The Molecular Basis of Impaired Follicle-Stimulating Hormone Action: Evidence From Human Mutations and Mouse Models

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Abstract

The pituitary gonadotropin follicle-stimulating hormone (FSH) interacts with its membrane-bound receptor to produce biologic effects. Traditional functions of FSH include follicular development and estradiol production in females, and the regulation of Sertoli cell action and spermatogenesis in males. Knockout mice for both the ligand (*Fshb*) and the receptor (*Fshr*) serve as models for FSH deficiency, while *Fshb* and *Fshr* transgenic mice manifest FSH excess. In addition, inactivating mutations of both human orthologs (*FSHB* and *FSHR*) have been characterized in a small number of patients, with phenotypic effects of the ligand disruption being more profound than those of its receptor. Activating human *FSHR* mutants have also been described in both sexes, leading to a phenotype of normal testis function (male) or spontaneous ovarian hyperstimulation syndrome (females). As determined from human and mouse models, FSH is essential for normal puberty and fertility in females, particularly for ovarian follicular development beyond the antral stage. In males, FSH is necessary for normal spermatogenesis, but there are differences in human and mouse models. The *FSHB* mutations in humans result in azoospermia; while *FSHR* mutations in humans and knockouts of both the ligand and the receptor in mice affect testicular function but do not result in absolute infertility. Available evidence also indicates that FSH may also be necessary for normal androgen synthesis in males and females.

Keywords

Follicle-stimulating hormone (FSH), FSH receptor, isolated FSH deficiency, *FSHB* gene, *FSHR* gene, *FSHB* knockout mouse, *FSHB* transgenic mouse, *FSHR* knockout mouse, *FSHB* mutations, *FSHR* mutations, ovarian hyperstimulation syndrome

Introduction

The coordination of the hypothalamic–pituitary–gonadal (HPG) axis is regulated by gonadotropin-releasing hormone (GnRH) action. Intriguingly, GnRH neurons originate outside the brain and migrate alongside olfactory nerves to reach the hypothalamus.^{1,2} The GnRH neurons from the arcuate nucleus within the hypothalamus secrete GnRH in a pulsatile manner into the hypophyseal portal system, where it acts on its cell surface receptor in the anterior pituitary.³ In turn, pituitary gonadotropes respond by increasing the production of the common α -subunit and specific β -subunits of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These assembled α - β dimers of FSH and LH exert their principal function in the gonads, where gametogenesis, folliculogenesis, and steroidogenesis occur. The steroid hormones, estradiol (E2) and progesterone (P4) in females, and testosterone in males, feedback to both the hypothalamus and pituitary to modulate the production of GnRH and the gonadotropins. The recently characterized gonadotropin inhibitory hormone (GnIH), also known as RFRP-3, inhibits gonadotropin expression and serum

levels and is likely to have an important regulatory function.⁴ The HPG axis is central to normal pubertal development and reproductive capacity, as the onset of puberty is accomplished through an activation of the GnRH pulse generator with FSH playing a vital role in reproductive maturation and function.³

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The FSH ligand is produced principally from the anterior pituitary and targets its receptor found on the cell surface of granulosa cells of the ovary and Sertoli cells of the testes.⁵ The ovarian response to FSH induces aromatase, converting androgens to estrogens and inducing follicular maturation. In addition, the hormone inhibin B is produced by the granulosa cells and exerts negative feedback upon FSH. In the testes, FSH upregulates Sertoli cells and along with testosterone plays a key role in spermatogenesis.⁵ Mutations of both the *FSH β* gene (*FSHB*) and its receptor (*FSHR*) have been reported in humans; and the corresponding knockouts (KOs) have been generated in mice. These recent discoveries have furthered the understanding of FSH signaling and metabolism, important for its effect upon sexual maturation and reproduction. The purpose of this review is to evaluate all of the existing literature on these very valuable “KOs of nature” in humans with mutations of *FSHB* and *FSHR*, as well as the corresponding KO and transgenic mice. Since activating *FSHR* mutations have also been described in humans, the phenotypic findings from excess FSH can be examined and compared with transgenic mouse overexpression models. Findings from these studies will then be incorporated into the understanding of FSH action in mammals as much has changed since we last summarized the literature more than a decade ago.⁵

The Human FSH Ligand

A dimeric pituitary glycoprotein hormone, FSH is composed of an α -subunit and a β -subunit, which are both expressed in the gonadotropes of the pituitary gland.⁶ The α -subunit is encoded by a single gene (*CGA*), which is common to FSH, LH, human chorionic gonadotropin (hCG), and thyroid-stimulating hormone (TSH).⁷ The α -subunit protein is noncovalently joined to the β -subunit to form a biologically active hormone, but it is the β -subunit that confers hormone specificity. The β -subunit is structurally similar to the members of the cysteine knot GF family.⁶

The 9.6 kb human *CGA* gene is localized to chromosome 6q14.3 and has 2 splice variants. Variant NM_001252383 represents the longer transcript and encodes the longer isoform, containing 5 exons, yielding a 147 amino acid protein that is N-linked glycosylated at positions 107 and 133 (Asn52 and Asn78 of the mature protein).⁶ The signal peptide is 24 amino acids and the mature protein 123 amino acids. The shorter variant NM_000735 encodes 116 amino acids and lacks an alternate in-frame exon 3 that the longer variant possesses. This protein has the same 24 amino acid signal peptide and a 92 amino acid mature protein. Tertiary structure of the α -subunit is provided by 5 disulfide bonds formed via 10 Cys residues, which are completely conserved across species.

The 4.26 kb human *FSHB* gene on chromosome 11p14.1 is composed of 3 exons (Figure 1), which encode a 129 amino acid FSH β -subunit (an 18 amino acid signal peptide and a 111 amino acid mature protein).⁸ The single *FSHB* gene possesses several unique characteristics compared to the other gonadotropin β -subunit genes.⁸ Alternative splicing of the first exon, which encodes the 5' untranslated regions, renders this small exon

Figure 1. The structure of the human *FSHB* gene is shown with all known mutations, all of which reside within exon 3. The 3 exons are indicated as to whether they are translated or untranslated. The blue circles beside each mutation indicate the number of times the allele has been reported in a family with isolated FSH deficiency (note: there may be more than 1 affected member within the family). FSH indicates follicle-stimulating hormone. See online version for color reference.

either 33 or 63 nucleotides in length.⁸ Exon 2 encodes the 18 amino acid signal peptide followed by the first 35 residues of the mature β -subunit, while the third exon encodes amino acids 54 to 129 and the 3' untranslated region. Therefore, 2 different-sized transcripts (*FSHB*-NM_000510 and NM_001018080) are produced from alternative splicing of exon 1 and the presence of 2 different potential sites of polyadenylation; however, each results in the translation of the same FSH β protein. The FSH β -subunit possesses 2 N-linked glycosylation sites at Asn25 and Asn42 of the total protein (Asn7 and Asn24 of the mature protein) as well as 12 highly conserved Cys residues that form 6 disulfide bonds including Cys21-Cys69, Cys35-Cys84, Cys38-Cys122, Cys46-Cys100, Cys50-Cys102, and Cys105-Cys112 of the full-length protein.⁸

Following transcription, translation, and glycosylation of individual α - and β -subunits, dimer formation occurs. The crystal structure of hCG was the first of the pituitary glycoprotein hormones to be reported⁹ but was subsequently accomplished for FSH⁶ and FSH complexing with its receptor.¹⁰ By lining up Cys residues from FSH β and hCG β of the mature protein, FSH β Cys20 and Cys104 (Cys 38 and Cys122 of the total protein) form a “seatbelt” that wraps around and stabilizes the α -subunit.^{6,9} Only the mature FSH dimer binds the FSHR.

The Human FSHR

The human FSH receptor (FSHR) is a glycosylated, heptahelical G-protein-coupled receptor (GPCR) with an exceptionally long extracellular domain (ECD) constituting more than half of the full-length protein (residues 1-357), a 7 transmembrane domain (TMD1: 358-390, TMD2: 395-429, TMD3: 440-472, TMD4: 482-506, TMD5: 525-557, TMD6: 565-597, and TMD7: 605-642), 3 short intracellular loops (ICL1: 391-394, ICL2: 473-481, and ICL3: 558-564), and 3 extracellular loops (ECL1: 430-439, ECL2: 507-524, and ECL3: 598-604), and an

Figure 2. The structure of the human FSHR protein is shown with the large extracellular domain (ECD) and the serpentine domain, which consists of 7 transmembrane domains (TMDs), 3 extracellular loops (ECLs); 3 intracellular loops (ICLs); and an intracytoplasmic tail carboxy-terminal (COOH) tail. Not all amino acids in the ECD are shown (including Tyr 335 in the hinge region) as no human mutations have been identified to affect it. Inactivating missense mutations are indicated by a black circle and white letter, corresponding to the location of the affected amino acid and mutant. Activating mutations are indicated by gray-shaded circles and a black letter, corresponding to the mutant amino acid residue. In both cases, the amino acid letter shown is the wild type. See online version for color reference.

intracellular tail (643-695; Figure 2).¹¹⁻¹³ The receptor has 2 functional domains—an ECD and a serpentine domain that includes the transmembrane domain as well as extracellular and intracellular loops.

Two transcript variants have been described in humans, a longer one (NM_000145) and a shorter one (NM_181446), which lacks an alternate in-frame exon 6 of the longer variant. The longer protein NP_000136 contains 695 amino acids in length and is encoded by a 192-kb gene on chromosome 2p16.3, which contains 10 exons. The first 9 exons range in size from 69 to 251 bp and encode the extracellular domain, while the last exon containing an open reading frame of 1234 bp encodes the entire transmembrane domain loops and intracellular domains of the receptor (Figure 2). The large ECD is made up of leucine-rich repeats (LRRs) and a horseshoe-shaped structure of α -helices and β -strands, conferring specificity and high-affinity binding.¹⁴ The rhodopsin-like domain comprising 7 hydrophobic transmembrane helices functions to activate the

receptor which then use G proteins for signal transduction. The FSHR is principally expressed in granulosa cells in females and in Sertoli cells in males but has also recently been found in the endothelium of a wide variety of tumor blood vessels.¹⁴

In addition to the 2 human FSHR isoforms, FSHR isoforms have been described in other species. Yarney et al¹⁵ identified an alternatively spliced ovine FSHR isoform, which is identical to the full-length FSHR for most of its protein, but diverges in sequence beginning at residue 625 and results in a shorter protein by 25 amino acids. As further shown by these investigators,¹⁶ this FSHR isoform was shown to possess dominant negative qualities since co-transfection of the isoform in stably transfected HEK293 cells abrogated hormone response. Kraaij et al¹⁷ identified 2 FSHR splice variants in rat, but both messenger RNAs (mRNAs) encode truncated proteins, and when co-transfected with the WT FSHR, signal transduction was not affected. Therefore, no clear gonadal function could

be attributed to these 2 isoforms. Similarly, Tena-Sempere et al¹⁸ identified 4 different mouse FSHR splice variants, but none were able to bind recombinant human FSH or elicit cyclic adenosine monophosphate (cAMP) or progesterone responses in vitro. More recently, Sairam et al¹⁹ characterized 4 ovine FSHR isoforms (R1-R4). R1 is the full-length FSHR comprised of 10 exons, while R2 has the 10 exons but has a different C-terminus (the dominant negative isoform mentioned above). R3 has exons 1-8, but is truncated, and appears to function as a cytokine/growth factor type receptor. R4 has only exons 1-4 and is a truncated receptor with uncertain function.¹⁹ Therefore, the complexity of the FSHR suggests diverse functions that will continue to be elucidated.

