

## Role of Kisspeptin in Female Infertility

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### Abstract

**Background:** Kiss1, a noble G protein coupled receptor designated as GPR54, was first identified in rat brain in 1999 and orthologue gene identified in human in 2001 the original niche for the function of kisspeptin was restricted to cancer biology for their ability to suppress tumor metastasis. However, kisspeptin has recently emerged as a key player in the field of reproductive endocrinology. **Method:** A systematic literature review was done by using PUBMED. Though there is lack of human data, used animal data also hold translational potential for human. **Results:** Inactivating mutation of GPR54 gene is linked with absence of puberty onset and idiopathic hypogonadotropic hypogonadism. Furthermore, recent studies support critical role of kisspeptin/GPR54 system on regulation of GnRH neurons, involvement of puberty onset and gonadal steroid feedback. **Conclusion:** This review will briefly discuss on cellular and molecular level of kisspeptin, their potential effects on human and clinical application of kisspeptin on human reproductive disorder.

### Introduction

Procreation is an indispensable part of every species. To initiate and control reproduction, coordination of neuronal networks play complex role and finally make a common pathway, which is well known as Hypothalamic Pituitary Gonadal (HPG) axis that synthesize gonadotrophic releasing hormone. Since its discovery, adequate pulsatile hypothalamic gonadotrophic releasing hormone (GnRH) secretion has been considered as key element for maintaining reproductive function.<sup>1-4</sup> During puberty, hypothalamus start synthesis and release of GnRH, which acts on anterior pituitary to secrete FSH and LH, as a result gonads begin to produce sex steroid and peptide hormone.<sup>5-7</sup>

Only a decade ago in 2003, discovery of kisspeptin and its role on regulation of the HPG

axis, revolutionized our current understanding on control of human reproduction.<sup>8</sup> It is believed that, kisspeptin and functional neural network KNDY (kisspeptin / neurokinin / dynorphin) system modulate GnRH pulse. Therefore, Kisspeptin is considered as a key regulator of gonadotropin secretion and responsible for many physiological phenomenon. Moreover, manipulation of KNDY neural network and regulation of LH pulse, subsequently control of gonadal hormonal secretion may open a new horizon in treatment of infertility. Recently reproductive scientists are working for future application of KNDY system by increasing LH pulse, like wise hypothalamic amenorrhea, hypogonadotropic hypogonadism or reduce LH secretion, such as in polycystic ovary syndrome.<sup>9</sup>

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In this review, briefly discussed on cellular and molecular level biology of kisspeptin and its potential effect on human, more precisely on female reproduction, and how future clinical application of kisspeptin may resolve neural reproductive disorder. A systematic literature review was done by using PUBMED. Though there is lack of human data, used animal data also hold translational potential for human.

### Discovery of Kiss1 Gene and Receptor

Kiss1, a noble G protein coupled receptor designated as *GPR54*, was first identified in rat brain in 1999.<sup>10</sup> This newly identified molecule catalogued as a suppressor of metastasis in melanoma cell line, therefore it is widely known as metastin. Hershey, (PA, USA) which was famous for Hershey kisses chocolate and birthplace of kiss I gene, also named as kiss after this exclusive sweets. However, 2 years later, in 2001, orthologue gene *AXOR12* and *hOT7T175* identified in human and termed as KISSIR.<sup>10-12</sup> Three independent research groups explore endogenous ligand of *GPCR* named as *GPR54*<sup>13</sup>, *AXOR12*<sup>11</sup>, *HOT7T175*<sup>12</sup> by using different model, CHO K1<sup>13</sup>, HEK 293<sup>11</sup> and B16-B26<sup>12</sup> consecutively. Therefore, international pharmacology association displaced metastin to kisspeptin considering structural similarities and origin of kiss1 derivative peptides.

### Biology of Kisspeptin

Kisspeptin, a noble neuromodulator, which is peptide in nature and encoded by the kiss 1 gene that activate G protein coupled receptor and upstream GnRH secretion. Kisspeptins are generated from a single precursor through

various proteolytic processing.<sup>14</sup> In human, kisspeptin precursor encodes 145 amino acid including a 54 amino acid region, named as kisspeptin 54 (formerly termed as metastin).<sup>12,15</sup> This segment can be further divided into low molecular weight forms which are termed as KP 54, KP13, KP 10 for 14, and 13 and 10 amino acid peptide respectively. In addition, all peptide fragment share c terminal sequence of kisspeptin 54, which is the characteristics feature of RF amide group of peptide, collectively known as kisspeptin.<sup>12</sup> Kiss 1R, upon binding its ligand kisspeptin activates phospholipase C and convert secondary intracellular messengers, inositol 1,4, 5 triphosphate (IP3) and diacyl glycerol, that induce calcium release and finally activation of protein kinase C to proceed kisspeptins function.<sup>11,16-17</sup>

