently bound to a common α-subunit. No human α-subunit mutations have been reported, although disruption of all the glycoprotein hormones, including TSH and human CG (hCG), would be expected. Because hCG plays an essential role in the maintenance of pregnancy in humans, a severe loss of function mutation in the α-subunit is unlikely to be found. A number of mutations in the FSHβ and LHβ subunits and the gonadotropin receptors have been reported (for review, see Ref. 21).

FSHβ and the FSH receptor. Mutations in *FSHβ* have been reported in three women with delayed puberty, absent breast development, and primary amenorrhea (22, 23). These homozygous or compound heterozygous mutations often affect the seatbelt region of the protein, and interfere with the synthesis and stability of the heterodimer complex. Consequently, patients were found to have undetectable serum FSH and elevated serum LH. Normal primordial follicles were detected and, in two patients, exogenous FSH treatment resulted in follicular maturation, ovulation, and fertility. This ovarian phenotype suggests that FSH is not necessary for primordial follicle development, but it is required for antral development and granulosa cell estrogen production, as reported following targeted disruption of FSHβ in female mice.

A block in folliculogenesis is also seen in female FSH receptor knockout mice, and a similar but less severe phenotype results from homozygous mutations in the FSH receptor in humans (24). The highest prevalence of these mutations is in Finland, where that A189V mutation is associated with variable pubertal failure and primary or secondary amenorrhea is reported. A milder clinical phenotype associated with a compound heterozygous mutation has also been described, suggesting that subtler clinical phenotypes exist (25).

The role of FSH in spermatogenesis is less clear. Male FSHβ and FSH receptor knockout mice are fertile despite having reduced testicular volume and partial spermatogenic failure, and men with FSH receptor mutations have variable spermatogenic defects (26). In contrast, *azoospermia* has been reported in two men with FSHβ gene mutations (27). Additional cases of FSHβ and FSH receptor mutations are needed, therefore, to clarify the role of FSH in spermatogenesis in humans.

LHβ and the LH receptor. Only one human *LHβ* gene mutation has been reported to date in a man with delayed puberty, low testosterone, and arrested spermatogenesis (28). This individual presented with elevated serum immunoreactive LH on presentation. However, a homozygous Q54R missense mutation in the long loop of *LHβ* impaired binding to its receptor. Long-term hCG treatment resulted in testicular enlargement, virilization, and an increase in sperm count, but fertility was not achieved. The normal development of male external genitalia in this patient likely reflects the *in utero*

action of hCG on the LH receptor to generate androgens during the critical period of genital development.

The clinical phenotype in this patient contrasts with that seen in patients with inactivating mutations in the LH receptor, who are unable to respond to either LH or hCG to produce sufficient testosterone [and dihydrotestosterone (DHT)] for virilization of the male external genitalia (29). Phenotypes associated with LH receptor mutations range from complete failure of virilization to hypospadias, micropenis, or absent puberty and infertility, reflecting variable residual receptor function. An absence of mature Leydig cells and an arrest of spermatogenesis at the spermatid stage has been reported, and this diagnosis should be considered in patients who are diagnosed with Leydig cell hypoplasia and infertility.

Women with homozygous LH receptor mutations have been identified within these families (30). These patients have oligomenorrhea or amenorrhea despite normal pubertal development, highlighting the importance of the LH receptor in ovulation. No homozygous *LHβ* gene mutations have been described in females, although heterozygous *LHβ* gene changes have been described in two women with infertility, and homozygous changes (W8R, I15T) with an extra glycosylation consensus site (Asn(13)-Ala-Thr) have been described as population polymorphisms. LHβ and the LH receptor therefore remain candidate genes for abnormalities in a subset of women with infertility.

Testis determination, differentiation, function, and spermatogenesis

The development of a 46XY fetus into a fertile, phenotypic male is a dynamic process that requires: development of the bipotential gonad into a testis (testis determination); differentiation of the Leydig and Sertoli cells within the testis; androgen biosynthesis by the Leydig cells to virilize the external genitalia and support development of Wolffian structures (*e.g.* prostate); anti-Mullerian hormone [AMH (Müllerian-inhibiting substance)] production by Sertoli cells to regress Müllerian structures (*e.g.* uterus and upper vagina); testicular descent; and migration of germ cells into the developing gonad leading to spermatogenesis at the time of puberty. Single gene mutations affecting each of these processes have been described in patients with disorders of sexual development or infertility (Fig. 2 and Table 2) (for reviews, see Refs. 31 and 32).