Follicle-Stimulating Hormone Signaling and Action

Dimeric FSH binds to the ectodomain of the FSHR in a hand-clasp fashion as β -strand residues of the receptor interact with the α - and β -subunits of the ligand.⁶ During this ligand binding, the FSHR dimerizes, which prompts changes as the 7-transmembrane domain then acts on a heterotrimeric G_s protein causing guanine nucleotide exchange and activation of adenylyl cyclase, which results in the production of protein kinase A.^{10,14} The resulting cAMP then initiates a signaling cascade culminating in steroid synthesis. In addition, there is evidence that FSH also activates other separate signaling cascades such as RAS,²⁰ PI3K (likely via an SRC tyrosine kinase),²⁰ and glycogen synthase kinase 3β (GSK3 β).²¹

Recently, the crystal structure of FSH binding to the entire ectodomain of the receptor has been reported by Jiang et al.²² The ectodomain of the FSHR is comprised of 12 LRR with a hairpin loop between the 11th and 12th LRRs. This hairpin loop contains an important amino acid residue, a sulfated tyrosine at codon 335 (sTyr), that forms the hinge region (the hinge region was previously thought to be a separate structural unit).²² The convex surface formed by the LRR and the hinge region resembles the fingers and thumb, respectively, of a right hand; and the binding of FSH with its receptor is similar to a right hand clasping an American football. Unexpectedly, FSH binding to the entire ectodomain forms a trimeric arrangement, which suggested an additional binding site on the FSHR outside the primary region (termed an exosite).²² These findings suggest a 2-step process of FSH binding to its receptor. First, the quiescent state FSHR exposes LRR 1-8 to recruit FSH to the hormone-binding region. In the second step, FSH accepts the hinge region of the FSHR with sTyr, which now prepares the FSHR to enter the active state with G_s or β -arrestin.²²

FSH functions to increase the differentiation and proliferation of granulosa cells and induces aromatase (*CYP19A1*) expression, converting androgens to estrogens, as well as *FSHR* and cyclin D2 (*CCND2*) expression.²³ A variety of growth factors, notably activins and inhibin, as well as members of the TGF β family are important in FSH action.²³ In males, FSH is involved in Sertoli cell proliferation and regulation of

spermatogenesis. Evidence from *Fshb* and *Fshr* KO mice and from humans with *FSHB* and *FSHR* gene mutations suggests additional roles for FSH function (see below).

Mutation of the Ligand

Females

The *Fshb* knockout mouse. Germline *Fshb* KO mice were generated using homologous recombination techniques.²⁴ Although *Fshb* +/− heterozygotes had normal estrous cycles and fertility, homozygous (*Fshb* −/−) KO female mice demonstrated low serum FSH levels, a lack of estrous cyclicity, and sterility.²⁴ Serum LH levels in the *Fshb* −/− mice were consistently elevated above baseline for all ages of development. Grossly, the uteri and ovaries of the *Fshb* −/− mice were markedly small, consistent with hypogonadism. Somewhat surprisingly, however, serum estradiol levels were normal in these *Fshb* −/− females, although it is notoriously difficult to discriminate low from low-normal estradiol by immunoassay.²⁵ Microscopically, the ovaries contained mostly primordial and primary follicles, with only occasional small, degenerating, antral follicles. No preovulatory mature follicles or corpora lutea were identified in female *Fshb* −/− mice.²⁴

These findings in *Fshb* −/− mice (Table 1) suggested that FSH is essential for estrous cyclicity and fertility in female mice. In addition, follicular development up to the primary and early antral stages in the ovary occurred in the absence of pituitary FSH.²⁴ The observed phenotypic manifestations in female *Fshb* −/− mice were all reversible by the administration of exogenous gonadotropins.³⁰

Burns et al³¹ used northern blot and in situ hybridization methods to more precisely characterize the alterations in ovarian gene expression in *Fshb* −/− mice. Ovarian *Fshr* expression doubled, but *Lhr* was 5-fold less than of WT and heterozygotes. Serum/glucocorticoid-inducible kinase (Sgk), which is implicated in transitioning granulosa cells from a proliferative to a terminally differentiated luteal state, was reduced more than 5-fold. Steroid enzyme machinery was also affected as expected. Without FSH, both P450 side chain cleavage (*Cyp11a*) and aromatase (*Cyp19*) were decreased by about 6-fold, while 17-hydroxylase (*Cyp17*) expression was doubled (and found to be localized to the theca).³¹ Expression of inhibin subunits revealed a 50% reduction in the α -subunit (*Inha*), and no evidence of expression of either of the β -subunits of inhibin (*Inhba* or *Inhbb*). Follistatin (*Fst*) expression did not change. Cell cycle genes were also altered: cyclin D2 (*Ccnd2*), which is important for granulosa cell proliferation, was decreased by 30%; whereas *Cdk2* and *Cdk6* (associated with granulosa cell cycle withdrawal, differentiation, and luteinization) were increased by 50% and 20%, respectively. Inhibitors of the cyclins were all reduced—p27^{Kip1} (*Cdkn1b*; 3-fold), p21^{Cip1} (*Cdkn1a*; 5-fold), and p15^{Ink4b} (*Cdkn2b*; >10-fold).³¹ The authors concluded that cyclin D2 loss could account for some of the defects in granulosa cell function, but the mechanism is different from cycle withdrawal at the time of luteinization.³¹

Table 1. Phenotype^a of *Fshb*²⁴ and *Fshr*^{26,27} Knockout Homozygotes (—/—) Mice are Shown, as Well as the Overexpressing *Fshb* Transgenic Mice.²⁸

Phenotype	<i>Fshb</i> —/—		<i>Fshr</i> —/—		High Copy Overexpressing <i>Fshb</i> Tg Mouse	
	Male	Female	Male	Female	Male	Female
FSH	Low	Low	High (3-fold)	High (15-fold)	Markedly increased	Markedly increased
LH	Normal	High	Normal ²⁶ ; elevated ²⁹	Normal	Normal	Normal
Testosterone	Normal	ND	65%-85% reduction	Increased	Elevated	Elevated
Estradiol		Normal ^b		ND; normal estrous cycles		Elevated
Progesterone		Reduced 50%				
Fertility	Fertile	Sterile	Fertile (reduced)	Sterile	Infertile	Infertile
Litter size	ND	0	Normal (7.3 ± 0.6 vs 8.1 ± 2.4 in controls)	0 ^c		
Ovarian follicles		Primordial mostly; few antral follicles; increased stroma		Primordial, primary, secondary; increased stroma		Hemorrhagic, cystic ovaries; no corpora lutea
Uterus		Small		Small		
Testes size	Decreased 50%	—	Decreased 20%-30%	—	Normal	—
Sperm concentration	Decreased 75%	—	Decreased 35%	—	75% increase	—
Sperm motility	Decreased 40%	—	Decreased 25%	—		
Sperm morphology	ND	—	Decreased 30%	—		
Sperm function	ND	—	Multiple parameters affected (see text)	—	ND	—
Other	Intratesticular testosterone and Leydig cell number ^b normal; Sertoli cell and germ cell number decreased 30%-39%	Steroid enzyme and cyclin expression abnormal	Intratesticular testosterone and Leydig cell number ^b normal at first, but both reduced in adulthood; Sertoli cell and germ cell number decreased 30%-39%	Increased adiposity, weight, and skeletal abnormalities	Seminal vesicle size doubled; histology of testes normal	No estrous cycles; Progesterone elevated; enlarged cystic kidneys and die 6-13 weeks of life from bladder obstruction

Abbreviation: ND, not determined.

^a Only the high copy overexpressing mice are shown, and low copy expression mice were normal.

^b Estradiol was measured as normal, although the phenotype indicates low levels.

^c Dierich et al²⁷ initially stated estrous was normal; Abel et al²⁶ found absent vaginal openings indicating no puberty.

Table 2. Reported Inactivating *FSHB* Mutations in Human Patients (Males and Females) for Which a Functional Effect Has Been Demonstrated.^a

#	Author	FSHB Mutation				Exon	Cells for In Vitro Analysis	Functional Effect In Vitro
		Old Nomenclature	New Nomenclature					
			Nucleotide	Protein				
1	Layman 1997 ³²	p.Val61X	c.236-237delTG	p.Val79fs or p.Val79fsX105	3	CHO	Absent I and B-FSH	
2	Layman 1997 ³²	p.Cys51Gly	c.205T>G	p.Cys69Gly	3	CHO	Absent I and B-FSH	
3	Layman 2002 ³³	p.Tyr76X	c.282C>A	p.Tyr94X	3	CHO	Absent I and B-FSH	
4	Clark 2003 ³⁴	p.Cys82Arg	c.298T>C	p.Cys100Arg	3	CHO	Absent I and B-FSH	
5	Kottler 2010 ³⁵	p.Ala79fs108X	c.289delG	p.Ala97fs or p.Ala97fsX127	3	CHO	Absent I and B-FSH	

Abbreviations: B, bioactive; CHO, Chinese hamster ovary cells; I, immunoactive.

^aNote that p.Val79fsX105 may also be indicated as p.Val79fs and p.Ala97fsX127 may be indicated as p.Ala97fs.

Human *FSHB* mutations. Only 5 different *FSHB* mutations have been found in humans (Table 2, Figure 1)—6 females (Table 3) and 3 males (Table 4). The first patient with an *FSHB* mutation was described by Matthews et al³⁶ in 1993. She was a 27-year-old female who presented with primary amenorrhea, low serum FSH, and high LH. At the age of 13, she had no breast development but was subsequently treated with estrogen. She later conceived with exogenous gonadotropin therapy. Upon DNA sequencing, the proband was found to possess a homozygous 2 base pair deletion at codon 61 (Val61X) of the mature protein. These investigators did not perform functional studies in vitro, but this frameshift mutation was predicted to alter amino acids 61 to 86, and then produce a stop codon, resulting in a truncated FSH β protein lacking amino acids 87 to 111.³⁶ It should be noted that for most of the described human *FSHB* mutations, the nomenclature only took into account the mature protein. However, in the now accepted mutation nomenclature, the 18 amino acid signal peptide should really be numbered as codon 1 as it represents the first in-frame ATG.⁴¹ In Table 2, the reported nomenclature of described *FSHB* mutations is shown, along with their corrected nomenclature. Therefore, this mutation is more correctly indicated as Val79fs or Val79fsX105, as it alters amino acids 79 to 104 and produces a stop codon truncating amino acids 105 to 129.

