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MINI REVIEW

Genetic variants of G-protein coupled receptors associated with pubertal disorders

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Abstract

Background: The human hypothalamic–pituitary-gonadal (HPG) axis is the regulatory center for pubertal development. This axis involves six G-protein coupled receptors (GPCRs) encoded by *KISS1R*, *TACR3*, *PROKR2*, *GNRHR*, *LHCGR*, and *FSHR*.

Methods: Previous studies have identified several rare variants of the six GPCR genes in patients with pubertal disorders. In vitro assays and animal studies have provided information on the function of wild-type and variant GPCRs.

Main Findings: Of the six GPCRs, those encoded by *KISS1R* and *TACR3* are likely to reside at the top of the HPG axis. Several loss-of-function variants in the six genes were shown to cause late/absent puberty. In particular, variants in *KISS1R*, *TACR3*, *PROKR2*, and *GNRHR* lead to hypogonadotropic hypogonadism in autosomal dominant, recessive, and oligogenic manners. Furthermore, a few gain-of-function variants of *KISS1R*, *PROKR2*, and *LHCGR* have been implicated in precocious puberty. The human HPG axis may contain additional GPCRs.

Conclusion: The six GPCRs in the HPG axis govern pubertal development through fine-tuning of hormone secretion. Rare sequence variants in these genes jointly account for a certain percentage of genetic causes of pubertal disorders. Still, much remains to be clarified about the molecular network involving the six GPCRs.

KEYWORDS
gene, G-protein coupled receptor, hypothalamic–pituitary-gonadal axis, puberty, variant

1 | INTRODUCTION

G-protein-coupled receptors (GPCRs) are integral membrane proteins that function as transmitters of extracellular stimuli to intracellular signaling pathways.[1–3] GPCRs consist of an extracellular N-terminus, seven transmembrane domains connected by intracellular and extracellular loops, and an intracellular C-terminus.[1,2] More than 800 GPCRs are encoded in the human genome.[4,5] Natural ligands of GPCRs include small peptides, lipids, ions, odorants, and large glycoproteins.[4] GPCRs play particularly important roles in the neurosensory and endocrine systems.[3] For example, the hypothalamic–pituitary-gonadal (HPG) axis in humans includes at least six GPCRs, that is, gonadotropin-releasing hormone (GnRH) receptor [GNRHR; alias, luteinizing hormone (LH)-releasing hormone receptor], kisspeptin receptor (KISS1R; formally known as GPR54), tachykinin receptor 3 (TACR3; alias, neurokinin 3 receptor), prokineticin receptor 2 (PROKR2; alias, GPR73b or GPR73L1), LH/chorionic gonadotropin receptor (LHCGR), and follicle-stimulating hormone

(FSH) receptor (FSHR). Genetic defects in the six GPCRs, either alone or in combination with other gene variants, lead to pubertal disorders.[6–11] This mini review summarizes the current understanding of naturally occurring GPCR variants associated with pubertal disorders.

2 | FUNCTIONS OF GPCRS

Upon binding to their ligands, GPCRs undergo conformational changes[4] and bind to heterotrimeric G proteins consisting of α, β, and γ subunits, as well as to other intracellular proteins.[5] This receptor-protein binding stimulates the production of second messengers and thereby activates various signaling pathways.[1,5] Subsequently, activated GPCRs are phosphorylated and undergo internalization and desensitization.[5] The desensitization process is mediated through binding to β-arrestin and other proteins.[12] Collectively, the function of GPCRs requires interactions with several other molecules.[13] Since many GPCRs act as oligomers, oligomerization appears to be one of the regulatory mechanisms of receptor activity.[4,14]

To date, more than 2000 variants in 55 GPCR genes have been reported as the causes of 66 human disorders.[15] Most of these variants are nucleotide substitutions or indels in the coding regions; however, other genetic abnormalities, such as copy-number variations of exons and mutations in the non-coding regions, were also reported.[15] The majority of known pathogenic variants are loss-of-function mutations in the germline that affect mRNA expression, ligand binding, signal transduction, or protein stability.[16] In addition, a few gain-of-function variants in the germline or somatic cells have been documented as the cause of 14 disorders.[6,15] These gain-of-function variants were shown to induce constitutive activity, broad ligand specificity, or high ligand sensitivity.[6,15] In addition, impaired desensitization and paradoxical activation of the co-existing wild-type receptor were also reported as novel mechanisms of gain-of-function of GPCRs.[6,17]

3 | GPCRS IN THE HPG AXIS

The HPG axis is the regulatory center for pubertal sexual maturation.[18–20] This axis comprises at least six GPCRs and their ligands, together with several other molecules such as FGFR1, ANOS1 (KAL1), and MKRN3.[4,20–22] The six GPCRs are GNRHR, KISS1R, TACR3, PROKR2, LHCGR, and FSHR (Table 1, Figure 1).

The kisspeptin-KISS1R and tachykinin 3 (TAC3)-TAC3R systems are predicted to reside at the top of the signaling cascade in the HPG axis (Figure 1).[10] In humans, kisspeptin neurons are present in the preoptic area and infundibular nucleus of the hypothalamus.[23] Kisspeptin neurons in the infundibular nucleus, which are designated as KNDy neurons, co-express TAC3 and dynorphin.[23–25] The kisspeptin-KISS1R and TAC3-TAC3R systems produce pulsatile secretion of GnRH into the portal circulation, which in turn stimulates gonadotropin secretion from the pituitary.[24] The prokineticin

TABLE 1 Known G-protein coupled receptors in the hypothalamic–pituitary-gonadal axis.

Name	Alias	Gene	Location	OMIM[a]	Amino acid length[b]	Major expression site	Cells with strong expression	Major binding protein	Major ligand
Kiss1 receptor	GPR54	*KISS1R*	19p13.3	*604161	398	POA and infundibular nucleus in the hypothalamus	GnRH neuron	Gq/11, (Gs, Gi)	Kisspeptin
Tachykinin receptor 3	Neurokinin 3 receptor, Neurokinin B receptor	*TACR3*	4q24	*162332	465	POA and infundibular nucleus in the hypothalamus	KNDy neuron, GnRH neuron	Gq/11	Tachykinin 3
Prokineticin receptor 2	GPR73b, GPR73L1	*PROKR2*	20p12.3	*607123	384	Hypothalamic regions close to GnRH neurons	PROKR2-expressing neuron	Gq/11, (Gs, Gi)	Prokineticin 2
GnRH receptor	LHRH receptor, GNRHR1	*GNRHR*	4q13.2	*138850	328	Pituitary	Gonadotroph	Gq/11, Gs, Gi	GnRH
LHCG receptor	LH receptor	*LHCGR*	2p16.3	*152790	699	Testis, Ovary	Leydig cell, theca cell	Gs	LH, hCG
FSH receptor		*FSHR*	2p16.3	*136435	695	Testis, Ovary	Sertoli cell, granulosa cell	Gs	FSH

Abbreviations: FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; KNDy neuron, kisspeptin-neurokinin B-dynorphin neuron; LH, luteinizing hormone; LHCG, luteinizing hormone/chorionic gonadotropin; LHRH, LH releasing hormone; POA, preoptic area.

