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MINI REVIEW

Role of Kisspeptin-GPR54 system in regulation of reproductive functions in human and other mammals

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Abstract: Kisspeptin is a 54- amino acid peptide that acts as a ligand of a receptor called GPR54 which is basically a transmembrane receptor that spins seven times across the cell membrane and coupled with G-protein. Kisspeptin regulates the development of reproductive functions and the onset of puberty in human and other mammals by acting at the brain, hypothalamus, pituitary and gonad levels of reproductive axis. Kisspeptin is also involved in regulation of trophoblastic invasion during pregnancy, ovulation, and sperm hyperactivation. Inactivating mutations in human kisspeptin gene (*KISS1*) cause idiopathic hypogonadotropic hypogonadism. Some mutations in human kisspeptin receptor gene (*KISS1R*) make the receptor inactive which result in idiopathic hypogonadotropic hypogonadism. Some mutations in human *KISS1R* gene make the receptor prematurely activated and result in the development of central precocious puberty. Central precocious puberty is also caused by some mutations in human *KISS1* gene that make the kisspeptin resistant to degradation. This leads to an increased basal kisspeptin level and subsequently the development of central precocious puberty. Higher kisspeptin level has been detected in the serum and plasma of central precocious puberty patients, which suggest that serum or plasma kisspeptin level can be used as a marker for diagnosis of central precocious puberty.

Keywords: Kisspeptin, GPR54, reproductive axis, pregnancy, trophoblastic invasion.

INTRODUCTION

Kisspeptin (KP) is a peptide encoded by the gene, *Kiss1*, which was initially known for its role in suppression of cancer (Ohtaki *et al*., 2001). In humans, this gene (*KISS1*) encodes a 145 amino acids peptide known as Preprokisspeptin which is proteolytically cleaved into fragments of 54 (KP-54), 14 (KP-14), 13 (KP-13) and 10 (KP-10) amino acids that share Arginine-Phenylalnine-NH2 (called RF-amide) at the C-terminus (Kotani *et al*., 2001) (fig. 1). The long KP isoform, KP-54, is very important biologically. The rest of the cleaved fragments also possess biological activity (Kirby *et al*., 2010).

According to the Human Genome Organization Gene Nomenclature Committee (HGNC), *KISS1* should be used for human kisspeptin gene and *Kiss1* for non-human Kisspeptin gene. The non-italicized versions of the gene nomenclature should be used for the peptide product of the genes e.g. KISS1 for human kisspeptin and Kiss1 for non-human kisspeptin. Similarly, these committees recommended the use of *KISS1R* for human Kisspeptin receptor gene or mRNA, and *Kiss1r* for non-human Kisspeptin receptor gene or mRNA. The non-italicized version of this gene nomenclature represents the receptor i.e., KISS1R (GPR54) in human and Kiss1r (GPR54) in non-human species (Gottsch *et al*., 2009).

Originally the receptor for kisspeptin was identified in rat brain as orphan receptor which shares 45% identity with gelanin receptor and was named as GPR54 as this receptor is coupled with G-protein (Lee *et al*., 1999). Kisspeptin receptor, GPR54, spins seven times across the cell membrane which has an intracellular -COOH terminus and an extra cellular -NH_2 terminus (Ohtaki *et al*., 2001; Gershengorn and Osman, 2001) (fig. 2). The role of KP-GPR54 interaction was determined during 2003 when mutations were detected in the kisspeptin receptor (GPR54) gene (*KISS1R*) in patients suffering from idiopathic hypogonadotropic hypogonadism (IHH) which was then confirmed in transgenic mice (Seminara *et al*., 2003). Human KP gene (*KISS1*) localization is chromosome 1q32 (West *et al*., 1998) and its expression occurs in different parts of hypothalamus, superior cervical ganglions and carotid body (Porzionato *et al*., 2011), testes, intestine, liver, pancreas (Lee *et al*., 1996; Muir *et al*., 2001; Ohtaki *et al*., 2001) and Kidney (Lee *et al*., 1996). Recently, *KISS1* expression has also been detected in female adipose tissues and spermatozoa (Cockwell *et al*., 2012; Pinto *et al*., 2012). Human KP receptor (GPR54) gene (*KISS1R*) localization is chromosome 19 p13.3 [Gene ID: 84634] and its expression has been identified in the hypothalamus and other brain parts (Lee *et al.,* 1999, Muir *et al*., 2001),

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carotid body (Porzionato *et al*., 2011), spinal cord (Kotani *et al*., 2001), intestine, liver (Lee *et al*., 1999), kidneys, skeletal muscles, heart, pancreas (Clements *et al.*, 2001) and placenta (Clements *et al*., 2001; Bilban *et al*., 2004). Recently Pinto *et al*. (2012) reported *KISS1R* expression in human spermatozoa.