Layman et al³² reported the first patient with an *FSHB* mutation that was confirmed by in vitro analysis. The proband was a 15 11/12 year-old female who presented with absent breast development, primary amenorrhea, low FSH, undetectable estradiol, elevated LH levels, and no pituitary tumor by MRI.³² She demonstrated compound heterozygosity for 2 different *FSHB* gene mutations, 1 being the previously described Val61X (Val79fs or Val79fsX105) inherited from her mother, while the other was a Cys51Gly (Cys69Gly, new nomenclature) missense mutation inherited from her father. When mutants versus controls were separately transfected into Chinese hamster ovary cells, both the Val79fs and the Cys69Gly proteins resulted in unmeasurable immunoreactive and bioactive FSH levels in the cellular media compared with controls.³² These in vitro data confirmed the phenotypic findings of the patient and indicated that FSH is necessary for

pubertal development and fertility in females. There were 5 heterozygotes within the family who were fertile, therefore suggesting that only biallelic *FSHB* mutations impair reproductive function.

Since these 2 patients were described, there have only been 4 more females with *FSHB* mutations reported (cases 3-6 in Table 3).^{33,37,38} Rather than completely lacking breast development as was present in the first 2 patients, these females all had partial breast development. Nevertheless, all 6 described females share certain phenotypic findings—primary amenorrhea, low serum FSH, low serum estradiol, and elevated LH (Table 3). When GnRH stimulation was performed in 5 of these patients, no FSH rise from baseline was observed, whereas there was a marked rise in serum LH in all 5 patients who were studied. These findings could be predicted since the absence of estradiol negative feedback should elicit an increase in GnRH secretion and a subsequent but appropriate rise in LH. However, no corresponding rise in FSH occurs because of the *FSHB* mutation, which likely interferes with dimer formation and subsequent secretion.³² As shown in Tables 2 and 3, only 5 different mutations have been reported in these 6 females—5 as homozygotes and 1 as a compound heterozygote. Two are frameshift; 1 is a stop mutation; and 1 is a missense involving the highly conserved Cys residue. All 4 reported *FSHB* mutations in females resulted in unmeasurable immuno and bioactive FSH in vitro, regardless of the clinical severity of the mutations (ie, the presence or absence of breast development).^{32,33}

Several interesting features of the patient with the compound heterozygous Val61X/Cys51Gly (Val79fs/Cys69Gly, new nomenclature) mutations deserve further mention.³² Although serum LH levels were elevated, this patient had a normal testosterone and no evidence of hirsutism.^{32,42,43} These findings were unexpected, considering that women with polycystic ovary syndrome (PCOS) demonstrate elevated LH levels and elevated androgens.⁴⁴ This suggested that perhaps FSH was necessary for the LH-induced theca cells to make androgens. In order to test this hypothesis, serial gonadotropin levels were obtained for 11 hours at 10-minute intervals. Consistent with the baseline levels, serum FSH was unmeasurable, while the mean LH level was elevated (49 mIU/mL) with 9 LH pulses

Table 3. The *FSHB* Mutations in Human Females.^a

#	Author	FSHB Mutation	Age	Race	Breast	Menses	Ht	Wt	BMI	FSH (1-10 mIU/mL)			LH (1-9 mIU/mL)			E2 (30-300 pg/mL)		InB (20-220 pg/mL)	Ultrasound	Histology	Other
										Basal	Peak	Basal	Peak	Basal	Peak	Basal	Peak				
1	Matthews 1993 ³⁶	p.Val79fsX105	18	Italian	T1	I ⁰ Am	176	73	23.6	< 0.1	< 0.1	< 0.1	28	104	27	U	ND	ND	After 15 days of rFSH, InhB and E2 levels rose just above detection; later FSH treatment resulted in pregnancy	ND: LH pulses similar to PCOS	
2	Layman 1997 ³²	p.Val79fsX105 and p.Cys69Gly	15.5	USA	T1	I ⁰ Am	155	ND	ND	0.6	0.8	21-33	170	< 25	ND	Multicystic ovaries—many small antral follicles	ND	Ovarian biopsy shows primordial follicles	ND	Laparoscopy: small uterus and ovaries	
3	Matthews 1997 ³⁷	p.Val79fsX105	17	Israeli	“Poor”	I ⁰ Am	163	ND	ND	< 3	ND	50-90	ND	< 10	ND	ND	ND	ND	ND	Treatment with rFSH resulted in hyperstimulation	
4	Layman 2002 ³³	p.Tyr94X	32	Brazil	T2-3	I ⁰ Am	156	ND	ND	< 0.01	< 0.01	51	159	12	ND	ND	ND	ND	ND	ND	
5	Berger 2005 ³⁸	p.Tyr94X	16	Brazil	T3	I ⁰ Am	158	48	19.2	< 1	< 1	30	98	< 13	ND	Small uterus (7 cc volume) normal ovaries	ND	ND	ND	ND	
6	Kottler 2010 ³⁵	p.Ala97fsX127	29	Spanish	T2	I ⁰ Am	165	ND	ND	< 0.05	< 0.05	49	170	7	U	Small uterus; Ovaries 1 and 1.6 mL volume (6.6 ± 1.9); few follicles <3 mm	ND	ND	ND	ND	

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; Am, amenorrhea; BMI, body mass index; E2, estradiol; ND, not determined; Ht, height in cm; InB, inhibin B; Ov Vol, ovarian volume; T, Tanner stage (T1, prepubertal and T5, adult); U, undetectable; Wt, weight in kg.

^aAll are homozygous except case 2 who has compound heterozygous mutations. All were 46, XX and had normal pituitary imaging.

Table 4. The *FSHB* Mutations in Males.

Pt.	Author	<i>FSHB</i> Mutation	Age	Nationality	Puberty	Testes (15-25 cc)				Ht	Wt	BMI	Sperm	FSH (1-10 mIU/mL)		LH (1-9 mIU/mL)		Testosterone (270-1100 ng/dL)	Testicular Biopsy
						3 and 6	No	No	No					Basal	Peak	Basal	Peak		
1	Lindstedt 1998 ³⁹	p.Cys100Arg	28	Serbian	Normal	3 and 6	No	No	No	171	181	62	Azoospermia	U	U	12	41	246	Leydig cells present but spermatogenic arrest
2	Phillip 1998 ⁴⁰	p.Val79 fs or p.Val79fsX105	18	Israeli	Absent	1 and 2	No	No	No	178	59	19	Azoospermia	< 0.5	< 0.5	24	75	130	ND
3	Layman 2002 ³³	p.Tyr94X	30	Brazilian	Normal	12 and 12	No	No	No	170	N	ND	Azoospermia	0.5	I	36	160	582	Leydig cell hyperplasia; small seminiferous tubules with germinal cell aplasia, peritubular fibrosis, and few Sertoli cells

Abbreviations: BMI, body mass index; Ht, height in cm; ND, not determined; T, testosterone; U, undetectable; Wt, weight in kg.

^aAll were homozygous. Normal endocrine values are indicated.

Figure 3. The LH pulses are shown for a female with an *FSHB* mutation (patient #2 in Table 3) who had serum LH levels drawn every 10 minutes overnight. Note the LH pulses indicated by arrowheads, which are similar to women with PCOS. Modified from Barnes et al.⁴² LH indicates luteinizing hormone; PCOS, polycystic ovary syndrome.

and an elevated amplitude (46 mIU/mL), as is shown in Figure 3.⁴² This pulsatile pattern of LH closely resembles that seen in women with PCOS.⁴⁴

This FSH-deficient female was further studied by Barnes et al.^{42,43} By ultrasound, she had multiple small antral follicles, similar to a PCO appearing ovary except there was no dense stroma.⁴² She was given dexamethasone to suppress the adrenal contribution of androgens for 4 days and then 5000 IU hCG was administered (endocrine studies were done at baseline and 24 hours later). Following a washout period of 1 month, baseline studies were obtained, and then she received 300 IU recombinant FSH (rFSH), and then hCG again 24 hours later.⁴³ Androgens increased somewhat in response to hCG alone, but there was a more dramatic rise in testosterone (which more than tripled) after rFSH followed by hCG. These data add further support to the hypothesis that LH-induced androgen production in the theca requires the presence of FSH for a normal physiologic response, perhaps by inducing inhibin, LH receptors, or growth factors.⁴³ In primates, there is a positive correlation between expression of the FSHR and the androgen receptor in healthy, growing follicles.⁴³

Similar studies were carried out in the Brazilian female with the p.Tyr76X (p.Tyr94X, new nomenclature) mutation by Lofrano-Porto et al.⁴⁵ By overnight sampling, she had 8 LH pulses/8 hours similar to the other patient. However, she had no response to hCG, rFSH, or hCG + rFSH, which could be an age-related effect since she was 41 at the time of the studies.⁴⁵

Males

The *Fshb* knockout mouse. FSH-deficient *Fshb* $-/-$ male mice had normal sexual development (Table 1); and serum levels of LH and testosterone were similar to control *Fshb* $+/+$ males. It is likely that testosterone's negative feedback in these

Fshb $-/-$ mice accounts for the quantitative difference in serum LH values between male and female *Fshb* $-/-$ mice. In contrast to female *Fshb* $-/-$ mice that are infertile, the male *Fshb* $-/-$ mice are fertile despite a decrease in testicular size and a 75% reduction in sperm. Microscopic examination of the testes reveals a relative diminution in all testicular elements, but progression through spermatogenesis as well as Leydig cell numbers appears to be normal. Unfortunately, however, the litter sizes were not provided. It seems possible that reduced litters or prolonged intervals between pregnancies would be observed because of the oligospermia, but this has not been reported. The combination of small testes and fertility in *Fshb* $-/-$ male mice suggests that FSH is necessary for testicular volume but may not be absolutely essential for spermatogenesis and normal fertility in mice.

Further support that these observed effects of *Fshb* $-/-$ mice are specifically caused by FSH deficiency comes from rescue of the *Fshb* $-/-$ mice of both sexes by the introduction of the human *FSHB* transgene,³⁰ which is selectively expressed in the pituitary. In both females and males, all of the phenotypic effects are corrected, indicating that they are caused by isolated FSH deficiency.³⁰

Since the original description of the *Fshb* $-/-$ mice, Wreford et al.⁴⁶ examined testicular function in more detail. Testicular weights were decreased about 60%, and Sertoli cell number and germ cell numbers were decreased 30% to 39%. The germ cell attrition was more pronounced for later stages of spermatogenesis, as manifested by a 46% reduction in spermatogonia and 60% decrease in round spermatids. The investigators suggested that the testicular function in *Fshb* $-/-$ male mice is due to the lack of FSH's action on Sertoli cell proliferation and the ability of Sertoli cells to nurture germ cells.⁴⁶

Baker et al.⁴⁷ studied *Fshb* $-/-$ male mice to determine the effects of absent FSH upon testicular function over time. Diminished testicular volume was apparent as early as 20 days of life and was about one-third the normal size in adults. Reflecting normal serum LH levels in *Fshb* $-/-$ male mice, Leydig cell number increased appropriately at days 1, 5, 20, and adult compared with control. Corresponding intratesticular testosterone was normal at 60 and 180 days. Leydig cell morphology was normal, but the reserve of the testis was impaired since hCG administration elicited an intratesticular response that approximated two-thirds of the WT animals.⁴⁷ Disordered testicular steroidogenesis was observed in adult *Fshb* $-/-$ males, however, as 17β -HSD type 3 (*Hsd17b3*) and StAR (*Star*) were increased. There were no changes in P450scc (*Cyp11a1*), 3β -HSD type 6 (*Hsd3b6*), and P450c17 (*Cyp17a1*).⁴⁷ Collectively, these findings indicate impairment of both endocrine and exocrine functions of the testes in the *Fshb* $-/-$ male mice.