* indicates OMIM numbers.

[a]OMIM, Online Mendelian Inheritance in Man (https://www.omim.org/).

[b]Amino acid lengths are based on the reference data in GeneCards (https://www.genecards.org/).

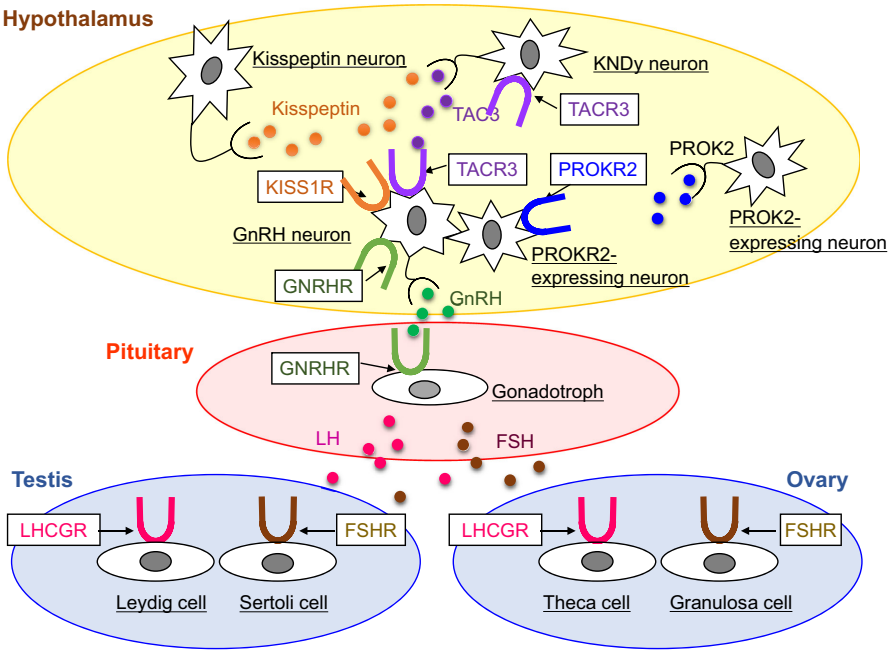

FIGURE 1 Schematic of the human hypothalamic–pituitary-gonadal axis. The six G-protein coupled receptors, that is, gonadotropin-releasing hormone receptor (GNRHR), kisspeptin receptor (KISS1R), tachykinin receptor 3 (TACR3), prokineticin receptor 2 (PROKR2), luteinizing hormone/chorionic gonadotropin receptor (LHCGR), and follicle-stimulating hormone receptor (FSHR), and their ligands are shown.

2 (PROK2)-PROKR2 system is also likely to be involved in the GnRH pulse generator.[26] Gonadotropins secreted from the pituitary gland bind to their receptors in the gonads to stimulate sex hormone production. There are positive and negative feedback loops of sex hormones on gonadotropin secretion.[25]

4 | PUBERTAL DISORDERS

Dysfunction of the HPG axis results in pubertal disorders, which are classified into gonadotropin deficiency (hypogonadotropic hypogonadism), hypergonadotropic hypogonadism (primary gonadal dysfunction), central precocious puberty (CPP), and gonadotropin-independent precocious puberty.[27] Hypogonadism leads to impaired pubertal development in patients of both sexes and can be associated with genital hypomasculinization in neonates with a 46,XY karyotype. Precocious puberty is characterized by early sexual maturation in childhood. Pubertal disorders occur either as isolated endocrinopathies or in combination with other clinical abnormalities. In particular, hypogonadotropic hypogonadism frequently couples with additional clinical features such as anosmia and hearing loss.

Pubertal disorders are multifactorial conditions resulting from various genetic and environmental factors.[28] In particular, CPP often arises from brain lesions.[29] To date, more than 60 genes have been reported as the causative genes of late/absent puberty, whereas only a few genes have been implicated in precocious puberty.[9,11,30,31] Of these, *FGFR1* and *MKRN3* variants are the most common causes of hypogonadotropic hypogonadism and CPP, respectively.[9,32–34] Variants in *KISS1R*, *TACR3*, *PROKR2*, and *GNRHR* were shown to lead to hypogonadotropic hypogonadism as an autosomal dominant, recessive, or oligogenic disorder (Table 2).[9,30] Thus, hypogonadotropic hypogonadism represents a typical oligogenic disorder.[9]

Identification of pathogenic variants in a patient with pubertal disorder enables genetic counseling for the family.[35] Moreover, such information is useful for predicting the prognosis and possible complications of patients. Table 2 and Figure 2 summarize the known pathogenic variants of the six GPCR genes.

5 | GENETIC VARIANTS OF GPCRS ASSOCIATED WITH PUBERTAL DISORDERS

5.1 | *KISS1R* variants associated with pubertal disorders

KISS1R is expressed in various brain tissues including the hypothalamus and pituitary.[36] The kisspeptin-KISS1R system is believed to act directly on GnRH neurons in the preoptic area and infundibular nucleus to produce pulsatile GnRH secretion.[23,36] Animal studies confirmed that most GnRH neurons express KISS1R (Figure 1).[36] In GnRH neurons, KISS1R is likely to act as a homodimer or a heterodimer with GNRHR.[37] The kisspeptin-KISS1R system plays a key role in the positive and negative feedback loops of sex steroids on gonadotropin secretion.[38] Moreover, the system is likely to mediate the functional interaction between the HPG axis and the hypothalamic–pituitary–adrenal axis.[39]

Germline loss-of-function variants of *KISS1R* typically result in normosmic hypogonadotropic hypogonadism and occasionally cause Kallmann syndrome (hypogonadotropic hypogonadism with anosmia).[26] *KISS1R* variants are relatively rare among the genetic causes of hypogonadotropic hypogonadism.[9] Usually, *KISS1R* variants cause the disease phenotype when they are combined with loss-of-function variants in other genes (oligogenicity).[9] However, in some cases, biallelic and monoallelic *KISS1R* variants were identified

TABLE 2 Representative variants in the six GPCR genes involved in human disorders.

