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Review of human genetic and clinical studies directly relevant to GnRH signalling

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Abstract

GnRH is the pivotal hormone in controlling the hypothalamic-pituitary gonadal (HPG) axis in humans and other mammalian species. GnRH function is influenced by a multitude of known and still unknown environmental and genetic factors. Molecular genetic studies on human families with hypogonadotropic hypogonadism over the past two decades have been instrumental in delineating the kisspeptin and neurokinin B signalling, which integrally modulates GnRH release from the hypothalamus. The identification of kisspeptin and neurokinin B ligand-receptor gene pair mutations in patients with absent puberty have paved the way to a greater understanding of the central regulation of the HPG cascade. In this article, we aim to review the literature on the genetic and clinical aspects of GnRH and its receptor, as well as the two ligandreceptor sets directly pertinent to the function of GnRH hormone signalling, kisspeptin/ kisspeptin receptor and NKB/NK3R.

Keywords

GnRH; hypogonadotropic hypogonadism; kisspeptin; neurokinins; puberty

1 | INTRODUCTION

GnRH is the key molecule in controlling gonadal function in humans, as in other mammalian species. Reproductive disorders stemming from abnormal GnRH neuron action

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CONFLICT OF INTEREST

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PEER REVIEW

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come to clinical attention primarily as either early or delayed puberty. GnRH function is influenced by a multitude of known and still unknown environmental and genetic factors.

To date, more than 50 genes have been proposed to modify GnRH neuron function either directly or indirectly.[1] In our understanding, the concept “puberty genes” exclusively refers to those directly pertaining to the regulation of GnRH secretory function. Currently, six genes encoding three ligand-receptor pairs (*GNRH1*/*GNRHR*,*KISS1*/*KISS1R*, and *TAC3*/*TACR3*) have been proven to directly affect GnRH secretion and action. Knowledge regarding these genes has been acquired mainly through performing molecular genetic studies in individuals exhibiting deviation from the expected timing of pubertal development. When these phenotypes are observed more than once in a family (multiplex pedigree), the condition is more likely to be inherited, and by extension, genetic in origin. The contributions from human genetics to our current understanding of GnRH neuron function have been enormous. The identification of kisspeptin and neurokinin B ligandreceptor gene pair mutations in human families with absent puberty have paved the way to the greater understanding of the central regulation of the hypothalamic-pituitary-gonadal (HPG) cascade. This reverse translational pathway of discovery, enhanced by the availability of whole genome sequencing, promises to continue to deliver even more insights into the central control of reproduction.

In this article, we aim to review the literature on the genetic and clinical aspects of GnRH and its receptor, as well as the two ligandreceptor sets directly pertinent to the function of GnRH hormone signalling, kisspeptin/kisspeptin receptor and NKB/NK3R.

2 | *GNRHR* AND *GNRH1*

2.1 | Genetics

In the past, clinicians and investigators used the term “GnRH deficiency” to describe patients with idiopathic hypogonadotropic hypogonadism (IHH). When treated with chronic intermittent GnRH via an exogenous, portable, programmable pump, the vast majority of patients with IHH demonstrate increases in LH and FSH secretion with the initiation of folliculogenesis and spermatogenesis. Thus, in the seeming absence of resistance to exogenous GnRH, the gene encoding the GnRH receptor (*GNRHR*) did not appear to be a strong candidate gene for absent pubertal development. However, not one but two groups proved this was precisely the case.

The GnRH receptor gene (*GNRHR*, 4q21.2) encodes a seventransmembrane domain G protein-coupled receptor but lacks the intracellular carboxyl-terminus typically seen in other members of this family.[2,3] In 1997, de Roux et al.[4] identified compound heterozygous mutations in *GNRHR* in two siblings with partial normosmic idiopathic hypogonadotropic hypogonadism (nIHH). Gln106Arg decreased the binding of GnRH to its receptor, while Arg262Gln decreased GnRH stimulated IP3 production. The male sibling exhibited normal levels of the gonadotropins (both mean levels and GnRH stimulated), normal LH pulse frequency but decreased LH pulse amplitude. The authors concluded that this individual’s partial phenotype was consistent with reduced GnRH activation of the receptor.[4] Just 2 months later, Layman et al.[5] reported a family with four siblings with IHH, each carrying

compound heterozygous mutations in *GNRHR* (Arg262Gln and Tyr284Cys). Both studies demonstrated that biallelic mutations in *GNRHR* could result in IHH without accompanying developmental defects or anosmia.[4,5] In a large study of 863 subjects, *GNRHR* variants were identified in 5.6% of patients with nIHH.[6] This relatively high prevalence of *GNRHR* was confirmed in subsequent studies.[7] To date, more than 60 different *GNRHR* mutations have been reported or listed in various databases.[1]