Human *FSHB* Mutations. Only 3 males with *FSHB* mutations have been described in humans (Table 4, Figure 1). Based upon the findings in *Fshb* $-/-$ mouse, the expected phenotype would be that of normal puberty (since LH-induced testosterone production should occur), but there could be varying

degrees of spermatogenic failure. The first male described by Lindstedt et al³⁹ with an *FSHB* mutation had exactly this presentation, with normal virilization and pubertal development although the testes were small (3 and 6 cc volume with normal being ~15-25 cc). His testosterone level was in the lower range of normal; LH was mildly elevated, but FSH was undetectable (Table 4). He demonstrated homozygosity for a Cys82Arg (p.Cys100Arg in new nomenclature) missense mutation, which was predicted to interfere with dimer formation but was not studied in vitro. Subsequently, Clark et al³⁴ demonstrated that this mutation resulted in absent immunoreactive and bioactive FSH (Table 2).

Phillip et al⁴⁰ reported the second FSH-deficient male that exhibited a more severe, and perhaps unexpected, phenotypic presentation. He had no evidence of pubertal development with very small testes (1 and 2 cc), a low testosterone (130 ng/dL; normal: 270-1070), elevated LH, and low FSH. He demonstrated homozygosity for the Val61X (p.Val79fs, new nomenclature),⁴⁰ previously shown to be completely devoid of immuno- and bioactivity.³² The third male with an *FSHB* mutation characterized by Layman et al³³ had a presentation similar to the first reported male. He had normal puberty, infertility, and azoospermia. His testicular biopsy demonstrated Leydig cell hyperplasia and sparse, small seminiferous tubules with germinal cell aplasia, peritubular fibrosis, and scant Sertoli cells.³³

All 3 men with *FSHB* mutations had in common the finding of azoospermia despite testicular size ranging from completely prepubertal to nearly normal (Table 4). Interestingly, none had gynecomastia or cryptorchidism. Also, in all 3 males, FSH was low and did not respond to exogenous GnRH administration. Similar to females with *FSHB* mutations, and unlike male *Fshb* $-/-$ mice, baseline LH levels were elevated and displayed an exaggerated LH response to GnRH. Inhibin B was low in the first 2 males, reflecting Sertoli cell dysfunction⁴⁸ (unfortunately, inhibin B was not studied in the third male).³³ Although only 2 had testicular biopsies, Leydig cells were present in 1 patient and numerous in the other, yet variable seminiferous tubule dysfunction was noted—germinal aplasia to spermatogenic arrest (Table 4).

It is intriguing that at least one of the males had low serum testosterone. Leydig cells do not express the FSHR, so direct evidence of FSH upon Leydig cells has not been demonstrated. However, several studies identified by Phillip et al⁴⁰ indicated that FSH may have an indirect role upon Leydig cell testosterone production. In fact, supernatants of Sertoli cells incubated with FSH have been reported to stimulate testosterone secretion by Leydig cells and testicular explants from rats,^{49,50} hamsters,⁵¹ and humans.²⁹ Therefore, human *FSHB* mutations in males clearly affect fertility by causing azoospermia and possibly could play a role in pubertal development. These mutations appear to be rare in eugonadal males with infertility, since none of 65 males studied, including 13 with nonobstructive azoospermia and 52 with oligozoospermia, had *FSHB* mutations (Clark AD and Layman LC, unpublished).

Inactivating Mutations of the Receptor

Females

The FSHR knockout mouse. Two different groups of investigators have knocked out the *Fshr* gene in mice.^{26,27} Similar to female *Fshb* $-/-$ mice, female *Fshr* $-/-$ mice, first described by Dierich et al,²⁷ demonstrated sterility (Table 1). As expected, serum FSH levels were markedly elevated (15-fold) in female *Fshr* $-/-$ mice versus WT *Fshr* $+/+$ mice.²⁷ However, serum LH levels were reportedly normal by these investigators,²⁷ which is in contrast to Abel et al²⁶ who found that serum LH was markedly elevated. Female *Fshr* $-/-$ mice had a small uterus and impaired follicular maturation with primordial, primary, and secondary follicles but absent mature follicles.^{26,27} Similar to the *Fshb* $-/-$ mice, stromal hypertrophy was present.²⁶ When *Fshr* $-/-$ mice were studied further, uterine, ovarian, and vaginal weights were decreased compared with WT and heterozygotes.⁵² As expected, serum estradiol and progesterone were markedly decreased, but interestingly, serum testosterone was elevated (Table 1).⁵² There was also no change in gene expression of aromatase (*Cyp19a1*), estrogen receptor- α (*Esr1*), estrogen receptor- β (*Esr2*) or their encoded proteins, but there was a 60% reduction of protein expression for both the A and B isoforms of progesterone receptor.⁵² In addition, the expression of the FSH-responsive gene cyclin D2 (*Ccnd2*) was unaltered in *Fshr* $-/-$ females.²⁷

When Abel et al²⁶ generated *Fshr* $-/-$ mice, overall, they reported similar findings. In addition, they found that the vagina was imperforate and that both ovarian and serum inhibins (A and B) were undetectable in *Fshr* $-/-$ mice versus heterozygote and wild-type animals. A notable finding by Dierich et al²⁷ was that the pituitaries of female (but not male) *Fshr* $-/-$ mice were enlarged, as both thyrotropes and gonadotropes were increased. Abel et al²⁶ found that pituitary content of FSH, but not LH, was significantly elevated in *Fshr* $-/-$ females. When these mice were treated with estradiol, serum LH was suppressed to the level of controls but FSH was only reduced by 50%. It has also been shown that transplantation of an ovary from WT animals into an *Fshr* $-/-$ mouse can correct hypoestrogenism and hyperandrogenemia, although it cannot rescue the effects of the FSHR loss in host granulosa cells.⁵³

Danilovich et al⁵² characterized additional features of *Fshr* KO mice involving body weight, adiposity, and the skeleton. Body weights of *Fshr* $-/-$ mice were significantly (20%) higher than WT, and there was a doubling of their adipose tissue (abdominal, inguinal, and retroperitoneal fat pads) versus matched age *Fshr* $+/+$ mice.⁵² Beginning about 4 to 5 months of age, *Fshr* $-/-$ females demonstrated a stooped posture kyphosis that was evident on X-ray; and the weight of the right femur was reduced in these females compared with wild-type mice.⁵² These skeletal changes are analogous to those in aging human females.

Interestingly, heterozygous female *Fshr* $+/-$ mice also had phenotypic findings that were intermediate between *Fshr* $-/-$

and *Fshr* $+/+$ mice. *Fshr* $+/-$ mice had a reduction in litter size by 50% and pups/litter by 43%, as well as a longer time between pregnancies.⁵² Body weight and weights of the reproductive organs did not differ in heterozygotes versus wild-type mice. Serum estradiol was not different, but progesterone was decreased and testosterone increased compared with *Fshr* $+/+$ mice.⁵²

Human inactivating FSHR mutations. Although inactivating mutations in the *FSHR* gene were predicted to cause 46, XX ovarian failure, no mutations were initially identified in a group of unrelated North American patients.⁵⁴ The first reported *FSHR* gene mutations were identified in Finnish kindreds with 46, XX hypergonadotropic hypogonadism (mutation 1 in Table 5 and case 1 in Table 6, Figure 2). Aittomaki et al⁵⁵ investigated all church records in Finland for families having women with primary amenorrhea under age 20, elevated gonadotropins, and a normal karyotype. Using linkage analysis of multiplex families, strong linkage at chromosome 2p21 was observed and the *FSHR* gene was found to be in linkage disequilibrium with this phenotype. DNA sequencing revealed a homozygous p.Ala189Val missense mutation in about one-third of all the Finnish ovarian failure patients studied. This mutation was shown to markedly reduce the binding capacity of the receptor in MSC-1 Sertoli cells, as well as to dramatically impair cAMP production in response to recombinant FSH (Table 5).⁵⁵

All Finnish women with this *FSHR* mutation had primary amenorrhea, but half had normal thelarche while the other half had impaired breast development.⁶² Ultrasound of the ovaries demonstrated follicles of varying sizes (Table 6). On histologic evaluation, only primordial follicles were seen in some patients, but in others, preantral or antral follicles were identified. In 1 patient, even a mature follicle was identified. Corpora lutea were not mentioned in the 9 women with *FSHR* gene mutations, suggesting that they did not ovulate, at least in the recent past. Stature of women with the *FSHR* gene mutations was less (160.5 cm) compared with ovarian failure patients without the mutation (165.8 cm). Although only a small sample size was studied, women with *FSHR* gene mutations (6 of 12) were more likely to have follicles on ultrasound than for those without mutations (1 of 11).⁶²

Similar to patients with *FSHB* mutations, the inheritance of inactivating *FSHR* gene mutations was autosomal recessive. Although the carrier frequency of this mutation was common in Finland, the allele was not found in 35 unrelated US women with 46, XX ovarian failure.⁶⁴ In a much larger international study, Jiang et al⁶⁵ showed that the prevalence of *FSHR* gene mutations was exceedingly low in other ethnic populations—only 1 of 1200 from Switzerland, 0 of 1100 from Denmark, and 0 of 540 from Singapore were heterozygous carriers of the Finnish missense *FSHR* allele.

The second *FSHR* mutation was identified in an Armenian woman with secondary amenorrhea and hypergonadotropic hypogonadism.⁵⁶ She had compound heterozygous *FSHR* missense mutations p.Ile160Thr/p.Arg573Cys (Tables 5 and 6).