	Germline loss-of-function variants			Germline gain-of-function variants				Somatic gain-of-function variants		
Name	Major phenotype	Mode of inheritance[a]	Representative variants[b]	Phenotype	Mode of inheritance	Representative variants[b]	Predicted mechanism	Phenotype	Representative variants[b]	Predicted mechanism
KISS1R	Normosmic HH, Kallmann syndrome	Oligogenic, (biallelic, monoallelic)	L102P, L148S, 150bp deletion, Y313H, R331*, *399R	CPP	Monoallelic	R386P	Impaired desensitization	No report		
TACR3	Normosmic HH	Monoallelic, biallelic, oligogenic	G93D, W208*, Y256H, W275*, P353S, c.737+1G>A	No report				No report		
PROKR2	Kallmann syndrome (normosmic HH)	Monoallelic, (oligogenic)	H20fs*43, R85H, M85C, V115M, R164Q, S188L, Q210R, G229R, M323I, T340S, R353H	CPP	Monoallelic	C242fs*305	Paradoxical activation of coexisting wild-type receptor	No report		
GNRHR	Normosmic HH (Kallmann syndrome)	Biallelic, (monoallelic, oligogenic)	N10K, Q11K, T32A, E90K, Q106R, L117R, A129D, M131T, R139H, S168R, A171T, c.523-1G>A, S217R, R262Q, T269M, T281I, Y284C, L314*, P320L	No report				No report		
LHCGR	Hypergonadotropic hypogonadism	Biallelic	Q18_P19insLLKLLLLLQLQ, R124*, C131R, V144F, E148*, F194V, C343S, E354K, L502P, Q525*, C543R, C545*, R554*, A593P, V609_L610del, S616Y, I625K	Male-limited precocious puberty	Monoallelic	L368P, A373V, M398T, L457R, I542L, D564G, A568V, M571I, A572V, I575L, T577I, D578G, D578Y	Increased constitutive activity	Leydig cell adenoma	D578H	Increased constitutive activity
FSHR	Spermatogenic failure, ovarian dysgenesis	Biallelic	I160T, A189V, V221G, D224V, P348R, A419T, P519T, R573C, A575V, P587H, L601V	Ovarian hyper-stimulation	Monoallelic	S128Y, T449I, T449A, I545T, D567N	Increased constitutive activity, broadened ligand sensitivity	No report		

Abbreviations: CPP, central precocious puberty; GPCR, G-protein coupled receptor; HH, hypogonadotropic hypogonadism.

* indicates the stop codon.

[a] Relatively minor forms are shown in parentheses.

[b] Representative variants that have been submitted to OMIM (Online Mendelian Inheritance in Man, https://www.omim.org/) and HGMD (Human Gene Mutation Database, https://www.hgmd.cf.ac.uk/ac/all.php) databases as pathogenic or likely_pathogenic are shown.