Testis determination and differentiation. The testis determining gene, *Sry*, was first identified and characterized just over a decade ago (31). Since then, mutations and deletions in several genes involved in the pathways of testis determination/differentiation have been described in patients with testicular dysgenesis and complete or partial 46XY sex reversal [*e.g.* Wilms' tumor 1, steroidogenic factor-1 (*SF1*), *SRY*, SRY-related HMG-box gene (*SOX9*), α-thalassemia/mental retardation syndrome, X-linked, desert hedgehog]. Associated features are often present (*e.g.* adrenal failure (*SF1*); renal dysfunction and tumors (*WT1*) (Denys-Drash syndrome or Frasier syndrome), campomelic dysplasia (*SOX9*); see Table 2). Gonadal dysgenesis (46XY karyotype) has also been described in patients with chromosomal rearrangements involving deletion of candidate genes for testis development (*e.g.* Doublesex- and MAB3-related transcription factor 1/2, *SOX8*) or duplication of loci containing antitestis genes (*e.g.* Dosage sensitive sex reversal-AHC critical region on the X chromosome, gene 1 (*DAX1*), Wingless-type MMTV integration site family, member 4) (33, 34). Taken together, these reports highlight the exquisite sensitivity of human gonadal development to gene dosage effects.

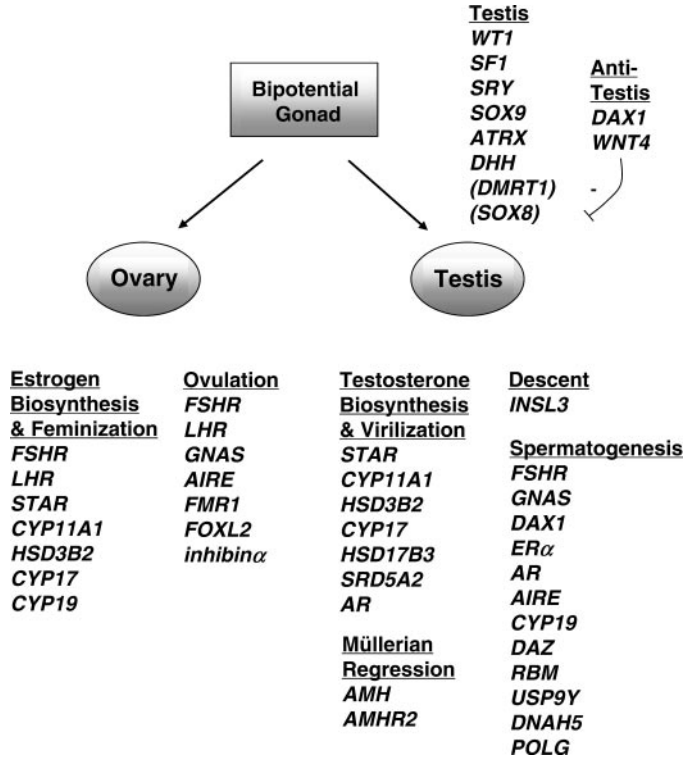

FIG. 2. Overview of the development and function of the gonad. Mutations in the genes listed in italics have been reported in humans. Overexpression of the antitestis genes, DAX1 and WNT4, affect testis development in 46XY individuals, whereas overexpression or inappropriate expression of the testis genes, SRY and SOX9, affect ovarian development in individuals with a 46XX karyotype.

Sex hormone biosynthesis and action. DHT is the most potent androgen implicated in virilization of the developing male fetus, and impaired masculinization has been reported in individuals with a range of steroid biosynthetic defects (*e.g.* mutations in steroidogenic acute regulatory protein, P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase type 2; 17α-hydroxylase/17,20-lyase, 3β-hydroxysteroid dehydrogenase type 3). Patients who are unable to convert testosterone to DHT due to mutations in the gene encoding 5α reductase (*SRD5A2*) usually show undervirilization at birth but may develop limited phallic growth in adolescence due to expression of an alternate isoform of the enzyme at this time.