Genotype-phenotype correlations are possible for certain types of mutations in *GNRHR*. Gianetti et al.[6] juxtaposed genetic burden (homozygous, compound heterozygous, or monoallelic missense) against clinical severity (ranging from complete IHH to partial forms including constitutional delay of growth and puberty (CDGP), functional hypothalamic amenorrhea, and adult-onset idiopathic hypogonadotropic hypogonadism [AOHH]). Although presumed receptor function correlate well with the phenotypic spectrum of the GnRH deficiency, patients harbouring monoallelic mutations in *GNRHR* demonstrate a wider range of clinical GnRH-deficiency, suggesting the coexistence of yet-to-be-identified genetic and/or nongenetic factors.[6]

Mutations in *GNRHR* can be associated with a broad range of hypogonadotropic phenotypes. For example, two sisters each carrying the homozygous missense mutation R139C located in the conserved DRS motif at the junction of the third transmembrane and the second intracellular loop of the GnRH receptor were found to have complete IHH.[8] The R139C mutation almost completely abolished plasma membrane expression, which could be rescued by a membrane-permeant, nonpeptide GnRH receptor antagonist IN3.[8] At the other opposite of the phenotypic spectrum, a heterozygous Gln106Arg mutation was found to be associated with AOHH[9] in which patients undergo normal puberty, even paternity, before the development of hypogonadotropism later in life. Homozygosity for the same variant (Gln106Arg) has also been associated with the fertile eunuch variant of IHH, another partial IHH form characterized by decreased virilization, eunuchoid proportions, hypogonadal testosterone levels but normal testicular size.[10]

Mutations in *GNRHR* have revealed differential sensitivities of LH and FSH to GnRH stimulation. In a female patient with IHH who was treated with exogenous pulsatile GnRH, a rightward shift of the dose-response curves to pulsatile GnRH was observed, resulting in low LH and oestradiol levels despite appropriate FSH secretion and follicular growth.[11] Similarly, increased doses of GnRH were found to effectively induce ovulation in some patients with *GNRHR* mutations.[12] Although the dose of GnRH of 100 ng/kg was adequate for folliculogenesis, a higher dose of 250 ng/kg was necessary for normal luteal function, demonstrating that higher doses of GnRH are required for normal luteal phase dynamics than for normal follicular phase function.

Long before the identification of human mutations in *GNRH1*, a spontaneous mouse model for GnRH deficiency had existed for decades. The *hpg* mouse carries a deletion of *Gnrh1*, resulting in the complete absence of GnRH synthesis.[13,14] Male and female *hpg* mice have complete IHH.[13] In the first example of gene therapy, the reproductive deficits of these mutant mice were rescued by the introduction of an intact GnRH gene into the genome of these animals.[14] Surprisingly, it took 12 years after the discovery of