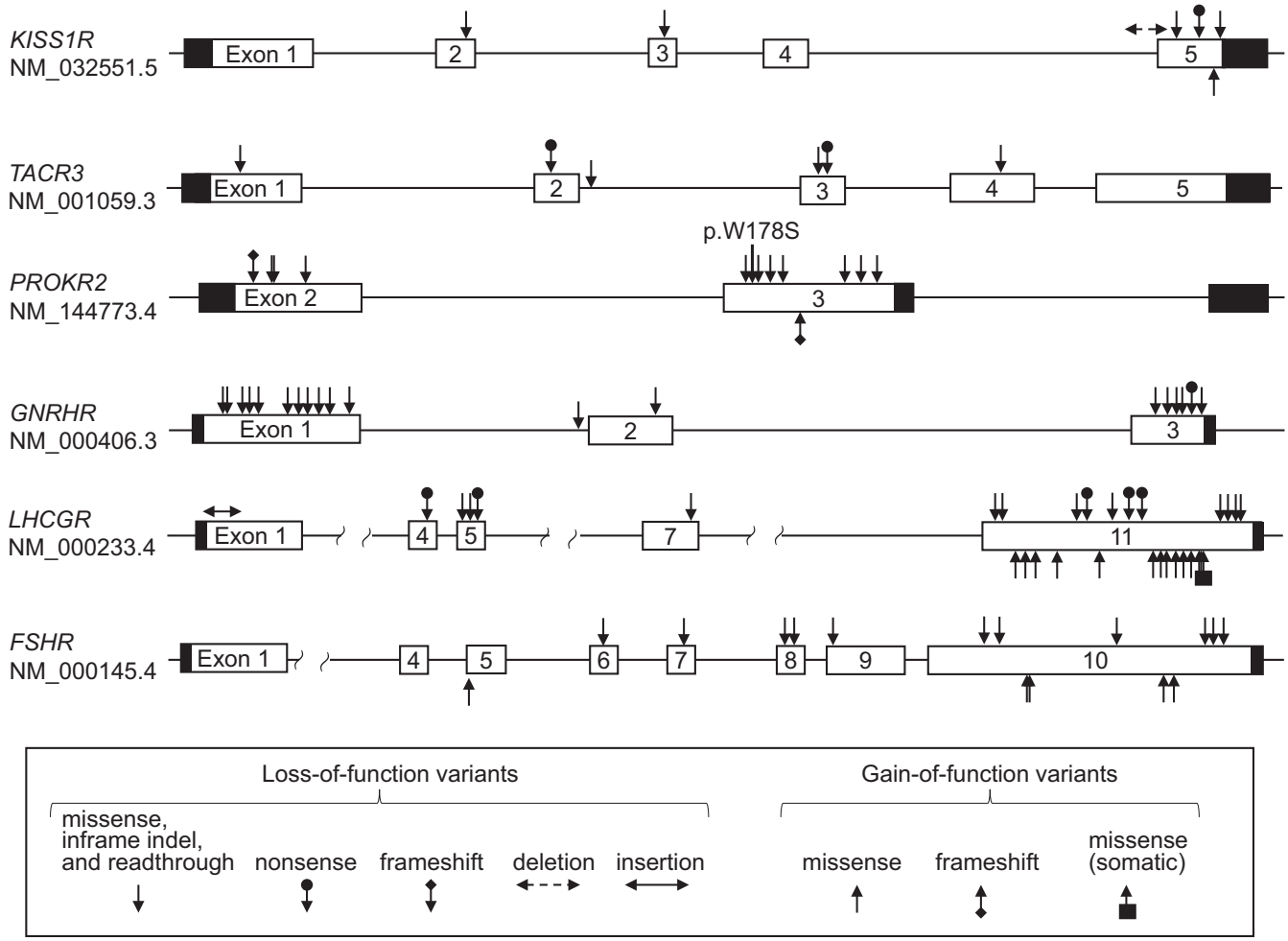

FIGURE 2 Representative pathogenic variants in the six G-protein coupled receptor genes. The "pathogenic" and "likely-pathogenic" variants submitted to Online Mendelian Inheritance in Man (https://www.omim.org/) and Human Gene Mutation Database (https://www.hgmd.cf.ac.uk/ac/all.php) are shown. The p.W178S variant in *PROKR2*, a founder mutation in China, is indicated. The black and white boxes depict the non-coding and coding regions, respectively. The sizes of the exons and introns are not drawn to scale.

as the sole genetic abnormalities.[9] Known loss-of-function variants were detected in various exons of *KISS1R* (Figure 2). Furthermore, loss-of-function variants of *KISS1* encoding the ligand of KISS1R have been reported as a rare cause of normosmic hypogonadotropic hypogonadism.[40]

A germline gain-of-function variant of *KISS1R* has been linked to CPP. In 2008, the heterozygous p.R386P variant of *KISS1R* was identified in a girl who presented with slowly progressive breast development from birth.[41] Her sexual maturation further accelerated from 7 years of age. In vitro assays showed that the *KISS1R* variant decreased receptor degradation, and thereby extended ligand-induced signaling. Subsequent studies identified a few additional rare variants of *KISS1R* in patients with CPP; however, the pathogenicity of these variants remains unknown.[42] Considering that only one variant was definitely linked to CPP, the role of *KISS1R* variants in the etiology of CPP appears to be small. Likewise, although some rare variants of *KISS1* have been identified in patients with CPP, the association between these variants and the phenotype is uncertain.[34,43]

5.2 | *TACR3* variants associated with pubertal disorders

TACR3 is expressed in the hypothalamus and other brain tissues.[10] Particularly strong expression of *TACR3* is detected in KNDy neurons.[10] TACR3 binds to TAC3 (also known as neurokinin B or neurokinin 3), and to a lesser extent, to neurokinin A and substance P.[44] The TAC3-TACR3 system is an integral part of the GnRH pulse generator.[10] Since the administration of exogenous kisspeptin was shown to increase LH secretion in patients with *TACR3* abnormalities, the TAC3-TACR3 system may reside upstream of the kisspeptin-KISS1R system.[24] However, the precise interaction between these two systems has yet to be clarified.

Germline loss-of-function variants of *TACR3* account for a certain percentage of the genetic causes of normosmic hypogonadotropic hypogonadism.[9,30] Autosomal dominant inheritance due to monoallelic variants appears to be the most common form of TACR3 deficiency, although biallelic and oligogenic variants were also reported.[9] Known pathogenic *TACR3* variants are widely distributed

across the gene (Figure 2). Interestingly, spontaneous improvement of reproductive function was documented in several male patients with pathogenic *TACR3* variants.[24] These findings may reflect the functional redundancy of tachykinin receptors. In addition, oligogenic loss-of-function variants of *TAC3* encoding the TACR3 ligand were identified in a few patients with hypogonadotropic hypogonadism.[9] The phenotype of patients with *TAC3* variants tended to be milder than that of patients with *TACR3* variants,[24] indicating compensatory roles of TAC1, TAC2, and TAC3. To date, there are no reports of gain-of-function variants of *TACR3* or *TAC3*.

5.3 | *PROKR2* variants associated with pubertal disorders

PROKR2 is strongly expressed in the central nervous system and weakly expressed in other tissues.[26] Particularly strong expression of *PROKR2* and *PROK2* was observed in hypothalamic areas where GnRH neurons accumulated.[26] *PROKR2* is also expressed in the neural precursor of the olfactory bulb.[26] These expression patterns are consistent with the significant roles of PROKR2 and PROK2 in the migration of GnRH neurons and the formation of olfactory bulbs. Notably, however, *PROKR2* expression is absent in mature GnRH neurons.[26] Hence, it remains unknown how the PROK2-PROKR2 system governs pulsatile GnRH secretion during puberty and reproductive ages. Moreover, the functional interactions among the PROK2-PROKR2, kisspeptin-KISS1R, and TAC3-TACR3 systems in the hypothalamus have yet to be elucidated.

Germline loss-of-function variants of *PROKR2* typically result in Kallmann syndrome and less frequently in normosmic hypogonadotropic hypogonadism.[9] Monoallelic variants of *PROKR2* appear to be sufficient to cause these phenotypes, although oligogenic variants were reported in some cases.[9] Pathogenic *PROKR2* variants account for a substantial fraction of the genetic causes of hypogonadotropic hypogonadism.[9,30] Reportedly, the frequency of *PROKR2* variants in patients with hypogonadotropic hypogonadism is particularly high in China, Japan, and Taiwan.[33] Of these, the p.W178S variant is regarded as a founder mutation in China.[33] Furthermore, loss-of-function variants of *PROK2* encoding the PROKR2 ligand cause hypogonadotropic hypogonadism in autosomal dominant, recessive, and oligogenic manners.[9] Notably, patients carrying pathogenic variants in *PROK2* or *PROKR2* frequently exhibit additional clinical features such as hearing loss, synkinesia, and obesity, suggesting multiple roles of the PROK2-PROKR2 system in the brain.[26,45] Moreover, gonadal dysfunction was frequently described in patients with PROKR2 abnormalities.[26] Indeed, *PROKR2* is strongly expressed in the testis and is likely to be involved in gonadal function.[26,45] Notably, some patients with pathogenic *PROKR2* variants exhibited a reversal of GnRH deficiency during sex hormone treatment.[26] However, the mechanism of this recovery remains unknown.