Mutations in the AR have been described in more than 200 patients with complete or partial forms of the androgen insensitivity syndrome (for the Androgen Receptor Database, see http://www.mcgill.ca/androgendb/). These mutations affect the actions of both T and DHT. Milder loss of function mutations have been reported in patients with im-

TABLE 2. Single gene disorders causing impaired gonadal development and function in humans

Gene	Locus	Inheritance	Associated features
Testis determination and differentiation (causing 46XY female or ambiguous genitalia)			
WT1	11p13	AD	DDS: diffuse mesangial sclerosis, Wilms' tumor, ambiguous FS: FSGS, early renal failure, gonadoblastoma, XY female
SF1 (NR5A1)	9p33	AD/AR	Primary adrenal failure, uterus
SRY	Yp11.3	Y	
SOX9	17q24.3–25.1	AD	Campomelic dysplasia
ATRX	Xq13.3	X-linked	Mental retardation, α-thalassemia
DHH	12q13.1	AR	Minifascicular neuropathy
Sex hormone biosynthesis and action (causing 46XY female or ambiguous genitalia)			
SF1 (NR5A1)	9p33	AD/AR	Primary adrenal failure, uterus
LHR	2p21	AR	Leydig cell hypoplasia
STAR	8p11.2	AR	Primary adrenal failure
CYP11A1	15q23–24	AD	Primary adrenal failure
HSD3B2	1p13.1	AR	Primary adrenal failure
CYP17	10q24–25	AR	Postnatal virilization, hypertension
HSD17B3	9q22	AR	Partial virilization at puberty
SRD5A2	2p23	AR	Partial virilization at puberty
AR	Xq11–12	X-linked	
Müllerian regression and testicular descent			
SF1 (NR5A1)	9p33	AD/AR	Primary adrenal failure, 46XY female
AMH	19p13.3–13.2	AR	Uterine structures
AMHR2	12q13	AR	Uterine structures
INSL3	19p13.2	AD	Undescended testes
Spermatogenesis			
FSHR	2p21-p16	AR	Variable impaired spermatogenesis
GNAS	20q13.1	AD(imprinted)	Pseudohypoparathyroidism, hormone resistance
DAX1	Xp21	X-linked	Primary adrenal failure, HH
ERα	6q25	AR	Tall stature, delayed epiphyseal fusion
CYP19	15q21	AR	Maternal virilization during pregnancy
AR	Xq11–12	X-linked/CAG	-
AIRE	21q22.3	AR	Autoimmune polyendocrinopathy syndrome type I
DAZ	Yq11	Y	
RBMY	Yq11	Y	
USP9Y	Yq11.2	Y	
DNAH5	5p15-p14	AR	Primary ciliary dyskinesia, left-right asymmetry
POLG	15q25	CAG	-
Ovarian development and function (not including steroid biosynthetic defects)			
FSHR	2p21-p16	AR	Variable pubertal development
LHR	2p21	AR	Normal puberty, amenorrhea
GNAS	20q13.1	AD(imprinted)	Pseudohypoparathyroidism, hormone resistance
AIRE	21q22.3	AR	Autoimmune polyendocrinopathy syndrome type I
FMR1	Xq27.3	CAG	
Inhibin-α	2q33–36	AR	
FOXL2	3q23	AD	Bletharophimosis-ptosis-epicanthus inversus

AD, Autosomal dominant; AR, autosomal recessive; CAG, differences in trinucleotide repeat sequences; DDS, Denys-Drash syndrome; FS, Frasier syndrome; FSGS, focal and segmental glomerulosclerosis; DHH, desert hedgehog; LHR, LH receptor; STAR, steroidogenic acute regulatory protein; CYP11A1, P450 side-chain cleavage; HSD3B2, 3β-hydroxysteroid dehydrogenase type 2; CYP17, 17α-hydroxylase/17,20-lyase; HSD17B3, 3β-hydroxysteroid dehydrogenase type 3; SRD5A2, 5α-reductase; AR, androgen receptor INSL3, insulin-like 3/relaxin-like factor; FSHR, FSH receptor; CYP19, aromatase.

paired spermatogenesis alone (36). Furthermore, abnormalities in AR specific coregulators may result in a similar clinical phenotype in patients with normal AR gene sequence and function (37).

The importance of estrogen for male fertility has been demonstrated by the reduced sperm viability seen in a patient with a mutation in the ERα (as well as ERα knockout mice), and potentially disordered testicular development and spermatogenesis in disorders of aromatase action (for review, see Ref. 38).

Müllerian regression and testicular descent. Mutations in *AMH* and the *AMHR2* have been reported in males with preserved Müllerian structures. Mutations in insulin-like 3/relaxin-like factor have been described recently in several patients with impaired testicular descent (39).