KP-GPR54 interaction

KP binding to its receptor (GPR54) in the hypothalamus stimulates the release of gonadotrophin releasing hormone (GnRH), which in turn stimulates gonadotrophin release i.e., follicle stimulating hormone (FSH) and luteinizing hormone (LH). In the hypothalamus, the neurons that release GnRH, express the KP-receptor, GPR54. In mouse, expression of GPR54 mRNA has been detected in 90 percent of the GnRH secreting neurons (Han *et al*., 2005). In primates and rodents, GnRH secreting neurons have been observed very close to KP neurons in the hypothalamus (Ramaswamy *et al*., 2009). Pompolo *et al*. (2006) reported that KP neurons colocalize to large number of GnRH- immunoreactive cells in the ovine preoptic area. The binding of the KP to GPR54 results in the activation of phospholipase-C through a pathway mediated by the G-protein (Liu *et al.,* 2008). The Phospholipase-C activation causes the hydrolysis of phosphatidylinositol 4, 5-bisphosphate into inositol triphosphate and diacylglycerol. Inositol triphosphate causes the release of Ca^{2+} from the intracellular Ca^{2+} stores, whereas the diacylglycerol activates the protein kinase-C (Constantin *et al.,* 2009). According to Zhang *et al.* (2008), the diacylglycerol and or Ca^{2+} activate transient receptor potential canonical - like channels and inhibit inwardly directed K^{+} channels which ultimately result in the release of GnRH. GnRH hormone is responsible for the secretion of gonadotropins i.e., LH and FSH in the anterior pituitary (Li *et al*., 1999). The LH and FSH promote follicle cells production, the synthesis of sex steroid hormones, spermatogenesis and ovulation (Ramaswamy and Weinbauer (2014).

GnRH is secreted both in pulse and surge modes. Pulse mode of GnRH secretion is essential for the development of reproductive system and onset of puberty (Millar *et al*., 2004). The pulse mode of GnRH secretion stimulates LH and FSH secretions which drives steroidogenesis, folliculogenesis, and spermatogenesis (Maeda *et al*., 2010), while the surge mode induces the pre-ovulatory gonadotropin discharge that initiates ovulation (Tsukamura and Maeda, 2011). Smith *et al*. (2010) studied the expression of KP mRNA in the hypothalamus of adult female rhesus monkey. They observed expression of KP mRNA in the caudal arcuate nucleus and pre-optic area of hypothalamus. Higher expression of *KISS1* gene was observed during the late follicular phase of menstrual cycle which prompts a surge-like pattern of GnRH release prior to ovulation (Smith *et al*., 2010).

Role of KP-GPR54 interaction in activation of reproductive axis in human

KP-54 administration has a stimulatory effect on human male gonadotrophins release (Dhillo *et al*., 2005). In women, subcutaneous injection of KP-54 causes increase in plasma LH level, with greatest effect before ovulation, which provides a mechanism for the manipulation of the female reproductive axis through administration of KP (Dhillo *et al*., 2007). In healthy men, intravenous injections of KP-10 at doses ranging from 0.01 µg/kg body weight to 3.0 µg/kg body weight elicited LH secretion and continuous administration of KP resulted in increased frequency and size of LH pulse and consequently increased testosterone level (George *et al*., 2011). Jayasena *et al*. (2012) reported a striking sexual dimorphism in the effects of KP-10 in humans. KP-10 stimulated gonadotrophin release in men and pre-ovulatory women, but failed to stimulate gonadotrophin release in follicular phase women. Thus KP has the potential to stimulate gonadotrophin release in humans and therefore represents a potential therapy for disorders of reproductive system. Recently, the expression of GPR54 mRNA has been detected in the head and neck of human spermatozoa (Pinto *et al*. (2012). Pinto *et al*. (2012) exposed the human spermatozoa to KP which caused an increase in the intracellular Ca^{2+} concentration, transient sperm hyperactivation and modulated sperm motility. This indicate the existence of KP-GPR54 interaction in spermatozoa of human. The findings of Pinto *et al*. (2012) represent the influence of KP in the control of fertility in male (Pinto *et al*., 2012).