The Ile160Thr mutation in the ECD resulted in dramatic functional effects: severely impaired binding of iodinated FSH in COS-7 cells and a 90% reduction of cAMP production in response to exogenous FSH (Table 5).⁵⁶ The other Arg573Cys allele in the third intracellular loop demonstrated normal binding affinity, but exhibited ~30% of the cAMP production upon FSH stimulation compared with the wild type (Table 5). The affected proband had normal breast development, but menstruated only 3 times in a year-and-a-half, and then became amenorrheic. Serum gonadotropins were elevated and estradiols were low/low normal (20–40 pg/mL), and she had a 46, XX karyotype (Table 6).⁵⁶ On transvaginal ultrasound, the ovaries were normal in size and contained numerous, small (3–5 mm in diameter) antral follicles (Table 6). The authors suggested that the compound heterozygous *FSHR* gene mutations in this patient produced a partially deficient state.⁵⁶ However, it is also true that women with the Finnish missense mutation displayed evidence of hypofunction rather than null since half of these women manifested breast development; and follicular maturation (antral and mature follicles) was observed in some patients.⁶²

After these 2 reports, 5 additional females with *FSHR* mutations, backed by in vitro and conformational studies, have been described (mutations 4–10 in Table 5 and cases 3–7 in Table 6).^{57–61} Of the 5, 4 had primary amenorrhea and hypergonadotropic hypogonadism (Table 6). It is also likely that the other patient also did as well; however, she had been treated with oral contraceptives at age 17 and then had a normal exam about 10 years later. When examining these 10 inactivating *FSHR* mutations, it can be seen that 9 were missense (Table 5, Figure 2). The ECD was the most common site involved (4 of 10), but mutations were also found in the TMDs ($n = 3$) and ECLs ($n = 2$)—none were located in the ICLs. One mutation (case #10 in Table 5) involved a deletion that was not completely mapped at the genomic level but would be annotated as chr2: g.(48 887 046–48 895 903)–(49 058 614–49 065 417)del (NCBI 36/hg18) at the genomic level. The minimal deletion would be 163 kb, involving exons 9 and 10, while the maximal deletion would consist of a 178 kb deletion, affecting exons 7 to 10. In both cases, all 7 TMD1–7, ICL1–3, and ECL1–3, and the intracellular domain (ICD) would be removed. The difference between the 2 would be in how much of the ECD would be deleted (see Table 5 legend for details). When mutations occurred in the ECD, ligand binding was affected in 3 of 4 (cases 1, 2, 5, and 7 in Table 5) but binding was only affected in 1 of the 6 mutations outside the ECD (the ECL2 mutation). Of the 6 mutations, 3 analyzed impaired receptor targeting, but all 9 mutations studied (regardless of the location) impaired FSH signaling manifested as a reduction in cAMP.

All of the patients with the Finnish p.Ala189Val *FSHR* mutations were homozygous (Table 6). Of the remaining 6 patients, 3 had compound heterozygous missense mutations,^{57–58} 1 had a homozygous missense mutation,⁶⁰ 1 had only 1 allele identified suggesting a possible unidentified mutation or deletion,⁵⁹ and 1 had a missense mutation on 1

Table 5. Reported Inactivating *FSHR* Mutations in Human Patients (Males and Females) Supported by Functional In Vitro Analysis.

Inactivating FSHR Mutations										
FSHR Mutation					In Vitro Analysis					
#	Author	Nucleotide	Protein	Exon	Protein Region	Cells	Receptor Binding Capacity	Receptor Binding Affinity	Receptor Targeting	Cyclic AMP Response to FSH
1	Aittomaki 1995 ⁵⁵	c.566C>T	p.Ala189Val	7	ECD	MSC-1	Marked reduction	Normal	ND	Negligible vs WT
2	Beau 1998 ⁵⁶	c.479T>C	p.Ile160Thr	6	ECD	COS-7	ND	Marked reduction	None seen on cell surface	10% of WT
3	Beau 1998 ⁵⁶	c.1717C>T	p.Arg573Cys	10	TMD6	COS-7	ND	Normal	Normal	30% of WT
4	Touraine 1999 ⁵⁷	c.1801C>G	p.Leu601Val	10	ECL3	COS-7	ND	Normal	Normal	12% of WT
5	Touraine 1999 ⁵⁷	c.671A>T	p.Asp224 Val	9	ECD	COS-7	ND	Barely detectable	Impaired (intracellular)	4% of WT
6	Doherty 2002 ⁵⁸	c.1255G>A	p.Ala419Thr	10	TMD2	COS-7	Normal	Normal	Normal	Absent cAMP
7	Allen 2003 ⁵⁹	c.1043C>G	p.Pro348Arg	10	ECD	COS-7	Normal	No different vs empty vector	ND	Absent cAMP
8	Meduri 2003 ⁶⁰	c.1555C>A	p.Pro519Thr	10	ECL2	COS-7	ND	Minimal	Impaired (intracellular)	Absent cAMP
9	Kuechler 2010 ⁶¹	c.1760C>A	p.Pro587His	10	TMD6	COS-7	ND, but predicted to cause a kink in TMD6 helix	ND	ND	Absent cAMP
10	Kuechler 2010 ^{61a}	ND precisely, Minimal deletion of 163 kb; Maximal deletion of 178 kb	Min del: p.Leu223-Asn695del; Max del: p.Leu175-Asn695del	Min del (9,10); Max del (7-10)	Part ECD; all TMD, ECL, ICL, and ICD	ND	ND	ND	ND	ND

Abbreviations: ECD, extracellular domain; ECL, extracellular loop; ICL, intracellular loop; ND, not determined; TMD, transmembrane domain; WT, wild type; AMP, adenosine monophosphate.

^a Kuechler et al found that the deletion was due to a 46, XX, t(2;8)(p16.3or21;p23.1), which was apparently balanced prior to chromosomal microarray analysis. The precise deletion breakpoints have not been determined. However, the minimal deletion would be 163 kb (chr2: 48 895 903-49 058 614) and the maximal deletion would be 178 kb (chr2: 48 887 046-49 065 417) based on the HG18 version. With the maximal deletion, the proximal breakpoint is within intron 6 of *FSHR* and so exons 7-10 would be deleted, encoding a truncated protein with the first 174 amino acids of full-length *FSHR*. In this case, almost of half of the ECD, all 7 TMD1-7, ICL1-3, and the first 222 amino acids of full-length *FSHR*. In this case, part of the ECD, all 7 TMD1-7, ICL1-3, and the intracellular domain (ICD) would be deleted, encoding a truncated protein with the first 222 amino acids of full-length *FSHR*. In this case, part of the ECD, all 7 TMD1-7, ICL1-3, and the intracellular domain (ICD) would be removed. The full mutation for case 10 is annotated as Chr2: g.(48 887 046-48 895 903)_(49 058 614-49 065 417)del (NCBI 36/hg18).

Table 6. Human-Inactivating *FSHR* Mutations in Females Supported by Functional Analysis.^a

#	Author	<i>FSHR</i> Mutation	Age	Race	Breast	Menses	Ht	Wt	BMI	FSH < 10	LH < 10	E2 (pg/mL)	lnB	US Ov Vol (cc) 6.6+0.19cc	Histology	Other
1	Aittomäki 1995 ^{53,62} Vaskivuo 2002 ⁶³ +	p.Ala189Val HMZ p.Arg573Cys	<20	Finn	Absent to normal	1 ⁰ Am	160.5 (150-172)	ND	ND	61 (42-176)	40 (17-119)	n = 12.16 (range: 8-81)	<10 (n=3) +	n = 12; 0.7 (0.3-4.8); 6 of 12 had follicles seen	N = 9; primordial and primary; 2/9 with preantral; 1/ 9 with mature	2 Females treated with FSH or FSH and hCG and no endocrine response; minimal GATA-4 or aroma- tase protein expression + FSHR protein expression normal but minimal LHR vs control
2	Beau 1998 ⁵⁶	p.Ile160Thr/ p.Arg573Cys	30	Arm	"N"	2 ⁰ Am	156	56	23	108	81	20-40	50	ROV: 8.4 cc; LOV: 3.4 cc many 3-5 mm antral follicles	Primary, primordial, secondary follicles	Protein expression of normal but minimal LHR vs control
3	Touraine 1999 ⁵⁷	p.Asp224Val p.Leu601Val	19	Cauc	"N"	1 ⁰ Am	174	73	24.1	63	26	11, 22	30	Ovaries stated "normal" with 10-12 follicles of 3 mm/ ovary	Primordial, primary, secondary follicles seen; 3 antral follicles	Protein expression of zp450SCC; weak staining of 3BHSD; no controls shown; stimulated with 5625 IU rFSH without any response ND
4	Doherty 2002 ⁵⁸	p.Ala419Thr/ p.Ala189Val	17	Finn	"N"	1 ⁰ Am	163	51	19.2	91, 78	39, 33	16	ND	"Small ovaries"	ND	ND
5	Allen 2003 ⁵⁹	p.Pro348Arg (1 allele from Dad); 2nd allele unknown	17	?	T3	1 ⁰ Am	160.6	50.8	19.6	105	36	21	ND	ND	Sreaks/ laparoscopy	MRI confirmed presence of follicles
6	Meduri 2003 ⁶⁰	p.Pro519Thr HMZ	26	Cauc	?	?	172	54	18.3	67	21	< 10	< 10	0.22 cc ovaries	Primordial and intermediary follicles (few primary follicles)	No follicles after 10,200 IU rFSH administration; c-Kit and PCNA labeling (no controls); no staining with SCC, Cyp17, Arom, or SFI
7	Kuechler 2010 ⁶¹	p.Pro587His/del by translocation 46, XX, t(2;8) (p16.3or21;p23.1)	17	Ger	T3	1 ⁰ Am	159	48	19	106, 122	48, 47	13, 11	ND	ND	Many primordial follicles, but no secondary or tertiary follicles	Laparoscopy—ovaries normal but small uterus

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; Am, amenorrhea; Arm, Armenian; Cauc, Caucasian; Finn, Finnish; Ger, German; LOV, left ovary; N, normal; ND, not determined; ROV, right ovary.

^a Reports 2-7 are single cases, whereas report 1 consists of 22 of 75 women screened from 13 different Finnish families had the same homozygous inactivating *FSHR* mutation. Breast development is by Tanner staging: T1 prepubertal; T5 adult). Height (Ht) and weight (Wt) are measured in cm and kg, respectively. LH and FSH are in mIU/mL; Inhibin B (lnB) is in pg/mL; ovarian volume (Ov Vol) is in cc. In case 6, spontaneous breast development and menses could not be determined because she was taking combined estrogen-progestin contraceptive pills at her first visit (this is indicated by a question mark). To convert pg/mL to pmol/L, multiply by 3.7. Normal follicular phase estradiol levels ~ 20 to 200 pg/mL.

allele and a deletion on the other allele caused by an autosomal translocation⁶¹ (Table 6). The phenotype is relatively severe in patients with *FSHR* mutations in that all described patients except one⁵⁶ had primary amenorrhea with elevated gonadotropins (Table 6). That patient, case #2 in Table 6 had secondary amenorrhea. These findings indicate that inactivating *FSHR* mutations in humans clearly cause the phenotype of hypergonadotropic hypogonadism.