In 2017, we identified a heterozygous p.C242fs*305 variant in *PROKR2* in a girl with CPP.[17] She manifested early breast budding and accelerated growth at 3.5 years of age. Blood examination showed increased responses of gonadotropins to GnRH stimulation. The mutant PROKR2 lacked the last two transmembrane domains and the C-terminal domain. In vitro assays revealed that, although the mutant PROKR2 had no signal transduction activity, cells co-transfected with the mutant and wild-type proteins exhibited higher ligand-induced signal activity than cells transfected with the wild-type protein alone. These data suggest that the mutant PROKR2 caused aberrant gonadotropin secretion through paradoxical activation of the co-existing wild-type protein. These findings suggest a novel gain-of-function mechanism of GPCRs. However, because the p.C242fs*305 variant was shared by the patient's mother who had no history of CPP, its pathogenicity needs to be confirmed in future studies. Thus far, no further gain-of-function variants of *PROKR2* have been identified in patients with CPP.

5.4 | *GNRHR* variants associated with pubertal disorders

GNRHR is a unique GPCR that lacks the intracellular carboxyl terminus.[10] *GNRHR* is strongly expressed in pituitary gonadotrophs and modulates the synthesis and secretion of LH and FSH.[10,37] Since continuous administration of GnRH reduces gonadotropin secretion,[46] pulsatile stimulation of GNRHR appears to be critical for pubertal development. *GNRHR* is also expressed in GnRH neurons in the hypothalamus.[37] In these neurons, GNRNR can heterodimerize with KISS1R.[37] Thus, the autocrine action of GnRH is assumed to contribute to pulsatile GnRH secretion.[37]

Germline loss-of-function variants of *GNRHR* are relatively common causes of normosmic hypogonadotropic hypogonadism.[9,33] In particular, *GNRHR* is the most frequently mutated gene in patients with normosmic hypogonadotropic hypogonadism in India.[33] *GNRHR* variants typically cause the phenotype as an autosomal recessive disorder, although autosomal dominant and oligogenic inheritances were observed in some cases.[9] *GNRHR* variants are associated with variable degrees of gonadotropin deficiency, and have also been identified in some cases with Kallmann syndrome.[10,30] Known pathogenic variants are widely distributed in exons (Figure 2). In vitro assays confirmed the impaired function of several mutant proteins. Interestingly, recent studies have suggested that more than half of the mutant variants of *GNRHR* cause protein misfolding and endoplasmic reticulum retention.[2] Genetic defects of *GNRH1* encoding the ligand of GNRHR were also reported to cause hypogonadotropic hypogonadism in some cases.[47] On the other hand, gain-of-function variants of *GNRHR* have not yet been reported.

5.5 | *LHCGR* variants associated with pubertal disorders

LHCGR is characterized by a large N-terminal extracellular domain containing several leucine-rich repeats.[48] *LHCGR* is expressed only in limited tissues, including the gonad.[49] The gene is clearly expressed

in Leydig cells of the testis, and theca, stromal, late-stage granulosa, and luteal cells of the ovary. LHCGR binds to both LH and human chorionic gonadotropin (hCG).[50]

Biallelic loss-of-function variants in *LHCGR* in the germline cause hypergonadotropic hypogonadism due to Leydig cell hypoplasia in men.[51,52] Usually, 46,XY patients with these variants exhibit hypomasculinization of the external genitalia at birth and impaired sexual maturation during puberty.[51] Hence, *LHCGR* represents one of the causative genes of 46,XY disorders of sex development.[52] The variant-positive patients exhibited markedly elevated blood LH levels indicative of LH resistance. Known loss-of-function variants in *LHCGR* include various nucleotide substitutions and indels widely distributed in the gene (Figure 2).[52] Women with such variants manifest normal female-type external genitalia and experience normal development of breast and pubic hair, although they frequently show amenorrhea or menstrual irregularity.[53,54] These clinical features are indicative of partial hypogonadism. Some *LHCGR* variants were identified in female individuals clinically diagnosed with empty follicle syndrome.[55]

Germline gain-of-function variants of *LHCGR* are known to cause early puberty in male individuals.[56] The characteristic symptom of boys with these variants is autosomal dominant male-limited precocious puberty due to Leydig cell hyperplasia.[57] This condition is designated as "testotoxicosis."[57] These patients usually develop pubertal signs from 3 or 4 years of age.[58,59] Most known gain-of-function variants of *LHCGR* are missense substitutions in exon 11 (Figure 2).[49] These variants are likely to cause ligand-independent activation of the receptor, by altering the secondary structure of the third cytoplasmic loop and the sixth transmembrane domain.[58] Such variants are assumed to increase the risk of malignant testicular germ cell tumor.[60] Furthermore, a specific gain-of-function variant of *LHCGR*. p.D578H was identified as a somatic mutation in Leydig cell adenoma.[61,62] This tumor causes male-limited precocious puberty. In female individuals, *LHCGR* gain-of-function variants do not lead to salient clinical abnormalities.[55] Indeed, mutation screening of *LHCGR* for girls with CPP detected no apparent pathogenic variants.[63]

5.6 | *FSHR* variants associated with pubertal disorders

FSHR is almost exclusively expressed in the ovary and testis and binds to FSH.[64] Particularly strong expression is observed in granulosa and Sertoli cells.[64] FSH signaling mediated by FSHR regulates the development and function of both the ovaries and testes.[65]

Biallelic loss-of-function variants of *FSHR* in the germline lead to ovarian dysgenesis in genetic females and spermatogenic failure in genetic males.[50] These variants are assumed to affect protein expression, ligand binding, and/or ligand-induced cAMP activation.[66] Women with these variants exhibit primary or secondary amenorrhea with elevated blood levels of gonadotropins.[67,68] Delayed puberty was reported in some cases.[69] Histological analyses showed streak gonads or hypoplastic ovaries with a reduced number of follicles.[70] Clinical severities of female patients are likely to correlate with the residual activity of the mutant proteins.[71] Male individuals with *FSHR* loss-of-function variants were reported to have small testes and various degrees of spermatogenic failure.[72] Since azoospermia and complete infertility are rarely seen in these male patients, FSHR function appears to be more important in the development of the ovary than that of the testis.[72]

Germline gain-of-function variants of *FSHR* do not result in precocious puberty. Instead, such variants lead to spontaneous ovarian hyperstimulation syndrome during pregnancy.[73,74] This condition is characterized by the development of multiple serous and hemorrhagic follicular cysts in the ovary. Known gain-of-function variants of FSHR reside in the transmembrane or extracellular domains (Figure 2) and are predicted to increase basal activity and sensitivity to hCG and/or thyroid-stimulating hormone.[75] A germline gain-of-function variant was identified in a man, who was hypophysectomized because of a pituitary tumor. The patient showed normal spermatogenesis despite low FSH levels.[76,77]