Role of KP-GPR54 interaction in activation of reproductive axis in other mammals

The critical role of KP in activation of reproductive axis and onset of puberty has been investigated in a range of mammals (Navarro *et al*., 2005; Castellano *et al*., 2006; Keen *et al*., 2008). Now it is a well-known fact that KP activates reproductive axis through eliciting the pubertal resurgence of pulsatile secretion of GnRH. Significant increase in KP-54 has been observed in the stalk-median eminence of female rhesus monkeys during puberty which correlated with increase in LH-releasing hormone-1 (also called gonadotrophin releasing hormone-1) release (Keen *et al*., 2008). In rats, central administration of KP stimulated LH secretion and it was concluded that the effect of KP on hypothalamic pituitary gonadal axis is mediated by LH-releasing hormone (Navarro *et al*., 2005). KP and GPR54 mRNAs have been detected in adult rat ovary along estrous cycle (Castellano *et al*., 2006). Rat ovarian KP mRNA rises with pre-ovulatory gonadotropin (LH) surge before ovulation, which suggests the role of ovarian KP in the regulation of ovulation (Castellano *et al*., 2006). Direct administration of KP-54 and KP-10 into the mice lateral cerebral ventricle stimulated LH and FSH secretions which reflected the role of KP-GPR54 interaction in activation

of mice reproductive axis (Gottsch *et al*., 2004). KP mutant mice fails to reach sexual maturation and possess small sized gonads and insufficient levels of circulating gonadotropin and sex steroid hormones (de Tassigny *et al*., 2007). In musk shrew (*Suncus murinus*), KP mediates mating-induced ovulation (Inoue *et al*., 2011). Exogenous Suncus KP administration in *Suncus murinus* mimicked the stimulus of mating that resulted in the maturation of ovarian follicles and subsequently in ovulation (Inoue *et al*., 2011). The role of KP in the reproductive function regulation has also been explored in ungulates. For example, Redmond *et al.* (2011) reported that intravenous administration of KP result in the elevation of frequency and amplitude of LH pulses and blood progesterone level. Suzuki *et al.* (2008) studied the direct effect of KP-10 *in*vitro on the release of LH from the cells of anterior pituitary gland of bovine and porcine. The direct application of KP-10 in concentration range of 1000 nM-10,000 nM on anterior pituitary cells of bovine and porcine resulted in the release of LH. It was concluded that KP-10 also acts directly on the anterior pituitary gland cells in bovine and porcine but its stimulating effect on anterior pituitary is far weak than the stimulating effect of GnRH on anterior pituitary cells. The role of KP in the stimulation of reproductive system has also been reported in equine (Magee *et al.,* 2009). Intravenous rodent KP-10 administration in diestrous mare resulted in elevated serum LH and FSH levels (Magee *et al.,* 2009).

Role of KP-GPR54 interaction in seasonal regulation of reproductive activity

Kisspeptin plays important role in regulation of reproduction in seasonally breeding species. Syrian hamster becomes active sexually during summer long days. In this mammal, *Kiss1* expression is significantly higher during long days as compared to short days (Revel *et al.,* 2007). In sheep, breeding is seasonal which is under the influence of light duration. In sheep, KP mRNA activity is significantly higher during short days (its breeding season) as compared to long days (Smith *et al.,* 2008). During non-breeding season, KP administration in ewe induces the release of ova from the ovary (Caraty *et al.,* 2007). According to De Bond *et al.* (2013), the introduction of a novel male sheep to seasonal anestrus female sheep results in the stimulation of reproductive axis, maturation of ovarian follicles and ovulation. They observed significantly higher expression of KP mRNA in the rostral and mid arcuate nucleus of anestrous female sheep after exposure to novel male and it was concluded that introduction of male sheep to seasonally anestrous female sheep activates KP neurons and other cells in the hypothalamus, leading to increased GnRH/LH secretion. Thus KP play important role in the activation of reproductive axis in seasonal breeders.

Mutation in human KP-GPR54 system and Idiopathic hypogonadotropic hypogonadism (IHH)

Inactivating mutations in human *KISS1* gene cause HH (Nimri *et al*., 2011). Recently Topaloglu *et al*. (2012) described an inactivating mutation in *KISS1* that caused IHH in human. IHH is characterized by absent puberty, infertility and low serum gonadotrophins in the absence of a pituitary tumor (Seminara *et al*., 1998). Impaired GnRH release, action or gonadotrophin release result in the development of IHH (Iovane *et al*., 2004). IHH may be with anosmia called Kallman syndrome or without anosmia called isolated IHH (Bouloux *et al*., 2002). Mutations in genes such as *Kal-1* (responsible for encoding Anosmin-1) and *FGFR-1*(responsible for encoding fibroblast growth factor receptor- 1) that result in impaired GnRH and olfactory neurons migration from the olfactory placode to the hypothalamus during embryonic development cause anosmic IHH (Kallman syndrome) (Bhagavath and Layman, 2007). Mutations in several other genes have also been linked with Kallman syndrome and IHH (Miura *et al*., 2004; Pitteloud *et al*., 2006; Falardeau *et al*., 2008; Bouligand *et al* 2009).