mutations in *GNRHR* for rare pathogenic variants in *GNRH1* to be identified in patients with IHH.[15,16] *GNRH1* encodes the preprohormone that is ultimately processed to produce the GnRH decapeptide. Screening a cohort of 310 patients with IHH, Chan et al.[16] found a homozygous 1-base deletion (c.87delA) leading to a frameshift mutation (p.G29GfsX12) in a male patient with severe congenital nIHH. This truncating mutation is predicted to disrupt the GnRH decapeptide.[16] Around the same time, Bouligand et al.[15] described a Romanian family with nIHH in which the affected family members carried a homozygous frameshift mutation of *GNRH1* (c.18–19insA) completely deleting the GnRH decapeptide sequence. Although the basal LH profile was apulsatile, pulsatile GnRH administration for 13 days resulted in synchronous LH pulses, increased circulating levels of oestradiol, and a single dominant ovarian follicle. These findings suggested a hypothalamic site of the hormonal defect consistent with underlying GnRH deficiency.[15] Although *GNRH1* is an obvious candidate gene, mutations are rare.[8,15–18] It has been estimated that the prevalence of *GNRH1* mutations is 0.33% based on 600 pedigrees screened by various groups.[19] Although it has been repeatedly observed that mutations are less frequent in the genes encoding ligands than in the gene encoding their corresponding cognate receptors,[19,20] the contrasting frequency of *GNRH1* and *GNRHR* mutations is nonetheless striking. Screening of another large cohort of nIHH patients revealed only two families with *GNRH1* mutations.[18] The proband from one of the families harboured the same mutation (c.87delA) as in one of the initial reports.[16] Comparison of phenotypes showed almost no differences; the severe phenotype of these patients (micropenis and cryptorchidism) is compatible with a complete loss of GnRH activity, with severely reduced androgen exposure during fetal life.[21] In the other family, the affected siblings carried a novel homozygous mutation of c.G92A leading to p.R31H, positioned at the 6th amino acid of the decapeptide. Previously, a heterozygous mutation changing arginine to cysteine at the same residue (p.R31C) was reported in nine patients from four families.[16,17] Because of the heterozygous nature of these mutations, it is unclear whether the nIHH phenotype is due to a dominant-negative effect or functional haploinsufficiency. To date, more than a dozen different *GNRH1* mutations have been published or listed in various databases.

2.2 | Clinical studies

GnRH was first isolated, characterized, and synthesized by Schally and Guillemin.[22–26] Within 7 years, the Knobil laboratory demonstrated in rhesus monkeys with hypothalamic lesions that abolish gonadotropic hormone release by the pituitary gland, intermittent administration of the synthetic GnRH once per hour could re-establish pituitary gonadotropin secretion. In contrast, a constant infusion of exogenous GnRH fails to restore sustained gonadotropin secretion.[27] These observations led to the widespread investigation of the therapeutic potential of GnRH, particularly for stimulation of the reproductive axis in hypogonadotropic states and suppression of the reproductive cascade when reversible medical castration is required.

Assessments of GnRH secretion in the human must be indirect. Therefore, in order to realize the promise of the therapeutic potential for GnRH, frequent sampling of peripheral blood to determine the normal patterns of gonadotropin release in both men and women was performed. With that information, treatment regimens utilizing exogenous, pulsatile

were designed to correct the abnormal/absent GnRH secretion observed in many disease states, thereby providing physiological GnRH pulse frequency and amplitude to patients, particularly those with hypogonadotropic hypogonadism (HH), which is characterized by abnormal release of endogenous GnRH. These regimens have now been employed in both clinical and research settings for over 30 years. In women, using an exogenous pulsatile GnRH pump, successful ovulation can safely be achieved in the majority of anovulatory patients with functional hypothalamic amenorrhoea, with a cumulative live birth rate over 80% and a low multiple pregnancy rate.[28] The pulsatile GnRH pump is a more physiological alternative to other means of ovulation induction as it results in monofolliculogenesis compared with injectable gonadotropins which frequently result in multiple gestation.[29,30] Pulsatile GnRH has been FDA approved for use in women with primary amenorrhoea. As in women, the exogenous pulsatile GnRH pump can restore testicular function and fertility in men with hypogonadotropic hypogonadism.[31]

Right after GnRH agonist administration, FSH and LH secretion increase, resulting in an initial transient rise in sex hormones (flare effect).[32] Subsequently, FSH and LH secretion decrease secondary to receptor downregulation resulting in a profound hypogonadal effect.[33] Long acting GnRH agonists are commonly used as androgen deprivation therapy of advanced prostate cancer as well as treatment of other benign conditions requiring hormonal inhibition such as endometriosis, uterine fibroids, and precocious puberty.[34–36] Whilst long acting GnRH agonists suppress gonadotropin release by pituitary desensitization, GnRH antagonists competitively block GnRH receptors.[37–39] Thus, endogenous GnRH is prevented (reversibly) from stimulating the secretion of LH and FSH, and by extension, gonadal sex steroids. The surge of gonadotropin release characteristic of GnRH agonists is prevented.[32] Therefore, both long-acting GnRH agonists and antagonists both ultimately lead to a hypogonadal state; however, GnRH antagonists are characterized by a more rapid onset and the lack of an initial surge in sex hormone release.