6 | OTHER GPCRS POSSIBLY INVOLVED IN THE HPG AXIS

The HPG axis may contain additional GPCRs. In particular, GPR147 encoded by *NPFFR1* has been reported as the receptor of RFamide-related peptide-3 (RFRP-3), which is designated as gonadotropin inhibitory hormone (GnIH) in birds.[78] Orthologs of RFRP-3 were shown to suppress gonadotropin secretion in quail and several mammalian species.[78] Since mRNA expression of *NPFFR1* is detected in the hypothalamus and pituitary gonadotrophs of humans,[79] the RFRP-3-GPR147 system may also play a role in the regulation of the human HPG axis.[80] However, mutation screening for 78 patients with CPP and 51 patients with hypogonadotropic hypogonadism failed to identify apparently pathogenic genetic variants in RFRP-3 or GPR147.[79] Thus, the contribution of these gene variants to the development of pubertal disorders appears to be limited. Lima et al. proposed that the RFRP-3-GPR147 system may play a secondary and modulatory role in the regulation of pubertal development.[79]

7 | FUTURE PERSPECTIVES

Multiple questions regarding GPCRs in the HPG axis remain unanswered. First, the trigger for normal pubertal onset is still unknown.[24] The first event of pubertal onset appears to be the activation of KNDy neurons by an unknown factor.[81] Second, although KISS1R, TACR3, and PROKR2 are known to constitute a major part of the GnRH pulse generator, functional interactions among these GPCRs remain largely unknown. Moreover, the signal network involving these GPCRs and other puberty-associated molecules such as FGFR1, ANOS1, and MKRN3, needs to be clarified in the future.

Conceptual modeling approaches and multicellular systems biology may be useful for addressing these issues.[82] Lastly, not only pathogenic variants, but also common single-nucleotide polymorphisms (SNPs) of the six GPCR genes may be of biological importance. For example, common SNPs in *KISS1R* or *KISS1* were linked to the risk of early puberty.[34] It is necessary to clarify the contribution of SNPs in each GPCR gene to the inter-individual variations in pubertal timing and reproductive activity.

8 | CONCLUSIONS

Genetic variants in the six GPCRs involved in the HPG axis are important causes of pubertal disorders. Several loss-of-function variants of the six GPCR genes were shown to cause absent/delayed puberty as autosomal dominant, recessive, or oligogenic disorders. In addition, a few specific variants of *KISS1R*, *PROKR2*, and *LHCGR* have been implicated in precocious puberty. The identification of pathogenic variants enables genetic counseling for patients with pubertal disorders. More importantly, the functional characterization of these GPCRs serves to understand the molecular network involved in the regulation of normal sexual maturation.

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CONFLICT OF INTEREST STATEMENT

The authors confirm that there are no conflicts of interest with the contents of this article. Because this is a mini-review, human rights, informed consent, animal care, and institutional ethical approval are not applied to this article.

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REFERENCES

1. Vassart G, Costagliola S. G protein-coupled receptors: mutations and endocrine diseases. Nat Rev Endocrinol. 2011;7(6):362–72.
2. Ulloa-Aguirre A, Zariñán T, Jardón-Valadez E. Misfolded G protein-coupled receptors and endocrine disease. Molecular mechanisms and therapeutic prospects. Int J Mol Sci. 2021;22(22):12329.
3. Yang LK, Hou ZS, Tao YX. Biased signaling in naturally occurring mutations of G protein-coupled receptors associated with diverse human diseases. Biochim Biophys Acta Mol Basis Dis. 2021;1867(1):165973.
4. Kleinau G, Müller A, Biebermann H. Oligomerization of GPCRs involved in endocrine regulation. J Mol Endocrinol. 2016;57(1):R59–80.
5. Gurevich VV, Gurevich EV. Molecular mechanisms of GPCR signaling: a structural perspective. Int J Mol Sci. 2017;18(12):2519.
6. Fukami M, Suzuki E, Igarashi M, Miyado M, Ogata T. Gain-of-function mutations in G-protein-coupled receptor genes associated with human endocrine disorders. Clin Endocrinol (Oxf). 2018;88(3):351–9.
7. Moise-Silverman J, Silverman LA. A review of the genetics and epigenetics of central precocious puberty. Front Endocrinol (Lausanne). 2022;13:1029137.
8. Balasubramanian R, Crowley WF Jr. Isolated gonadotropin-releasing hormone (GnRH) deficiency. In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 2022.
9. Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, et al. Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. Am J Hum Genet. 2013;92(5):725–43.
10. Seminara SB, Topaloglu AK. Review of human genetic and clinical studies directly relevant to GnRH signaling. J Neuroendocrinol. 2022;34(5):e13080.
11. Tajima T. Genetic causes of central precocious puberty. Clin Pediatr Endocrinol. 2022;31(3):101–9.
12. Magalhaes AC, Dunn H, Ferguson SS. Regulation of GPCR activity, trafficking and localization by GPCR-interacting proteins. Br J Pharmacol. 2012;165(6):1717–36.
13. Ritter SL, Hall RA. Fine-tuning of GPCR activity by receptor-interacting proteins. Nat Rev Mol Cell Biol. 2009;10(12):819–30.
14. Ferré S, Casadó V, Devi LA, Filizola M, Jockers R, Lohse MJ, et al. G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. Pharmacol Rev. 2014;66(2):413–34.
15. Schöneberg T, Liebscher I. Mutations in G protein-coupled receptors: mechanisms, pathophysiology and potential therapeutic approaches. Pharmacol Rev. 2021;73(1):89–119.
16. Stoy H, Gurevich VV. How genetic errors in GPCRs affect their function: possible therapeutic strategies. Genes Dis. 2015;2(2):108–32.
17. Fukami M, Suzuki E, Izumi Y, Torii T, Narumi S, Igarashi M, et al. Paradoxical gain-of-function mutant of the G-protein-coupled receptor PROKR2 promotes early puberty. J Cell Mol Med. 2017;21(10):2623–6.
18. Kaiser U, Ho K. Pituitary physiology and diagnostic evaluation. In: Melmed S, Koenig R, Rosen C, Auchus R, Goldfine A, editors. Williams textbook of endocrinology, 14e. Netherlands: Elsevier; 2019. p. 217–8.
19. Lanfranco F, Ghigo E, Strasburger CJ. Hormomes and athletic performance. In: Melmed S, Koenig R, Rosen C, Auchus R, Goldfine A, editors. Williams textbook of endocrinology, 14e. Netherlands: Elsevier; 2019. p. 1167–9.
20. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol. 2016;4(3):254–64.
21. Noel SD, Kaiser UB. G protein-coupled receptors involved in GnRH regulation: molecular insights from human disease. Mol Cell Endocrinol. 2011;346(1–2):91–101.
22. Manotas MC, González DM, Céspedes C, Forero C, Rojas Moreno AP. Genetic and epigenetic control of puberty. Sex Dev. 2022;16(1):1–10.
23. Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum Reprod Update. 2014;20(4):485–500.
24. Lehman MN, He W, Coolen LM, Levine JE, Goodman RL. Does the KNDy model for the control of gonadotropin-releasing hormone pulses apply to monkeys and humans? Semin Reprod Med. 2019;37(2):71–83.
25. Goodman RL, Herbison AE, Lehman MN, Navarro VM. Neuroendocrine control of gonadotropin-releasing hormone: pulsatile and surge modes of secretion. J Neuroendocrinol. 2022;34(5):e13094.