Males

The *Fshr* knockout mouse. First reported by Dierich et al,²⁷ the male *Fshr* $-/-$ mouse exhibited oligospermia with reduced fertility and a ~ 3 -fold elevation of serum FSH, normal LH, and decreased testosterone levels (Table 1).²⁷ This reduction in testosterone ranged from 65% to 85% that of wild-type mice and was unlike male *Fshb* KO mice, which had normal testosterone levels (Table 1).^{27,66} However, Abel et al²⁶ found elevations of both LH and FSH in the serum of *Fshr* $-/-$ mice, but the pituitary content of either gonadotropin was not different from wild-type animals. These investigators also found that intratesticular inhibins A and B were not different in *Fshr* $-/-$ mice compared to wild type or heterozygotes (although inhibin A was lower than inhibin B in all groups).²⁶

Fshr $-/-$ mice had small testes and seminiferous tubules and were initially reported by Dierich et al²⁷ to have normal accessory structures (similar to male *Fshb* $-/-$ mice). Subsequent studies by this same group quantified the reduction in testes weight by 50%, the seminal vesicle by 23%, and the epididymis by 30%.⁶⁶ In addition, the tubal and luminal diameter of the seminiferous tubules and tubal diameter of the caput epididymis was reduced.⁶⁶ These morphologic changes were likely related to the drastic reduction in serum testosterone.⁶⁶

Sperm concentration, motility, and morphology were all reduced by 20% to 35% in *Fshr* $-/-$ mice.²⁷ In a subsequent, in-depth analysis by Krishnamurthy et al,⁶⁶ there was no disruption or alteration of any of the steps of spermatogenesis, but 2 stages of spermatogenesis (VII and XI) were quantified. At 3 months of age, flow cytometric analysis indicated a 28% increase in spermatogonia and testicular somatic cells (2C fraction) and a 25% reduction in elongated spermatids in *Fshr* $-/-$ mice.⁶⁶ There was no difference in BrdU incorporation of S-phase cells indicating no increase in actively dividing spermatogonia. The expression of c-kit ligand (*Kit*), which stimulates DNA synthesis in type A spermatogonial cells, was doubled compared with WT. However, the expression of the FSH-responsive gene cAMP responsive element modulator (*Crem*), which is necessary for spermiogenesis, was unaltered.²⁷ Elongated spermatids had reduced nuclear compaction, and there was evidence for inadequate condensation of sperm chromatin and poor sperm quality.⁶⁶ Despite these abnormalities in sperm, litter size of *Fshr* $-/-$ males was not different from wild-type or heterozygous mice as reported by Abel et al,²⁶ although Dierich et al²⁷ found that matings between heterozygous males and females resulted in a 35% to 50% litter size. Overall, the fertility of *Fshr* $-/-$ male mice was more impaired than that of *Fshb* $-/-$

— mice and was thought to be due to the reduced testosterone levels in *Fshr* $-/-$ mice.²⁷

Interestingly, *Fshb* $-/-$ and *Fshr* $-/-$ males both have small testes, but *Fshb* $-/-$ males have normal serum testosterone and LH levels, while *Fshr* $-/-$ males have reduced testosterone and elevated LH levels. Baker et al⁴⁷ compared Leydig cell function in these 2 KOs. Although *Fshb* $-/-$ males had normal intratesticular testosterone and Leydig cells over time, *Fshr* $-/-$ males had a reduction in intratesticular testosterone at 60 and 180 days (about one-third of the controls) and a reduction in Leydig cells at 20 (30% of controls) and 60 days (60% of controls). Similar to *Fshb* $-/-$ males, *Fshr* $-/-$ males demonstrated an impaired intratesticular testosterone response to hCG. There was a reduction of P450_{scc} (*Cyp11a1*), 3 β HSD6 (*Hsd3b6*), and StAR (*Star*) expression, but no change in P450c17 (*Cyp17a1*) or 17 β HSD3 (*Hsd17b3*).⁴⁷ They hypothesized that constitutively active FSHR in *Fshb* $-/-$ male mice could regulate Leydig cell function and explain the different phenotypes. When the WT *Fshr* was transfected into a Leydig cell line, cAMP and progesterone were both upregulated 4-fold. These authors suggested that FSH signaling is not needed for the fetal population of Leydig cells (starting $\sim$ day 5-10), as evidenced by normal Leydig cell number for both *Fshb* $-/-$ and *Fshr* $-/-$ at days 1 and 5.⁴⁷ However, the adult population of Leydig cells was diminished by day 20 and 60 in *Fshr* $-/-$ males (and not *Fshb* $-/-$ males), but the constitutive action of the WT FSHR in *Fshb* $-/-$ mice permitted sufficient signaling to effect normal function. Since only Sertoli cells (and not Leydig cells) express the FSHR, this is likely a permissive action of FSH upon Leydig cell function.⁴⁷

Human inactivating FSHR mutations. Tapanainen et al⁶⁷ reported the phenotypic findings of 5 men homozygous for the Finnish p.Ala189Val *FSHR* gene mutation who were identified from studies of ovarian failure families described above (Table 7, Figure 2). They all had normal puberty, with normal to small testes ranging from 4 to 15 cc (normal: 15-25 cc) and normal testosterone levels. All 5 had elevated FSH levels, with normal to elevated LH levels. In all, 2 males each had 2 children, but all 5 had abnormal sperm parameters with concentrations ranging from < 0.1 million/mL to 42 million/mL (normal > 20 million/mL). Another 151 Finnish men with moderate-to-severe spermatogenic failure were studied for *FSHR* gene mutations but no homozygotes were identified. These authors suggested that FSH function may not be essential for spermatogenesis since fertility occurred in some men despite oligospermia.⁶⁷ No other inactivating *FSHR* mutations have been described in men (<http://omim.org/entry/136435>).

Activating Mutations of the Receptor

Overexpression of FSH Using Transgenic Mice

In order to study how a gain of FSH function affects reproduction, transgenic mice were generated that ectopically produce FSH.²⁸ In these experiments, metallothionein 1 promoter-

Table 7. Inactivating *FSHR* Mutations in Males.^a

Author	<i>FSHR</i> Mutation	Pt.	Age	Testes (15-25 cc)	Sperm			FSH (1-10.5 mIU/mL)	LH (1-8.4 mIU/mL)	Testosterone (8.2-34.6 nmol/L)	Inhibin B (76-447 ng/L)	Fertility
					Sperm Conc.	Volume (mL)	% Normal					
Tapanainen 1997 ⁶⁷	p.Ala189Val	1	29	8.6 and 6	42×10^6	1.5	16%	40	11	14.7	53	Not tested
		2	42	8 and 8	$<1.0 \times 10^6$	2.5	16%	21	16	26.2	54	Not tested
		3	45 (30) ^b	4 and 4	$<0.1 \times 10^6$	4.8	Low	24	16	14.5	<15	Infertile
		4	47	15 and 13.8	5.6×10^6	3	Low	13	6	8.8	33	2 children
		5	55	13.5 and 15.8	$<0.1 \times 10^6$	3.3	Low	15	4	15.8	62	2 children

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone.

^a All were homozygous and stated to be normally masculinized.^b Semen analysis was done at the stated age for all except patient #3 who was of age 30 when the semen analysis was performed. Height, weight, presence of gynecomastia, and sperm motility were not mentioned in the publication. To convert ng/dL to nmol/L multiply by 0.03.

driven expression of *Cga* in some mice and *Fshb* in other mice was accomplished. These overexpressing mice were then crossed to produce mice that could form dimeric FSH. Some strains of mice—the weak FSH expressers—had no phenotypic effects but had mildly elevated FSH levels (FSH = 48 mIU/mL in males; and 116 mIU/mL in females). In contrast, the high copy FSH expressers did manifest recognizable reproductive abnormalities. Male transgenic mice were infertile, despite having normal sized testes and increased sperm production (by ~75%). In these mice, FSH levels were markedly elevated (150 000 mIU/mL), as were serum testosterone levels (18-fold increase compared to wild type). No obvious testicular defects were noted on histologic evaluation, but the seminal vesicle size was more than doubled. The infertility in these transgenic mice was thought to be due to either high testosterone levels or altered reproductive behavior. There is precedence for this as it is well known that the administration of exogenous testosterone to human males may act as a contraceptive agent by suppressing LH to decrease sperm concentration.⁶⁸

In contrast, female high copy FSH expressers had markedly elevated FSH levels (360 000 mIU/mL), elevated estradiol, progesterone, and testosterone levels and demonstrated hemorrhagic, cystic ovaries similar to human ovarian hyperstimulation syndrome. Of interest, these female transgenic mice were infertile but usually died because of extreme bladder and kidney enlargement with obstruction.²⁸

Activating Human FSHR Mutations

Males

The first activating *FSHR* mutation was reported by Gromoll et al⁶⁹ who characterized a p.Asp567Gly missense mutation in a hypophysectomized man who fathered children solely with testosterone replacement (Figure 2). The patient had undetectable serum FSH and LH, though his testicular volume was normal and semen analysis parameters were essentially normal. In vitro analysis of the mutant *FSHR* revealed cAMP production that was increased by approximately 1.5-fold without ligand.⁶⁹ These investigators⁷⁰ then performed additional innovative studies involving the hypogonadal (hpg) mouse, which is gonadotropin deficient secondary to a partial deletion of *Gnrh1*.⁷¹ They created the p.Asp567Gly transgene and directed its expression using the rat androgen binding protein (*Abp*) gene promoter into the Sertoli cells of hpg mice. The insertion of the transgene resulted in a 2-fold-increased testicular weight, which contained mature Sertoli cells and premeiotic germ cells (absent in controls). Isolated Sertoli cells had a 2-fold increase in basal cAMP production and 1.5-fold increase in FSH-stimulated cAMP levels. Intriguingly, this transgene also resulted in increased testicular production of testosterone, suggesting a Sertoli-cell-mediated paracrine effect upon Leydig cells of the hpg mouse.⁷⁰ These findings provide strong support for constitutive activation of mutant Asp563, a conserved residue that is also susceptible to mutation in the luteinizing hormone receptor (*LHR*) and *TSHR*.⁷⁰ It is interesting that transgenic mouse models have reproduced the clinical phenotype of this missense *FSHR* mutation.^{70,78}

Females

Similar to the phenotype of the overexpressing FSH mouse, human females with *FSHR* mutations manifest spontaneous ovarian hyperstimulation syndrome (sOHSS), previously only thought to be an iatrogenically induced condition in infertile females receiving exogenous gonadotropins for infertility treatment (Table 8). A hyperresponsiveness to gonadotropins in these patients results in excess follicular maturation, supranormal estradiol levels, with resultant ovarian enlargement, ascites, and hydrothorax if severe, as well as an increased risk for congestive heart failure, oliguria, and thrombotic events.⁷⁹ These phenotypic findings are thought to be at least partially due to enhanced vascular endothelial growth factor action as well as alterations in cytokines and renin.⁷⁹ Spontaneous OHSS is generally thought to be rare.

The first 2 descriptions of affected females with activating *FSHR* mutations were reported in back-to-back publications in 2003 (Tables 8 and 9, Figure 2).^{72,73} Vasseur et al⁷² described a Moroccan family in which 3 females manifested sOHSS. The proband was a 25-year-old woman who developed sOHSS in 4 of her 5 pregnancies, 1 of which was severe OHSS with hydrothorax and ascites. Two of her sisters had multiple occurrences of sOHSS during some, but not all of their pregnancies, while a third sister gave no such history. These investigators found that all 3 affected sisters demonstrated heterozygosity for a p.Thr449Ile missense mutation in TMD3 of the *FSHR* gene which was absent in the unaffected sister (Tables 8 and 9).