### Distribution of Kisspeptin

In 2001, different independent study group isolated kiss1 mRNA from placenta, spinal cord, pancreas, and pituitary gland by using reverse transcriptase polymerase chain reaction.<sup>11-13</sup> In addition, northern blotting test has revealed kiss1 and *GPR54* genes in peripheral area, such as heart, liver, kidney, placenta and Immune Reactivity (IR) found in different areas of the brain.<sup>18,19</sup> Thus in 2005, specific population of kiss1 neuron has been recognized in hypothalamus.<sup>11-12</sup> Infundibulum as source of kiss1 neuron has been isolated from autopsy samples of premenopausal and post-menopausal women.<sup>20</sup> Furthermore another study done in 2010 including both male and female autopsy sample support the location of most dense Kisspeptin neuron area as infundibulum and

second most dense as pre-optic area.<sup>21</sup> As highlighted above, Kisspeptin neuron has been detected in the Infundibular / Arcuate Nucleus in all species. However, availability of Kisspeptin neuron in rostral pre-optic area varies from species to species.<sup>21-25</sup> For example, in rat model Kisspeptin is located in rostral periventricular region of the third ventricle of its brain whereas, it is absent in human brain.<sup>26-27</sup> Only infundibulum in human, homologous to arcuate in rodent express all three KNDY neuron.<sup>36</sup>

### **Physiological Action of KNDY Neuron**

GnRH pulse is mainly mediated by gonadal steroid, (steroid sensitive receptor).<sup>30</sup> In a study by Goodman et al revealed that Kisspeptin / Neurokinin / Dynorphin are in same functional neuronal network, collectively known as KNDY network which is steroid sensitive and play as a crucial regulator for GnRH pulse.<sup>29</sup> In a schematic overview published by Skoroupskate et al 2014 to correlate KNDY-GnRH pathway and sex steroid feedback system<sup>9</sup> which is adapted in this article. (Fig:1) It was not until 2003, that *GPR54* mutation in men was identified to be associated factor for hypogonadotropic hypogonadism and subsequently absent/delayed puberty, made a revolutionary understanding in role of Kisspeptin and KNDY neuron GnRH pulse. Furthermore, subsequent research has revealed that KNDY subpopulation in various species range from rodent to human play a key role in GnRH secretion by controlling neuro activity of KNDY cells.<sup>30-32</sup> These reciprocally inter connected KNDY cells are very sensitive to steroid and act by direct contact with GnRH cell bodies and neuro secretory terminal (in human)

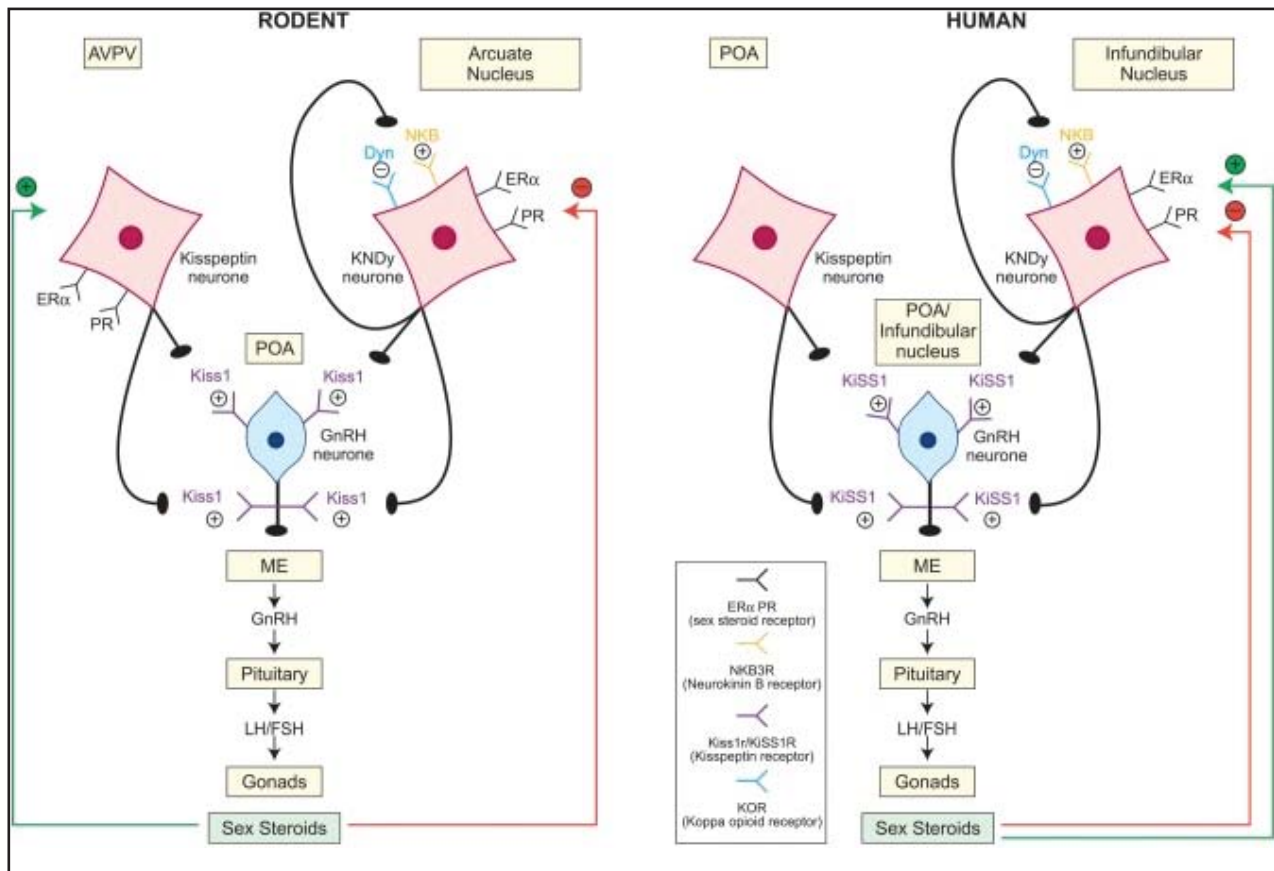
on to the median eminence (in rodent, sheep & monkey).<sup>22,23,25,33</sup> In 2009, Navarro et al discovered that LH secretion was inhibited by dynorphin and neurokinin B, which act auto synaptically on pulsatile release of Kisspeptin and drive pulsatile release of GnRH and LH.<sup>34</sup>