Spermatogenesis. Spermatogenesis is a complex process that requires normal germ cell migration, testicular development, and Sertoli cell function, as well as appropriate maturation, motility, and penetration of the sperm. Impaired spermatogenesis and infertility is a feature of many transgenic animals. Thus, a host of candidate genes exist for impaired spermatogenesis in humans. Microdeletions of the Y chromosome (including RNA-binding motif protein and Deleted in azoospermia) have been found in a significant proportion of men with azoospermia (for review, see Ref. 40), and specific point mutations in the Ubiquitin-specific protease 9, Y

chromosome have been described (41). In addition, mutations in dynein, axonemal, heavy chain 5 and dynein, axonemal, intermediate chain 1 have been reported recently in patients with infertility associated with primary ciliary dyskinesia and left-right asymmetry (42), and differences in the length of a trinucleotide repeat within the mitochondrial gene *POLG* may be linked to spermatogenic function in the population.

Ovarian development and function

Although specific ovarian determining genes are yet to be identified, overexpression (or inappropriate expression) of testis-specific genes (*e.g. SRY*, *SOX9*) in 46XX individuals can result in the development of testicular tissue and virilization. Disorders of steroid biosynthesis that affect ovarian estrogen production will interfere with pubertal development and ovulation. However, in some situations (*e.g.* mutations in *STAR*) sufficient estrogen can be synthesized to allow partial pubertal development (43). No ER (α/β) mutations have been reported in females, although these would be expected to impair reproductive function.

Premature ovarian failure (POF) is occasionally familial, suggesting a genetic basis in some cases. POF loci have been identified at Xq13.3-q21.1 (POF2) and Xq26.2-q28 (POF1), possibly involving breakpoint deletions in fragile site mental retardation 2 or *DIAPH2*. Premature ovarian failure may also be associated with trinucleotide repeat differences (premutations) in fragile site mental retardation 1 (*FRAXA*) and mutations/polymorphisms in the inhibin-α subunit (44), as well as due to point mutations in the forkhead transcription factor foxl2 in the blepharophimosis-ptosis-epicanthus inversus syndrome (45), the autoimmune regulator 1 gene in autoimmune polyendocrinopathy syndrome type I, and guanine nucleotide-binding protein α-stimulating activity polypeptide 1 in pseudohypoparathyroidism (for a review of primary hypogonadism in females, see http://www.uptodate.com/html/J Clin Endocrinol Metab./december/topics/48781). It is likely that many more mutations in genes involved in oogenesis, follicular maturation, and cell survival/apoptosis will be identified in women with infertility.

Nuclear receptors expressed throughout the reproductive axis: SF1 and DAX1

SF1 (NR5A1) and DAX1 (NR0B1) are two orphan nuclear receptors that are expressed in the hypothalamus, gonadotropes, gonads, and adrenals, and regulate the development and function of the HPG axis at multiple levels. Studies in mice as well as humans have confirmed the crucial role that these transcription factors play in reproductive function and fertility.

SF1. SF1 regulates the transcription of an array of genes involved in male sexual differentiation, Müllerian regression, testicular descent, steroidogenesis, and reproduction (47). Homozygous deletion of *Sf1* (−/−) in mice results in complete gonadal and adrenal agenesis, phenotypic XY sex-reversal with persistence of Müllerian structures in males, abnormalities in the development of the VMH and gonadotropes, and obesity. Heterozygous animals have a milder adrenal phenotype. Furthermore, mice with gonadotrope-specific deletion of *Sf1* have impaired gonadotropin release and hypogonadism but respond to gonadotropin treatment, confirming the role of Sf1 in pituitary function.

SF1 mutations have been reported in two patients with complete XY sex-reversal, testicular dysgenesis, Müllerian structures, and primary adrenal failure. In the first patient, a *de novo* heterozygous G35E mutation was identified in the P-box of the first zinc finger of SF1 (48). This motif is known to be a crucial determinant of DNA binding specificity, and the mutation affects SF1 binding and transactivation of many target genes (49). Recently, a homozygous R92Q mutation was identified in the A-box of SF1, a region known to stabilize binding by nuclear receptors that function as monomers (50). Heterozygous carriers have normal fertility. These cases confirm that SF1 plays a major role in the development and function of the HPG axis in humans and reveal the importance of functional gene dosage when one factor regulates many genes in the reproductive axis (49, 50). However, the precise role of SF1 in human puberty is less clear as the first patient had a gonadectomy, and the second patient did not survive beyond infancy.