When this mutant *FSHR* was transfected into COS-7 cells, cell surface expression was normal and there was no constitutive activation basally (without ligand), and there was no difference in cAMP production after FSH administration compared to WT. However, the Thr449Ile mutant demonstrated an increased cAMP response to hCG. The wild-type *FSHR* had little or no activation of cAMP to increasing doses of hCG, but there was a profound increase (>20-fold) in maximal cAMP production of the mutant receptor compared to wild type. This occurred despite the observation that I¹²⁵-labeled hCG did not bind to the mutant receptor; and there was no displacement of I¹²⁵-labeled FSH by hCG. Additionally, TSH had no effect upon this receptor (Table 8). Therefore, this mutant receptor was not constitutively active, but exhibited a loss of binding specificity as it bound hCG in addition to FSH.⁷² It is interesting that although the ECD is responsible for ligand binding, activation of this mutant receptor by hCG without binding suggests an interplay between the 2 regions of the receptor resulting in decreased binding specificity.⁷²

The other activating *FSHR* mutation was reported by Smits et al⁷³ who found a heterozygous p.Asp567Asn in a 40-year-old woman with sOHSS in each of her 4 prior pregnancies (Table 8). This affected residue was located within the sixth transmembrane domain (TMD6) and interestingly was the same wild-type amino acid affected as in the hypophysectomized man (although it was a different mutation). Functional studies revealed that despite a 31% decrease in cell surface

Table 8. Reported Activating FSHR Mutations in Human Patients (Males and Females) Supported by Functional In Vitro Analysis.^a

#	Author	FSHR Mutation			In Vitro Analysis						Constitutively Active and Other
		Nucleotide	Protein	Exon	Protein Region	Binding Capacity	Binding Affinity	Receptor Targeting	Cyclic AMP		
1	Gromoll 1996 ⁶⁹ ; Haywood 2002 ⁷⁰	c.1700A>G	p.Asp567Gly	10	TMD6	ND	ND	ND	Increased 1.5 fold over WT in COS; Tg mouse Sertoli cells of hpg mice increased 2-fold basally and 1.5 fold after FSH	Yes; 2-fold increase in testes weights; increased testosterone in absence of FSH/LH	
2	Vasseur 2003 ⁷²	c.1346C>T	p.Thr449Ile	10	TMD3	Normal	Normal	Normal; No direct hCG binding; no displacement of labeled FSH to hCG	Normal basally and after FSH vs WT; >20-fold increase to hCG; No effect by TSH	No	
3	Smits 2003 ⁷³ ; Olatunbosun 1996 ⁷⁴	c.1699G>A	p.Asp567Asn	10	TMD6	ND	Normal	Decreased cell surface expression	3-fold increase basally without hormone; Increase similarly to WT with FSH; increase in response to hCG (1/1000 as sensitive as LHR); 3-fold increase to TSH	Yes	
4	Montanelli 2004 ⁷⁵	c.1455A>G	p.Thr449Ala	10	TMD3	ND	ND	Mutant had 2-fold increased receptor expression	2.5-fold constitutive increase in cAMP; response to rFSH similar to WT; increase in response to hCG (1/1000 as sensitive as LHR); increase to TSH by 2-fold	Yes	
5	De Leener 2006 ⁷⁶	c.1634T>C	p.Ile545Thr	10	TMD5	ND	Markedly decreased	23% reduction in cell surface expression	30% increase in basal cAMP (no hormone); 2-fold increase in cAMP with hCG or TSH	Yes	
6	De Leener 2008 ⁷⁷	c.383C>A	p.Ser128Tyr	5	ECD	ND	ND	64% reduction of WT FSHR expression	No difference in cAMP without hormone; not sensitive to TSH; decreased specificity of the mutant FSHR toward hCG with an increase in sensitivity and affinity toward hCG	No	

Abbreviations: ECD, extracellular domain; ECL, extracellular loop; ICL, intracellular loop; ND, not determined; TMD, transmembrane domain; WT, wild type; hCG, human chorionic gonadotropin; TSH, thyroid-stimulating hormone; cAMP, cyclic adenosine monophosphate; rFSH, recombinant follicle-stimulating hormone.

^a Case 1 is a male, whereas cases 2 to 6 are females. Hpg, hypogonadal mouse, which has gonadotropin deficiency secondary to a deletion in *Gnrh1*. All experiments were performed using COS-7 cells unless otherwise noted.

Table 9. The Phenotypes of the Females With Activating FSHR Mutations Supported by Functional Analysis.

#	Author	FSHR Mutation	Age	Race	Pregnancies	Pregnancy Outcome	Other	Family History
1	Vasseur 2003 ⁷²	p.Thr449Ile	25	Moroccan	4/5 pregnancies affected 1. Ascites and bilateral ovarian masses after TAB at 8 weeks 2. SAB at 6 weeks—no OHSS 3. Diagnosed 8 weeks and lasted all pregnancy 4. TAB for OHSS at 9 weeks 5. 8 weeks; 14 cm ovaries, which stayed large all pregnancy	TAB SAB 2450 g female SGA TAB Delivered normal female	hCG normal, FSH appropriately suppressed; estradiol high only in first TM; normal free T; inhibin B markedly elevated, but declined with hCG drop Normal ovarian and adrenal hormones No fetal anomalies; ovaries normal 12 weeks postpartum; polycystic appearing ovaries Multiple paracenteses yielded 11 L of ascites; normal TSH, hCG, Hct; ovaries regressed to normal size 8 weeks after D&C Infertility evaluation showed PCO appearing ovaries; CD#3 FSH = 7mIU/mL; LH = 18 mIU/mL	Two affected sisters: 1) 4/7 pregnancies and always began in 1st TM; 2) 2/4 pregnancies affected; One unaffected sister had 2 normal pregnancies
2	Smits 2003 ⁷³	p.Asp567Asn	40	Canadian	4/4 pregnancies affected 1. Age 24, multicystic, enlarged ovaries, acne; theca lutein cysts 2. Age 24, multicystic, enlarged ovaries 3. Age 29, at 9 weeks, hydrothorax, ascites, bilateral multicystic ovaries 4. Age 31, infertility evaluation showed PCO appearing ovaries	Delivered term healthy 2635 g female Death in utero 41.5 weeks SAB at 10.5 weeks		
3	Montanelli 2004 ⁷⁵ ; Di Carlo 1997 ⁸⁰	p.Thr449Ala	24	Italian	1. Age 24, pain and enlarged multicystic ovaries at 10 weeks; uncertain if ascites 2. Age 26, pain and dyspnea at 10 weeks; 11X10X9 cm ROV; 13X11X10 LOV; ascites, hemoconcentration (severe) 1. Normal pregnancy	Delivered term healthy 3050 g female Delivered a term healthy 3220 gram male Healthy male	25 days to resolution	Affected sister with severe OHSS who delivered 3470 g male
4	De Leener 2006 ⁷⁶ ; Chae 2001 ⁸¹	p.Ile545Thr	35	Korean	2. 12 weeks, she had severe OHSS 15 × 11 × 11 cm ROV; 12 × 9 × 12 cm LOV—both ovaries multilobulated 7 cystic; ascites was managed with 1000 mL of 5% dextrose/sodium; 100 mL of 20% albumin and 500 mL of dextran Presented with lower abdominal pain, distention, nausea, shortness of breath, and anuria for 24 hours; 11 week IUP with massive ascites and bilateral multilobulated cystic ovaries both 12 × 9 cm. Hemoconcentration, low sodium; high potassium; slightly elevated serum hepatic transaminases	Healthy 3045 gram female at 41 4/7 weeks Normal term vaginal delivery of male	Symptoms regressed in 2nd and 3rd TM and resolved postpartum; 22 1/7 weeks ROV was 9 × 4 × 5 cm; LOV was 8 × 4 × 6 cm, and small amount of ascites Treated with normal saline (1 L/d) and 20% albumin (200 mL/day); felt better within 2 days and discharged on 10th day; Ovaries were normal in 3 months.	Unremarkable

Abbreviations: ND, not determined; LOV, left ovary; ROV, right ovary; SAB, spontaneous abortion; TAB, therapeutic abortion; TM, trimester; OHSS, ovarian hyperstimulation syndrome; hCG, human chorionic gonadotropin; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone.

expression, the mutant exhibited a 3-fold basal increase in cAMP production relative to the wild type. Binding affinities were similar to the WT with increasing doses of exogenous FSH, as were the maximal responses for the mutant and wild-type receptors. However, there was an enhanced response of the mutant receptor, but not WT, to hCG. This response to hCG was only about 1 of 1000 as sensitive as the cAMP response of the LHR to hCG. Unlike p.Thr449Ile, the p.Asp567Asn mutant displayed constitutive activity, and an increased sensitivity to TSH (Table 8).⁷³

Since these 2 studies, 3 additional cases were reported, each of whom had heterozygous activating *FSHR* mutations (Tables 8 and 9, Figure 2).⁷⁵⁻⁷⁷ The p.Thr449Ala in TMD3 described by Montanelli et al⁷⁵ was constitutively active and displayed increased sensitivity to hCG and TSH (Table 8). Montanelli et al⁸³ further studied each the reported activating *FSHR* mutations (p.Thr449Ile, p.Asp567Asn, and p.Thr449Ala) and showed that these 3 mutations affect the interhelical bonds between transmembrane helices which are known to keep the receptor in an inactive state.

In a patient with first trimester sOHSS and normal hCG levels, in 2006 De Leener et al⁷⁶ characterized a p.Ile545Thr in TMD5 with a modest (30%) increase in basal cAMP activity, which was further increased by hCG or TSH (case 5 in Table 8). The sOHSS patients with reported *FSHR* mutations to this point all had normal hCG levels during pregnancy. These investigators⁷⁶ studied 7 additional sOHSS patients with elevated hCG ($n = 4$) or TSH ($n = 3$) levels in pregnancy but did not find *FSHR* mutations, suggesting a natural stimulation of the WT receptor to high concentrations of the hormones. These studies indicate that not all patients with sOHSS have activating *FSHR* mutations or that they were not identified using current methodology. Whether these patients might possess mutations in regulatory regions of the receptor or have intragenic deletions or rearrangements not detectable by PCR-based DNA sequencing remains to be determined.

All reported activating *FSHR* mutations were localized to the transmembrane regions of the FSHR until 2008 when De Leener et al⁷⁷ discovered the first mutation in the ligand binding domain—a heterozygous p.Ser128Tyr missense mutation (Tables 8 and 9, Figure 2). Functional analysis of the p.Ser128Tyr mutation resulted in a 64% reduction in cell surface expression, and there was no constitutive activation. However, the mutant receptor had increased affinity and sensitivity to hCG but not to TSH (Table 8).

When the identified heterozygous, activating *FSHR* mutations in 5 females with sOHSS are examined, it can be seen that 4 are in the serpentine region of the receptor and only 1 is in the ECD (Figure 2, cases 2-6 in Table 8). In fact 4 of the 6 activating *FSHR* mutations (when the 1 male is also included) occur in either the p.Asp567 (changing it to either a Gly or Asn) or a Thr449 (changing it to either an Ile or Ala). In both cases mutation of p.Asp567 resulted in a constitutively active protein, while Thr449Ala was constitutively active but Thr449Ile was not (Table 8). Overall, 2 of the 5 activating mutations in females (1 in TMD3 and 1 in the ECD) were not

constitutively active. However, the common feature is that all 5 activating *FSHR* mutations, causing OHSS have reduced specificity such that they respond to the increasing hCG levels that occur during normal pregnancy. Perhaps this would be expected in the ascertainment of a phenotype exacerbated in pregnancy. A total of 3 also had an increased response to TSH further, demonstrating the decreased specificity of the mutant receptor.