### **Role of steroid on Kisspeptin**

Studies with human and various animal models suggest that pulsatile GnRH secretion followed by LH secretions is controlled by steroid feedback.<sup>35</sup> However, GnRH neuron located in hypothalamus are devoid of estrogen receptor that suggests another group of neuron is essential to convey message of ovulation induction to GnRH neuron.<sup>9</sup> After discovery of physiological role of KNDY it is suggested as <missing link> of gonadal steroid feedback.

Various animal data collection suggests that estrogen derived negative feedback has been mediated by Kisspeptin neuron and neurokinin B of arcuate nucleus. In ovariectomised animal model (rodents, sheep, monkey etc.)<sup>32</sup> express high level of kiss1 mRNA in arcuate nucleus, which supports high level of kiss1 mRNA and neurokinin B in infundibulum of post menopausal women than in premenopausal women.<sup>20</sup>

In the late follicular phase estrogen feedback switches to positive, ultimately induce LH surge for ovulation. Though arcuate Kisspeptin (widely known as KNDY) mediates negative feedback, the positive steroid feedback mediated Kisspeptins are species specific (figure). Apart from Kisspeptin, other KNDY neuron originating from arcuate nucleus do not participate in positive feedback.<sup>31,34</sup>



**Fig. 1: Illustrative figure of KNDY system and GnRH neurons explain kisspeptin-GnRH pathway in humans and rodents.**

Kisspeptin neurons send impulse directly to the GnRH neurons of kisspeptin receptor (Fig. 1). Kisspeptin neurons located in the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus in rodents, and within the preoptic area (POA) and the infundibular nucleus in humans. KNDY neuron regulates kisspeptin pulse through their reciprocal connection via neurokinin B receptor and kappa opioid peptide receptor where neurokinin B acts as stimulatory and dynorphin inhibitory. Negative (red) and positive (green) sex steroid feedback is mediated via distinct kisspeptin populations in rodents, via the AVPV and the arcuate nucleus, respectively. In humans KNDY neurons in the infundibular nucleus relay both negative (red) and positive (green) feedback. The role of the POA kisspeptin population in mediating sex steroid feedback in humans is incompletely explored.

(ME, median eminence; +, stimulatory; -, inhibitory; ERα, estrogen receptor alpha; PR, progesterone receptor; Kiss1/KISS1, kisspeptin; NK3R, neurokinin B; dynorphin)

\*\* Slight modified and adapted from: The kisspeptin-GnRH pathway in human reproductive health and disease.