A heterozygous mutation in SF1 has also been described in a girl with primary adrenal failure (51). The presence of ovaries in this patient suggests that SF1 is not necessary for ovarian differentiation. Whether this mutation in SF1 will impair estrogen biosynthesis, thereby preventing the development of puberty, remains to be seen.

DAX1. Mutations or deletions in this atypical orphan nuclear receptor cause X-linked adrenal hypoplasia congenita (AHC). DAX1 mutations have now been described in almost 100 families with AHC (52). Affected boys usually present with primary adrenal failure in infancy or early childhood. Although the HPG axis is often intact in infancy, HH becomes apparent at the expected time of puberty. Several studies have shown that the HH associated with this condition represents defects at the level of the pituitary gonadotropes as well as the hypothalamus, consistent with the expression of DAX1 in both of these structures (53). Consequently, pulsatile GnRH therapy is often ineffective. In addition, studies of the adrenal hypoplasia congenita and hypogonadism (*Dax1*) knockout mouse have revealed a crucial role for Dax1 in testis development and spermatogenesis. Limited data from patients with X-linked AHC suggest that DAX1 is involved in spermatogenesis in humans too, and the response to gonadotropin therapy is usually ineffective (54, 55).

Most patients with X-linked AHC have nonsense or frameshift mutations that truncate the carboxyterminus of DAX1 and severely impair its function as a transcriptional repressor. Missense mutations in DAX1 are less common, and cluster within the putative ligand-like binding domain (56). Several patients with variant phenotypes associated with DAX1 mutations have been reported. For example, extreme delayed puberty has been described in the female carriers of DAX1 mutations in one family (54), and HH in the absence of adrenal dysfunction has been reported in a woman who is homozygous for a truncation mutation in DAX1 through gene conversion (57). Although analysis of *DAX1* in more

than 100 patients with idiopathic HH failed to detect any mutations (58), two patients have now been reported who presented with partial HH (4–5 ml testes) and delayed-onset adrenal failure in adulthood (55, 59). The missense mutations found in these patients (Y380D, I439S) show partial loss of function in transient gene expression assays, consistent with the mild clinical phenotype. These reports again highlight the importance of gene dosage effects in the reproductive axis (60) and indicate that patients with variant phenotypes may present after childhood.

Summary

The genetic mutations described in patients with reproductive disorders have provided important insight into the transcription factors, receptors, and hormones that regulate the HPG axis in humans. These mutations can affect development and function of the HPG axis at many levels. Laboratory investigations and associated features can help to focus on a gene of interest in some cases, but it is likely that these reports represent the most severely affected individuals; less severe loss of function mutations may be manifest as milder clinical phenotypes (*e.g.* DAX1, LH receptor). Thus, the true prevalence of these genetic abnormalities in patients with reproductive dysfunction or infertility is not known.

A major challenge in this field is that mutations lead to infertility, thereby limiting the investigator's ability to use traditional genetic linkage and association studies to identify candidate genes. However, the human genome project is starting to have a major impact on strategies used to identify genetic mutations. The density of polymorphic markers, such as single nucleotide polymorphisms, is increasing rapidly, allowing better gene mapping. In addition, easy access to the structure of genes known to be involved in reproductive disorders is allowing high-throughput screening of candidate genes. A large number of genes involved in reproduction are being identified in transgenic and gene knockout mice. As these phenotypes are characterized more thoroughly, it may be possible to better predict candidate genes in humans based on characteristic hormonal and histologic features of particular mutations. Gene microarrays have the potential to provide gene expression fingerprints associated with specific types of genetic disorders.

Finally, it is important to translate advances in genetics into improved clinical management. In addition to genetic counseling, it may be possible to direct selected patients to various forms of assisted reproduction such as intracytoplasmic sperm injection for spermatogenic defects or *in vitro* fertilization for ovulatory dysfunction. The paradigm of using reproductive physiology and pathophysiology to develop new treatments has a track record of success. In a relatively short period of time, our understanding of the physiologic role of gonadotropins has been used to create recombinant gonadotropins, which are now commonly used to facilitate reproduction. By analogy, the discovery of additional key regulators of gonadal development and gametogenesis may provide additional therapeutic tools for enhancing reproductive function.

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