When the phenotypes of the affected sOHSS women are examined, 2 of the 5 patients had other affected members, indicating autosomal dominant inheritance, while 3 were sporadic (Table 9). Mutations seem to affect different ethnicities. Interestingly, 2 of the patients described had 4 of 5 and 4 of 4 pregnancies affected with sOHSS, but 2 of 5 had some pregnancies that were not affected with OHSS. Therefore, the presence of an activating *FSHR* mutation did not preclude the possibility of an unaffected pregnancy. Overall, in these 5 patients, sOHSS occurred in 12 of 14 pregnancies with varying pregnancy outcomes. All 5 patients had at least 1 healthy live-born child during the cycle of hyperstimulation. Of the 12 pregnancies complicated by sOHSS, 1 resulted in a spontaneous abortion, 2 were electively aborted, 1 resulted in a death in utero at 41½ weeks, and 8 resulted in healthy live-born children—3 males and 5 females (Table 9).

Human FSHB and FSHR Polymorphisms

Single-nucleotide polymorphisms (SNPs) have been identified in the *FSHR* and *FSHB* genes, and there have been a number of studies attempting to associate them to fertility potential. Simoni et al⁸⁴ studied men with infertility and did not find any causative *FSHR* mutations but did characterize 2 SNPs: p.Asn680Ser (rs6166) and p.Thr307Ala (rs6165). These missense variants had no functional effects in vitro; and the prevalence did not differ in fertile versus infertile men.⁸⁴ Since this study, a number of investigators have conducted association studies of *FSHR* polymorphisms, with often contradictory results (reviewed elsewhere^{85,86}). Perez Mayorga et al⁸⁷ first found that the Ser680 allele is associated with higher serum FSH levels and the need for additional exogenous FSH in cycles of controlled ovarian hyperstimulation. In a review and pooling of data from 4 publications regarding Asn680Ser (the best studied of the *FSHR* SNPs), Moron and Ruiz⁸⁶ concluded that the *FSHR* genotype is associated with response to exogenous gonadotropin therapy. The 3 studies showing association with the *FSHR* genotype enrolled patients in Spain,⁸⁸ Greece,⁸⁹ and the Ukraine,⁹⁰ while no association was seen in the Netherlands population.⁹¹ Although promising, the clinical utility of the better studied *FSHR* SNPs, as well as *FSHB* SNPs, requires further investigation.

Immunoneutralization of FSH—Effects Upon Reproductive Phenotype

A number of investigators have shown that continuous bioneutralization of endogenous FSH may affect reproduction in

males and females from a variety of species including rat, monkey, and human. Either passive or active immunization in fertile adult male monkeys has been shown to result in oligospermia and infertility.^{92,93} Moudgal et al⁹³ showed that active immunization with ovine FSH (oFSH) isolated from sheep pituitaries elicited an antigenic response in adult male bonnet monkeys, but it did not affect the health, libido, or testosterone levels of the animals. However, there was a marked decrease (75%-100%) in the number of spermatozoa in seminal ejaculates by 150 days. These monkeys also manifested infertility, which was reversed when booster injections were discontinued.⁹³ Similarly, blockade of FSH receptor in male monkeys by receptor antibody formation also resulted in testicular dysfunction and infertility.⁹⁴ Moudgal et al⁹⁵ injected human males with oFSH, and they produced specific antibodies capable of binding and neutralizing FSH bioactivity for 1 complete sperm cycle (~65 days). Seminal plasma transferrin, a marker of Sertoli cell and seminiferous tubular function, was markedly reduced (30%-90%) and sperm counts were reduced (30%-74%) in some patients.⁹⁵

To examine the spermatogenic dysfunction in more detail, Meachem et al⁹⁶ used immunoneutralization with a polyclonal rat FSH antibody in male rats and demonstrated a significant reduction in the number of type A and B spermatogonia, pachytene spermatocytes, round spermatids, and elongated spermatids after 8.5 days of treatment. Krishnamurthy et al⁹⁷ studied bonnet monkeys and normal, healthy fertile men that were actively immunized with oFSH and showed that sperm exhibited defective chromatin packaging and had a reduction in acrosomal glycoprotein content. Interestingly, these alterations in sperm quality occurred well in advance of decreased sperm counts in the ejaculate.⁹⁷

Most of these studies have involved males. However, Ferro et al⁹⁸ conjugated a variety of FSH β peptides to tetanus toxoid, which were used to immunize female rats. All animals showed reduced estradiol levels and had disrupted estrous cycles. Similarly, Butterstein et al⁹⁹ generated antibodies against human FSH β that were administered to mature Sprague-Dawley rats. Continuous exposure to immunoneutralizing antibodies disrupted estrous cycles in all animals, but only animals with titers high enough to completely block binding of FSH to its receptor demonstrated infertility.⁹⁹ Collectively, these findings provide additional support that FSH is essential for gonadal function in murine and primate species.

Synthesis of Human and Mouse Findings

Inactivating Mutations

Females. A small number of inactivating mutations in both the *FSHB* and the *FSHR* genes in humans have been described. The effect of mutations of the *FSHB* gene in human females is very similar to that described in *Fshb* $-/-$ KO mice. These women had irreversibly delayed puberty and infertility with low serum FSH levels and elevated LH levels. *Fshb* $-/-$ mice also were sterile with low FSH and elevated LH. Estradiol

levels were low in the human patients; and although reportedly normal, they were likely low in the *Fshb* $-/-$ mice, particularly given the appearance of the reproductive organs. Histologically, these mice had predominantly primordial follicles, although some degenerating antral follicles were present. Human females with *FSHB* mutations demonstrated primordial and small antral follicles. However, both mice and humans responded to exogenous FSH, indicating that even with endogenous depletion of the FSH ligand, the ovaries have the capacity to activate FSH signaling (except in the 1 patient who was older).

The phenotypes of women with inactivating *FSHR* mutations and the *Fshr* $-/-$ KO mice were quite similar. The mice were sterile, while the women all had primary amenorrhea (with or without breast development). Both humans and mice had elevated serum gonadotropin levels. Most women with *FSHR* mutations had low or undetectable estradiol levels. The female *Fshr* $-/-$ KO mice had low estradiol and progesterone, but elevated testosterone (not present in the humans with *FSHR* mutations); and these mice had no vaginal openings (indicating no puberty). The mice demonstrated primordial, primary, and secondary follicles, but antral follicles were not observed. Rarely, humans with *FSHR* mutations also had further follicular development with at least 1 of the Finnish patients having mature preovulatory follicles.

Women with inactivating *FSHB* or *FSHR* gene mutations possess mostly primordial follicles but may also have antral follicles. Therefore, FSH does not appear to be necessary for follicular development up to the small antral follicle stage but seems to be required for further follicular development. Since women with *FSHB* gene mutations have elevated LH levels but do not have hyperandrogenism, FSH may have some requisite function for ovarian thecal androgen production possibly by stimulating production of CYP17, LHR, inhibin, or other growth factors. These findings suggest that FSH is important for normal pubertal development and fertility.

Males. The phenotypic effects of males (human and mice) deficient in FSH are somewhat disparate, particularly for the ligand. Male *Fshb* $-/-$ KO mice demonstrated smaller testes with a 75% reduction in sperm concentration and 40% reduction in motility, but they were fertile. Serum FSH was low, but LH was normal as was testosterone. All 3 human males with *FSHB* mutations had low FSH, elevated LH, and azoospermia. Interestingly, 2 males had small testes, but one of them also had low testosterone, suggesting that FSH deficiency could play some role in androgen production in the male.

Mutations in the receptor, whether in the human or mouse, had no demonstrable effect upon fertility in males. *Fshr* $-/-$ KO male mice had a somewhat decreased testes size and a modest reduction in sperm concentration, motility, and morphology. As expected, serum FSH was elevated, but perhaps unexpectedly, serum testosterone was reduced by 65%. There have only been 5 human males with *FSHR* mutations—all ascertained in Finnish families with the p.Ala189Val mutation found in women with ovarian failure. They all had elevated serum FSH, normal to elevated LH, and normal testosterone

levels. Their ages and sperm parameters were quite variable, but 2 of the 3 attempting fertility had children.

These findings in males suggest that perhaps there are species-specific differences between mice and men. In humans, mutations in the ligand gene are more severe than those of the receptor, while the opposite is true for the mouse. The endocrine function of human *FSHB* mutants may be more adversely affected than the human *FSHR* or either of the mouse genes since 1 of the 3 males with an *FSHB* mutation had low testosterone and delayed puberty. In the mouse, the *Fshb* $-/-$ mouse may have a less severe phenotype because of the constitutively active *FSHR*, which may result in some FSH function without the presence of the ligand. However, both the *Fshb* $-/-$ and *Fshr* $-/-$ mice have decreased germ cell and Sertoli cell numbers (30%-40%). These studies also suggest that FSH is necessary in both males and females to augment LH-induced synthesis of androgens.

Inactivating *FSHR* and *FSHB* gene mutations in humans are both inherited as autosomal recessive traits, and they produce a somewhat similar phenotype in that they result in hypogonadism. However, both gonadotropins are elevated in patients with *FSHR* mutations (hypergonadotropic hypogonadism), while patients with *FSHB* mutations manifest reduced FSH levels (isolated FSH deficiency) and elevated (or normal) LH levels. Women with *FSHR* or *FSHB* mutations may have completely absent pubertal development or partial development. The difference lies in the reproductive potential of these patients with treatment as women with *FSHB* mutations have normal ovarian function and an excellent chance to conceive with exogenous gonadotropins; while women with *FSHR* mutations have impaired ovarian function and a poorer prognosis, as do other women with ovarian failure from any cause.

Activating *FSHR* Mutations

The creation of transgenic mice overexpressing human *Fshb* produced variable phenotypes depending upon the level of expression and the sex of the animal. With low *Fshb* expression, elevated FSH protein levels were observed similar to humans with gonadal failure, but the phenotype was normal in both male and female mice. However, with high *Fshb* expression, females demonstrated infertility and had enormously elevated levels of FSH with hemorrhagic ovarian cysts similar to iatrogenic human OHSS. Subsequently, human females with activating *FSHR* mutations were shown to have the same phenotype as the mouse. Although they are usually constitutively active, activating human *FSHR* mutations causing sOHSS have uniformly altered the receptor such that it responds to hCG, which is why ovarian enlargement is restricted to pregnancy. This sOHSS is inherited as an autosomal dominant trait. Therefore, hypofunction of both the ligand and the receptor are inherited as autosomal recessive disorders, while hyperfunction of the receptor is autosomal dominant, similar to the genetics of human *LHR* mutations. Lastly, when the single activating *FSHR* mutation in a human male was inserted into Sertoli cells of the hpg mouse,

testosterone production increased. These data, in addition to that discussed above, provide further support that FSH action is necessary for LH-dependent androgen production in males as well as females.

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