### **Clinical Aspects of Kisspeptin in Reproductive Health**

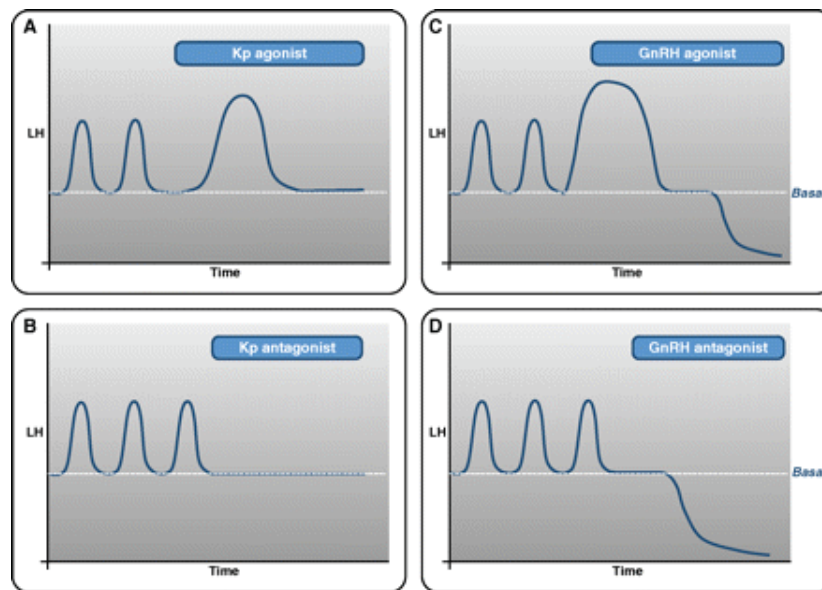
Since discovery of inactivating point mutation in gene GPR54 is associated with impaired puberty and idiopathic hypo gonadotrophic hypogonadism in 2003<sup>8,37</sup> it is believed that dysfunction in Kisspeptin neuronal network may be associated with many other clinical disorders. In addition, Teles et al identified activating mutation (Arg 386 Pro) in 2008, which is associated with precocious puberty.<sup>38,42</sup> Furthermore missense mutation of Kisspeptin has been identified as precocious puberty in three individual cases, which suggests that mutation gene resist *in vitro* degradation followed by high bio-availability, ultimately causing precocious puberty.<sup>39</sup>

One neuro endocrine disorder is hypothalamic amenorrhea, which is characterized by low GnRH pulse and subsequently declined LH, FSH and failure in follicular development. In this disease, sustained gonadotrophic secretion at normal physiological level was achieved with Kisspeptin inject in subcutaneous twice daily (6.4 mmol/kg) for 8 weeks.<sup>40</sup> Though folliculogenesis was not restored in initial studies, the ability of Kisspeptin to increase GnRH secretion and subsequent effect on FSH and LH to restore menstrual cycle will play a major role in the therapeutic approach of Kisspeptin.<sup>42</sup>

Another common disorder in women is PCOS,

which is characterized by high level of LH due to neuro endocrine feedback defect and relative high insulin lead to metabolic defect.<sup>43</sup> KNDY neurons are believed to be potential regulator of steroid mediated negative feedback. In 2003, Meneilly et al also suggested that GnRH secretion mainly depends on LH level, therefore, reduction of GnRH pulses may restore normal LH secretion. Therefore therapeutic manipulation can be achieved by using Kisspeptin and neurokinin B receptor antagonist or dynorphin agonist through stimulating /inhibitory action of dynorphin. Moreover, ovarian hyper stimulation syndrome (OHSS) in PCO cases can be significantly reduced by kisspeptin.<sup>9</sup>

Kisspeptin may be a good therapeutic option in certain gynecological disorder, for example: endometriosis, uterine fibroid, where partial suppression of gonadotropin secretion is more useful than marked suppression, and significantly reduce side effect of complete suppression.<sup>44</sup> Data obtained from animal model revealed that kisspeptin antagonist /agonist reduce LH pulse without affecting basal LH secretion (Fig. 2). In contrast, repeated administration of GnRH agonist/antagonist results in marked suppression of gonadotropins, more precisely LH response. Therefore, kisspeptin may be a key player in treatment of reproductive endocrine diseases and In vitro fertilization (IVF).



**\*\*Fig 2: Schematic figure of tentative LH secretion pattern after continuous administration of kisspeptin agonist (A), antagonist (B), GnRH agonist (C) and GnRH antagonist (D).**

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\*\* Adapted from Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Review Kisspeptins and reproduction: physiological roles and regulatory mechanisms. *Physiol Rev.* 2012 Jul; 92(3):1235-316

### Conclusion

In the last couples of years, kisspeptin is considered as key regulator of HPG axis and coordinator of GnRH secretions. Recent understanding on regulation of LH pulse by kisspeptin creates a new opportunity in treatment modalities of reproductive endocrinology and infertility. Now it is believed that consequent effect of complete GnRH suppression may minimize by ensuring basal LH secretion through kisspeptin analogue. Therefore, it will play a master role in the treatment of future female infertility.

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