GnRH agonists and antagonists have traditionally required parenteral administration. More recently, orally-active small-molecule GnRH antagonists targeting the GnRH receptor have been synthesized and approved for use. The ability to administer these drugs orally reduces the burden of reconstituting subcutaneous formulations and performing injections. Elagolix, an orally bioactive nonpeptide GnRH antagonist, has been approved by the FDA for the management of moderate to severe pain associated with endometriosis.[40] This and other antagonists have also been approved for the management of heavy menstrual bleeding associated with uterine fibroids in premenopausal women[41,42] as well as the treatment of advanced prostate cancer.

3 | *KISS1* AND *KISS1R*

3.1 | Genetics

Despite the primacy of GnRH, investigators have long sought the afferent inputs that regulate GnRH neurons guiding the tempo of sexual maturation, modulating secretion, controlling seasonal breeding, and pausing reproductive activity under adverse conditions. In 2003, kisspeptin was thrown into the international spotlight as a key regulator of GnRH neuronal function. Mutations were identified in a then little-known G protein coupled

receptor (*GPR54*, later termed the kisspeptin receptor *[KISS1R]*) and subsequent clinical neuroendocrine studies led, in short order, to the elucidation of kisspeptin as the most critical stimulus of GnRH secretion at the time of puberty.

Two groups utilized homozygosity mapping in families with IHH leading to the initial identification of mutations in *KISS1R*. One group, led by de Roux et al.,[43] utilized an inbred family to uncover a large deletion within this gene. This deletion encompassed the splicing acceptor site of intron 4-exon 5 junction and part of exon 5. Utilizing a different family from Saudi Arabia, Seminara et al. identified homozygous c.T443C variant, resulting in the missense change, p.L148S, in the second intracellular loop of KISS1R. In an unrelated African American proband, two heterozygous variants were identified [991C→T (R331X)] [1195T→A (X399R)].[44] Mutant constructs representing each nucleotide change were assembled and their deleterious effects on receptor function were demonstrated by in vitro functional assays. In addition, mice with targeted deletions of Kiss1r were found to be phenocopies of the human hypogonadotropic phenotype, confirming the important role of this ligand-receptor family in the control of puberty and reproductive function across mammalian species.[44]

Similar to *GNRHR*/*GNRH1*, several years passed between the identification of mutations in *KISS1R* and the subsequent discovery of mutations in the gene encoding the ligand for this receptor, *KISS1*. The existence of *KISS1* mutations was presaged by the identification of heterozygous rare variants, some pathogenic, in a large cohort of patients with IHH, although the total complement of these variants did not meet statistical burden when compared to a small control population.[45] However, in 2012, Topaloglu et al.[46] identified a deleterious homozygous missense variant p.N115K in a large consanguineous family with nIHH, demonstrating that *KISS1* variants can underlie IHH.

Mutations in *KISS1* and *KISS1R* may be rare because kisspeptin's roles in placentation and/or metastasis suppression create purifying selection to remove deleterious alleles within families. Therefore, it is important to seek genotype/phenotype correlations whenever possible. For example, mutations in *KISS1*/*KISS1R* may play an important role in the mini-puberty of infancy. A patient with compound heterozygote mutations in *KISS1R* had laboratories consistent with hypogonadotropic hypogonadism (with low levels of gonadal sex steroids and gonadotropins) documented during his early months of life.[47] This finding is notable because the first 6 months to 2 years of life is normally characterized by robust activity of the hypothalamic-pituitary–gonadal cascade. Therefore, dampened gonadotrophin and sex steroid levels at this time in life are not only abnormal but may also serve as a harbinger of pubertal delay.