26. Martin C, Balasubramanian R, Dwyer AA, Au MG, Sidis Y, Kaiser UB, et al. The role of the prokineticin 2 pathway in human reproduction: evidence from the study of human and murine gene mutations. Endocr Rev. 2011;32(2):225–46.
27. Huhtaniemi IT. The role of mutations affecting gonadotrophin secretion and action in disorders of pubertal development. Best Pract Res Clin Endocrinol Metab. 2002;16(1):123–38.
28. Palmert MR, Boepple PA. Variation in the timing of puberty: clinical spectrum and genetic investigation. J Clin Endocrinol Metab. 2001;86(6):2364–8.
29. Brito VN, Canton APM, Seraphim CE, Abreu AP, Macedo DB, Mendonca BB, et al. The congenital and acquired mechanisms implicated in the etiology of central precocious puberty. Endocr Rev. 2023;44(2):193–221.
30. Amato LGL, Montenegro LR, Lerario AM, Jorge AAL, Guerra Junior G, Schnoll C, et al. New genetic findings in a large cohort of congenital hypogonadotropic hypogonadism. Eur J Endocrinol. 2019;181(2):103–19.
31. Gohil A, Eugster EA. Delayed and precocious puberty: genetic underpinnings and treatments. Endocrinol Metab Clin North Am. 2020;49(4):741–57.
32. Liu J, Li T, Peng M, Luo M, Gui Z, Long S, et al. The key roles of Makorin RING finger protein 3 (MKRN3) during the development of pubertal initiation and central precocious puberty (CPP). Curr Mol Med. 2022. (in press). https://doi.org/10.2174/1566524022666220624105430 Accessed 30 Jan 2023.
33. Patil VA, Lila AR, Shah N, Arya S, Ekbote AV, Sarathi V, et al. Regional genotypic variations in normosmic congenital hypogonadotropic hypogonadism: our experience and systematic review. Pituitary. 2022;25(3):444–53.
34. Neocleous V, Fanis P, Toumba M, Gorka B, Kousiappa I, Tanteles GA, et al. Pathogenic and low-frequency variants in children with central precocious puberty. Front Endocrinol (Lausanne). 2021;12:745048.
35. Maione L, Dwyer AA, Francou B, Guiochon-Mantel A, Binart N, Bouligand J, et al. Genetics in endocrinology: genetic counseling for congenital hypogonadotropic hypogonadism and Kallmann syndrome: new challenges in the era of oligogenism and next-generation sequencing. Eur J Endocrinol. 2018;178(3):R55–80.
36. Xie Q, Kang Y, Zhang C, Xie Y, Wang C, Liu J, et al. The role of kisspeptin in the control of the hypothalamic-pituitary-gonadal axis and reproduction. Front Endocrinol (Lausanne). 2022;13:925206.
37. Krsmanovic LZ, Hu L, Leung PK, Feng H, Catt KJ. The hypothalamic GnRH pulse generator: multiple regulatory mechanisms. Trends Endocrinol Metab. 2009;20(8):402–8.
38. Trevisan CM, Montagna E, de Oliveira R, Christofolini DM, Barbosa CP, Crandall KA, et al. Kisspeptin/GPR54 system: what do we know about its role in human reproduction? Cell Physiol Biochem. 2018;49(4):1259–76.
39. Ozawa H. Kisspeptin neurons as an integration center of reproductive regulation: observation of reproductive function based on a new concept of reproductive regulatory nervous system. Reprod Med Biol. 2021;21(1):e12419.
40. Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, et al. Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med. 2012;366(7):629–35.
41. Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, et al. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med. 2008;358(7):709–15.
42. Pagani S, Calcaterra V, Acquafredda G, Montalbano C, Bozzola E, Ferrara P, et al. MKRN3 and KISS1R mutations in precocious and early puberty. Ital J Pediatr. 2020;46(1):39.
43. Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, et al. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab. 2010;95(5):2276–80.
44. Nussdorfer GG, Malendowicz LK. Role of tachykinins in the regulation of the hypothalamo-pituitary-adrenal axis. Peptides. 1998;19(5):949–68.
45. Cole LW, Sidis Y, Zhang C, Quinton R, Plummer L, Pignatelli D, et al. Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. J Clin Endocrinol Metab. 2008;93(9):3551–9.
46. Anderson RA, Millar RP. The roles of kisspeptin and neurokinin B in GnRH pulse generation in humans, and their potential clinical application. J Neuroendocrinol. 2022;34(5):e13081.
47. Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, et al. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med. 2009;360(26):2742–8.
48. Ascoli M, Fanelli F, Segaloff DL. The lutropin/choriogonadotropin receptor, a 2002 perspective. Endocr Rev. 2002;23(2):141–74.
49. Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr Rev. 2000;21(5):551–83.
50. Casarini L, Simoni M. Recent advances in understanding gonadotropin signaling. Fac Rev. 2021;10:41.
51. Themmen AP, Verhoef-Post M. LH receptor defects. Semin Reprod Med. 2002;20(3):199–204.
52. Kremer H, Kraaij R, Toledo SP, Post M, Fridman JB, Hayashida CY, et al. Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. Nat Genet. 1995;9(2):160–4.
53. Toledo SP, Brunner HG, Kraaij R, Post M, Dahia PL, Hayashida CY, et al. An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46. XX Female J Clin Endocrinol Metab. 1996;81(11):3850–4.
54. Arnhold IJ, Latronico AC, Batista MC, Carvalho FM, Chrousos GP, Mendonça BB. Ovarian resistance to luteinizing hormone: a novel cause of amenorrhea and infertility. Fertil Steril. 1997;67(2):394–7.
55. Qiao J, Han B. Diseases caused by mutations in luteinizing hormone/chorionic gonadotropin receptor. Prog Mol Biol Transl Sci. 2019;161:69–89.
56. Shenker A, Laue L, Kosugi S, Merendino JJ Jr, Minegishi T, Cutler GB Jr. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature. 1993;365(6447):652–4.
57. Kawate N, Kletter GB, Wilson BE, Netzloff ML, Menon KM. Identification of constitutively activating mutation of the luteinising hormone receptor in a family with male limited gonadotrophin independent precocious puberty (testotoxicosis). J Med Genet. 1995;32(7):553–4.
58. Laue L, Chan WY, Hsueh AJ, Kudo M, Hsu SY, Wu SM, et al. Genetic heterogeneity of constitutively activating mutations of the human luteinizing hormone receptor in familial male-limited precocious puberty. Proc Natl Acad Sci U S A. 1995;92(6):1906–10.
59. Wu SM, Leschek EW, Brain C, Chan WY. A novel luteinizing hormone receptor mutation in a patient with familial male-limited precocious puberty: effect of the size of a critical amino acid on receptor activity. Mol Genet Metab. 1999;66(1):68–73.
60. Kooij CD, Mavinkurve-Groothuis AMC, Kremer Hovinga ICL, Looijenga LHJ, Rinne T, Giltay JC, et al. Familial male-limited precocious puberty (FMPP) and testicular germ cell tumors. J Clin Endocrinol Metab. 2022;107(11):3035–44.
61. Liu G, Duranteau L, Carel JC, Monroe J, Doyle DA, Shenker A. Leydig-cell tumors caused by an activating mutation of the gene encoding the luteinizing hormone receptor. N Engl J Med. 1999;341(23):1731–6.
62. d'Alva CB, Brito VN, Palhares HM, Carvalho FM, Arnhold IJ, Mendonca BB, et al. A single somatic activating Asp578His mutation of the luteinizing hormone receptor causes Leydig cell tumour in boys with gonadotropin-independent precocious puberty. Clin Endocrinol (Oxf). 2006;65(3):408–10.