The placenta releases high levels of kisspeptin into the maternal circulation during pregnancy. Kisspeptin may represent a signal that plays a physiological role in placental health and islet cell biology. Although much more work needs to be done to understand the role of human genetic variation within this pathway during pregnancy, a female patient homozygous for the L148S mutation in *KISS1R* had the following phenotypic features: (1) physiological responses to exogenous GnRH and gonadotropin stimulation, (2) multiple conceptions, (3) two uncomplicated pregnancies leading to the delivery of healthy children,

suggesting a functional placenta in each pregnancy, (4) spontaneous uterine contractions signalling the initiation of labour, and (5) initiation and maintenance of breast feeding for several months post-partum.[48]

3.2 | Clinical studies

Just as GnRH was found to have stimulatory properties when given intermittently and repressive properties when given continuously, the same principles apply to kisspeptin. Studies in numerous mammalian species have demonstrated that kisspeptin stimulates the secretion of gonadotropins from the pituitary by stimulating the release of GnRH after the activation of KISS1R.[49–51] The enormous momentum in numerous physiological studies paved the way for clinical studies in the human.

Kisspeptin has been administered using different isoforms (kisspeptin 68–121 [54-mer], kisspeptin 112–121 [decapeptide]); sites of administration (iv, sc); time periods of administration (single bolus, continuous); total duration of administration (single bolus, multiple doses); and research subject participants (healthy males and females, patients with reproductive disorders). The first human study was performed in healthy males in 2005, demonstrating that kisspeptin is a powerful stimulus for GnRH-induced LH secretion, as it is in lower species.[45,52] Shortly thereafter, kisspeptin was administered to women,[53] and again, was found to stimulate GnRH induced LH secretion, most notably across the luteal and periovulatory phases of the menstrual cycle. Building on these observations, kisspeptin was subsequently shown to be capable of serving as an ovulatory agent in infertility cycles.[54]

Although a single injection of kisspeptin is a powerful stimulus of the reproductive cascade in healthy men and women, repeated administration can cause tachyphylaxis.[55] Reducing the frequency of kisspeptin administration to twice weekly can maintain gonadotropin stimulation over an 8 week period but does not bring about any change in baseline levels of hormones, follicle number or follicle growth.[56] In contrast to patients with hypothalamic amenorrhoea, subjects with congenital, abiding hypogonadotropic hypogonadism do not demonstrate a GnRH-induced LH response to a single or even multiple boluses of kisspeptin.[57] This failure to respond has been observed in research subjects carrying several different genotypes, including mutations in *ANOS1* (formally known as *KAL1*), *FGFR1*, *GNRHR*, *KISS1*, *PROKR2*. It is possible that the subjects who participated in these studies were carriers of *KISS1R* mutations, preventing them from responding to exogenous kisspeptin. However, all participants were screened for such variants, and at least at the level of the exome, did not carry them. Although the aetiology for the lack of response to exogenous kisspeptin remains unknown, the inability to respond appears to supersede the specific genetic signatures.

Contrary to adults with hypogonadotropism (congenital or acquired), the response to kisspeptin administration in children with pubertal delay is heterogeneous.[58] Some children show a robust response and others show little to none. However, in these children, the response to kisspeptin administration appears to predict future pubertal entry.[59] Specifically, in a longitudinal cohort study, all children with pubertal delay who had responded to kisspeptin with a rise in LH of ≥0.8 mIU/ml progressed through puberty ($n = 8$) but all participants whose LH response was ≤0.4 mIU/ml did not ($n = 8$). Thus, responses

to kisspeptin may accurately predict later pubertal outcomes, raising the possibility that kisspeptin can play a diagnostic role in the conditions characterized by pubertal delay.

Just as continuous occupancy of GnRH receptors leads to an immediate and reversible inhibition of the secretion of gonadotropins, continuous administration of kisspeptin also results in desensitization of its receptor, as initially shown in the non-human primate.[51] Initially, parallel experiments performed in human subjects using an intravenous kisspeptin infusion (22.5 h) did not cause downregulation of gonadotropin and sex steroid hormone secretion in men.[60] However, continuous infusion of a novel kisspeptin analogue, TAK-448, resulted in sustained testosterone suppression in healthy males,[61] consistent with receptor downregulation. Administration of a kisspeptin antagonist, peptide 234, has been shown to inhibit spontaneous GnRH pulses in the nonhuman primate[62] and reduce LH pulses in ovariectomized sheep,[63] suggesting that kisspeptin is required for GnRH pulsatile secretion. However, studies in lower species have showed that the synthesis and release of endogenous GnRH is not completely controlled by kisspeptin. For example, in rats, peptide 234 was shown to inhibit *kisspeptin-induced* LH secretion. However, this antagonist did not reduce the baseline levels of LH, suggesting that GnRH secretion is not completely dependent on kisspeptin.[63] These findings further expand the potential clinical application for kisspeptin antagonists, as it is possible that they may have a role in serving as an alternative option for the treatment of hormone-related diseases without inhibiting sex hormones.

4 | *TACR3* AND *TAC3*

4.1 | Genetics

Neurokinins are members of the tachykinin family of ancient signalling peptides found both in vertebrates and invertebrates.[64] In humans, the main tachykinins are substance P (SP) and neurokinin A (NKA), both encoded by the same gene, *TAC1*, and neurokinin B, encoded by *TAC3*.[65] These neurokinins are broadly expressed throughout the CNS. They employ three closely related Gq-coupled receptors to transmit their effects. The genes *TACR1*, *TACR2*, and *TACR3 encode for* neurokinin receptors 1, 2, and 3 (NK1R, NK2R, and NK3R), respectively.[66–68] All tachykinins have varying degrees of action on each of the three receptors. However, there is a significant degree of selectivity for NKB on NK3R in vivo.[69,70] Unlike the identification of *KISS1R* mutations, a role for neurokinin B in the control of reproduction had been postulated for nearly 20 years, although most findings had been negative or conflicting. However, studies from the Rance laboratory demonstrated dramatically increased NKB expression in infundibular nuclei from postmortem postmenopausal female hypothalami.[71] Moreover, estrogen treatment of ovariectomized monkeys was shown to reduce kisspeptin expression.[72] Subsequently, the Goodman laboratory discovered that both kisspeptin and NKB are coexpressed in the ovine arcuate nucleus,[73] suggesting important interactions between these two peptides in the modulation of reproductive function.

Similar to the discovery of *KISS1R* mutations, autozygosity mapping (utilizing genome-wide SNP genotyping) was performed in 10 multiplex families with IHH. In half of these families, homozygous nonsynonymous mutations were identified within *TACR3* (4 families) and *TAC3* (1 family).[74] The ligand mutation was predicted to change

the terminal methionine of the mature NKB decapeptide to threonine,[74] disrupting the canonical tachykinin motif Phe-X-Gly-Leu-Met-NH2, which is universally conserved among tachykinins.[75] This mutation also compromises the post-translational amidation of the C-terminal which is necessary for full peptide activity.[76] Although mutations in *TAC3* are rare in patients with normosmic IHH, mutations in *TACR3* are relatively common.[20,7,77] To date, number of mutations in *TAC3* (n = 9) and *TACR3* (>40) have been published or listed in various databases.

Micropenis and cryptorchidism in male infants with IHH implies that intact fetal gonadotropin secretion is essential for proper testicular size, descent, and penile growth.[21] Particularly, micropenis has been noted at birth in the majority of infants who have *TACR3* mutations, suggesting that intact NKB signalling is required for normal fetal gonadotropin secretion.[20,74,77,78] As with patients who harbour mutations in *GnRHR*, *GNRH1*, *KISS1R*, and *KISS1*, the phenotype patients with mutations in *TAC3* or *TACR3* appears to be restricted to the reproductive system. Initial reports suggested that *TAC3* and *TACR3* mutations were associated with fully penetrant nIHH and an autosomal recessive pattern of inheritance. However, a large study of 345 probands with normosmic IHH uncovered many heterozygous cases and a significant rate of clinical recovery.[77] Several of these variants were predicted to be truncating mutations which would lead to haploinsufficiency. It is also possible that these patients might carry an as-yet-unidentified mutation on the opposing allele of *TACR3*, or a mutation in another IHH gene. In fact, the presence of more than one IHH-associated-mutant gene in a patient/pedigree (oligogenic aetiology) has been proposed to account for 10%–20% of all IHH cases.[79–82] With the increasing use of unbiased genetic studies that are facilitated by whole-exome sequencing, it is now appreciated that oligogenic inheritance is more common than previously appreciated in Mendelian disorders.[83] Further studies will be necessary to determine the true prevalence of oligogenicity in IHH.