63. Jeong HR, Lee HS, Hwang JS. LHCGR gene analysis in girls with non-classic central precocious puberty. Exp Clin Endocrinol Diabetes. 2019;127(4):234–9.
64. Bhartiya D, Patel H. An overview of FSH-FSHR biology and explaining the existing conundrums. J Ovarian Res. 2021;14(1):144.
65. de Pascali F, Tréfier A, Landomiel F, Bozon V, Bruneau G, Yvinec R, et al. Follicle-stimulating hormone receptor: advances and remaining challenges. Int Rev Cell Mol Biol. 2018;338:1–58.
66. Desai SS, Roy BS, Mahalea SD. Mutations and polymorphisms in FSH receptor: functional implications in human reproduction. Reproduction. 2013;146(6):R235–48.
67. Beau I, Touraine P, Meduri G, Gougeon A, Desroches A, Matuchansky C, et al. A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. J Clin Invest. 1998;102(7):1352–9.
68. Kuechler A, Hauffa BP, Köninger A, Kleinau G, Albrecht B, Horsthemke B, et al. An unbalanced translocation unmasks a recessive mutation in the follicle-stimulating hormone receptor (FSHR) gene and causes FSH resistance. Eur J Hum Genet. 2010;18(6):656–61.
69. Meduri G, Touraine P, Beau I, Lahuna O, Desroches A, Vacher-Lavenu MC, et al. Delayed puberty and primary amenorrhea associated with a novel mutation of the human follicle-stimulating hormone receptor: clinical, histological, and molecular studies. J Clin Endocrinol Metab. 2003;88(8):3491–8.
70. Aittomäki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, et al. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. Cell. 1995;82(6):959–68.
71. Aittomäki K, Herva R, Stenman UH, Juntunen K, Ylöstalo P, Hovatta O, et al. Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. J Clin Endocrinol Metab. 1996;81(10):3722–6.
72. Tapanainen JS, Aittomäki K, Min J, Vaskivuo T, Huhtaniemi IT. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. Nat Genet. 1997;15(2):205–6.
73. Smits G, Olatunbosun O, Delbaere A, Pierson R, Vassart G, Costagliola S. Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. N Engl J Med. 2003;349(8):760–6.
74. Vasseur C, Rodien P, Beau I, Desroches A, Gérard C, de Poncheville L, et al. A chorionic gonadotropin-sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial gestational spontaneous ovarian hyperstimulation syndrome. N Engl J Med. 2003;349(8):753–9.
75. de Leener A, Caltabiano G, Erkan S, Idil M, Vassart G, Pardo L, et al. Identification of the first germline mutation in the extracellular domain of the follitropin receptor responsible for spontaneous ovarian hyperstimulation syndrome. Hum Mutat. 2008;29(1):91–8.
76. Santi D, Potì F, Simoni M, Casarini L. Pharmacogenetics of G-protein-coupled receptors variants: FSH receptor and infertility treatment. Best Pract Res Clin Endocrinol Metab. 2018;32(2):189–200.
77. Gromoll J, Simoni M, Nieschlag E. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. J Clin Endocrinol Metab. 1996;81(4):1367–70.
78. Tsutsui K, Ubuka T. Gonadotropin-inhibitory hormone (GnIH): a new key neurohormone controlling reproductive physiology and behavior. Front Neuroendocrinol. 2021;61:100900.
79. Lima CJ, Cardoso SC, Lemos EF, Zingler E, Capanema C, Menezes LD, et al. Mutational analysis of the genes encoding RFamide-related peptide-3, the human orthologue of gonadotrophin-inhibitory hormone, and its receptor (GPR147) in patients with gonadotrophin-releasing hormone-dependent pubertal disorders. J Neuroendocrinol. 2014;26(11):817–24.
80. Ubuka T, Morgan K, Pawson AJ, Osugi T, Chowdhury VS, Minakata H, et al. Identification of human GnIH homologs, RFRP-1 and RFRP-3, and the cognate receptor, GPR147 in the human hypothalamic pituitary axis. PLoS One. 2009;4(12):e8400.
81. Livadas S, Chrousos GP. Molecular and environmental mechanisms regulating puberty initiation: an integrated approach. Front Endocrinol (Lausanne). 2019;10:828.
82. Yvinec R, Crépieux P, Reiter E, Poupon A, Clément F. Advances in computational modeling approaches of pituitary gonadotropin signaling. Expert Opin Drug Discov. 2018Sep;13(9):799–813.

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