Clinical reversibility, which is evident by the spontaneous initiation of pubertal development (often after a period of exogenous sex steroid exposure), is observed in 10%–20% of unselected IHH patients.[84,85] Patients with mutations in several genes, including *ANOS1*, *GNRHR*, *PROKR2*, *FGFR1*, *CHD7*, *TAC3*, and *TACR3*, have been reported to recover as evidenced by increases in testicular volume, spontaneous menstruation, normalization of sex steroid levels, and fertility in the absence of fertility medications;[77,86,87] In one family that was studied in detail, three of four sisters with IHH each of whom carried a novel homozygous null mutation in *TAC3* (c.61_61delG p.A21LfsX44) demonstrated reversal as evidenced by pregnancy or spontaneous menstrual periods.[88] Unfortunately, their reversal was not sustained and they reverted back to IHH. In fact, IHH reversibility was observed in 10 of 12 IHH patients (83%) carrying *TAC3*/*TACR3* mutations, suggesting that the role of the NKB pathway in GnRH secretion may be less critical in adult life than during late gestation and the early neonatal period.[77]

With this variably high rate of reversibility, some have hypothesized that CDGP may be a mild form of IHH caused by *TAC3*/*TACR3* mutations. Although a Finnish cohort of CDGP was devoid of mutations,[89] *TAC3*/*TACR3* variants were found to be enriched in a different CDGP/delayed puberty cohort, suggesting both CDGP and IHH share a common underlying

mechanism.[90] In addition, an SNP immediately upstream of *TACR3* (rs3733631) was found to be significantly associated with the age at menarche.[91]

Complementary clinical studies have provided valuable insight into the role of NKB in the biology of reproduction. First, Young et al.[78] were able to produce pubertal levels of gonadotropins and gonadal sex steroids with repeated administration of GnRH in patients with null *TAC3* mutations, indicating that the site of NKB action is upstream of the GnRH neuron. Second, two sisters with homozygous *TAC3* mutations showed clinical evidence of reproductive recovery and conceived spontaneously. Moreover, one of the pregnancies proceeded successfully to term. These observations indicate that NKB, although expressed at high levels in the placenta and other peripheral reproductive organs, is not required for placentation nor pregnancy.[77]

4.2 | Clinical studies

Initially, activation of the NKB receptor in vivo gave conflicting results across species. Intravenous administration of 100 μg (approximately 30 nmol/kg) NKB was shown to stimulate LH secretion 3-fold in agonadal juvenile male monkeys.[92] However, NKB appeared to have both excitatory and inhibitory effects on gonadotropin secretion in rodents, depending probably on the hormonal milieu.[66,93–102] NKB had no significant effect on reproductive hormone (LH, FSH, testosterone, or oestradiol) secretion or LH pulsatility in healthy men, healthy reproductive-age women, and post-menopausal volunteers.[103,104]

Short-term administration of NKB receptor antagonists, on the other hand, reduced LH secretion and or pulsatility in healthy women. Fezolinetant (ESN364), an NK3R antagonist, produced dose-dependent decreases in LH (with no significant effect on FSH) in healthy female volunteers with regular ovulatory menstrual cycles.[105] Another NK3R antagonist, MLE4901 (Pavinetant), reduced basal LH secretion, without changing pulse frequency, and delayed the LH surge by 7 days in healthy women with regular menses.[106] Elinzanetant is a dual NK1,3R antagonist and therefore has the potential to reduce GnRH pulsatility by blocking the endogenous effects of NKB and SP on the reproductive axis. Elinzanetant has recently been shown to dose-dependently lower serum LH, oestradiol, and luteal-phase progesterone in healthy women. At the highest dose tested, Elinzanetant prolonged the cycle length by a median of 7.0 days.[107] Collectively, these data demonstrate the involvement of NKB-NK3R signalling in the physiological regulation of GnRH/LH secretion in women.

Unlike *KISS1R* or *GNRHR*, loss-of-function mutations in *TACR3* present differential effects on gonadotropin levels, such that plasma LH levels are profoundly diminished while FSH levels are not significantly different from those of healthy individuals,[78] suggesting that this phenotype may arise from a lowered GnRH pulse frequency.[108] In female monkeys, the NK3R antagonist ESN364 prolonged the LH interpulse interval but did not change baseline FSH levels, analogous to the phenotype in patients with deleterious mutations in *TAC3* or *TACR3*.[109] These observations may reflect the fact that the regulation of FSH secretion is multifactorial while LH release is exclusively dependent upon GnRH.

NK3R antagonists have been studied as candidate therapeutic agents for clinical disorders pathophysiologically associated with increased LH secretion, most notably, postmenopausal hot flushes and PCOS. The results so far are promising.

4.2.1 | Postmenopausal hot flushes—About 70% of women globally suffer from postmenopausal hot flushes. Current clinical practice proposes estrogen replacement in severe cases, which is associated with a variety of untoward effects. As mentioned above, the hypertrophied neurons in the hypothalamus of postmenopausal women and ovariectomized monkeys overexpress kisspeptin and NKB; this overexpression is reversed by estrogen replacement.[71,110–112] KNDy neurons project to the TACR3-expressing median preoptic nucleus within the hypothalamus, an important centre for thermosensory heat-defence.[111,113] Based on these observations, the Rance laboratory has shown that KNDy neuron ablation in rats results in reduced tail-skin temperature, indicating that KNDy neurons facilitate cutaneous vasodilatation, a major component of a hot flush. Three independent clinical studies have suggested that NK3R antagonists may be candidates to treat postmenopausal hot flashes. In a randomized, double-blind, placebo-controlled, crossover study, peripheral infusion of NKB intravenously to healthy premenopausal women induced typical hot flushes.[114] Then, a phase 2, randomized, double-blind, placebo-controlled, 4-week crossover trial of an oral neurokinin 3 receptor antagonist, MLE4901 (Pavinetant), formerly also known as AZD4901, demonstrated that this agent significantly reduced the number of hot flushes by 45% compared with the placebo.[115] MLE4901 also showed beneficial effects on sleep quality and mood. MLE4901 was well tolerated with a mild to moderate elevation in liver transaminases. Similarly, in another study, treatment with MLE4901 for 7 days reduced menopausal hot flashes by twothirds.[116] A different compound, Fezolinetant (ESN364), when given orally for 12 weeks, significantly reduced total vasomotor symptoms (VMS) score versus placebo by about one-half and decreased mean frequency of moderate/severe VMSs by five episodes per day versus placebo. Remarkably, the severity and frequency of moderate/severe VMSs were reduced from the first day of treatment. Improvements were achieved in all quality-of-life measures. Mild to moderate transient serum ALT elevations were also observed in this study. As in other studies, NK3R antagonist treatment resulted in lower LH levels while not affecting those of FSH, thus reducing LH/ FSH ratio. Fezolinetant is now in phase 3 development for the treatment of vasomotor symptoms in postmenopausal women.[117]

4.2.2 | Polycystic ovary syndrome (PCOS)—Polycystic ovary syndrome affects approximately 10% of reproductive-aged women globally, making it the most common endocrine disorder. PCOS is the leading cause of anovulatory infertility.[118,119] The diagnostic criteria for PCOS include clinical or biochemical hyperandrogenism, which is proposed to be pathophysiogically linked to high-frequency pulses of LH, elevated serum LH, and a high LH/FSH ratio.[120,121] In premenopausal women, NK3R antagonism decreases the GnRH pulse frequency leading to reduced basal LH secretion, lower LH/FSH ratio, and the modulation of the temporal dynamics of ovarian sex hormone production over the menstrual cycle.[106] The NK3R antagonist MLE4901 was demonstrated to reduce LH pulse frequency, as well as serum LH and testosterone levels, in women with PCOS.[122] These hormonal findings were echoed in a recent study utilising Fezolinetant; however,

there was no improvement in menstrual cycle regularity or PCOSQ scores. The investigators argued that a 12-week duration of treatment in this trial may be inadequate to change these parameters as positive clinical outcomes in PCOS clinical trials are typically detected after 6–9 months of treatment.[117,123]

Taken together, neurokinin receptor antagonism may negate differential hypersecretion of LH (i.e., excessive secretion of LH in comparison to FSH), and by extension, prevent excessive ovarian androgen production, a cardinal feature of PCOS.

In summary, the NKB signalling is an integral part of the GnRH pulse generation. Various NK3R antagonists have been shown to selectively reduce the pituitary LH secretion while being neutral on FSH. This differential effect has been translated into the pharmacological agents to treat the two most common reproductive health problems of the women globally: menopausal hot flushes and PCOS. The results from these clinical studies have been promising.

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