Mutations in human *KISS1R* gene that make the receptor inactive also result in IHH. De Roux *et al*. (2003) detected homozygous deletion in a family in which five siblings were affected with IHH. The deletion was identified in part of exon 5 and splicing acceptor site at the junction of intron 4 and exon 5 which resulted in truncated GPR54. Such truncated receptor was inefficient in stimulating the G-protein mediated signaling pathway and thus resulted in IHH (Gether, 2000). Lack of puberty in members of a consanguineous family was due to mutation in *KISS1R* that resulted in substitution of serine at the place of normal leucine at position 148 (Seminara *et al*., 2003). This mutation was responsible for impaired signaling of the GPR54 which was the cause of IHH. During 2005, a homozygous sequence variation in *KISS1R* was identified that caused an increase of 43 amino acids in the intracellular domain of GPR54 in which proline residues was maximum. Such mutant GPR54 was not able to stimulate the G-protein mediated signaling pathway which was the reason of IHH male (Lanfranco *et al*., 2005). Another homozygous mutation in *KISS1R* gene was identified in which Thymine to Cytosine changeover was detected at nucleotide 305, exon 2 (Tenenbaum-Rakover *et al*., 2007). The mutation resulted in substitution of leucine with proline in the first extra cellular loop of GPR54. The substitution of leucine with proline resulted in IHH patients during 2007 (Tenenbaum-Rakover *et al*., 2007). This substitution completely inhibited GPR54 signaling that resulted in delayed puberty (Tenenbaum-Rakover *et al*., 2007). Recently Nimri *et al*. (2011) detected homozygous mutation in *KISS1R* gene in six individuals who were suffering from normosmic IHH. The patients were belonging to two families of Israeli Muslim of Arab

origin. The mutation caused the substitution of phenylalanine with serine that resulted in inhibition of KP-GPR54 signaling pathway and consequently in IHH (Nimri *et al*., 2011). Semple *et al*. (2005) reported an IHH patient who was compound heterozygote for two missense mutations in *KISS1R* where one mutation resulted in the substitution of cysteine with arginine at position 223 in the fifth transmembrane helix, while the second mutation caused the substitution of arginine with leucine at position 297 in the third extra cellular loop. During 2012, Topaloglu *et al*. (2012) for the first time described an inactivating mutation in *KISS1R* in a large consanguineous family that resulted in failure of pubertal development and concluded that an inactivating mutation of *KISS1R* caused complete isolated IHH.

Mutation in human KP-GPR54 system and precocious puberty

Precocious puberty (PP) is characterized by the development of secondary sexual characteristics before the age of 8 years in girls and 9 years in boys (Papathanasiou and Hadjiathanasiou, 2006). If PP is driven by premature activation of GnRH secretion, then the phenomenon is called central precocious puberty (CPP). CPP has a predominance among girls, and most of these cases are idiopathic (Papathanasiou and Hadjiathanasiou, 2006). Mutations in *KISS1R* that make the receptor prematurely activated cause central precocious puberty. For example, Teles *et al*. (2008) reported dominant mutation in *KISS1R* gene at nucleotide position 1157 in exon 5 in a girl with idiopathic CPP that resulted in the substitution of proline for arginine at position 386 (Arg386Pro) in the carboxy-terminus of GPR54. The CPP in girl was linked with the activation of G-protein mediated signaling pathway for abnormally long time in response to KISS1 (Teles *et al*., 2008). Silveira *et al*. (2010) identified two novel missense mutations in the *KISS1* gene during a study in three unrelated children with CPP. One mutation was detected in heterozygous state in a boy of one year age who was suffering from CPP. This mutation resulted in the substitution of proline with serine at position 74 of prepro-kisspeptin. This mutation made the KP resistant to degradation which resulted in an increased basal KP level and consequently in CPP. The second mutation was detected in the homozygous state in two unrelated girls with sporadic CPP. This mutation resulted in the substitution of a histidine by an aspartic acid at position 90 of prepro-kisspeptin which was the cause of sporadic CPP. In each case, the mutation was detected in the –NH2 terminal region of mature KP-54 (Silveira *et al*., 2010). Higher serum KP level has been reported in Korean girl with CPP and it was suggested that serum KP level can be used as a marker of CPP (Rhie *et al*., 2011). Demirbilek *et al*. (2012) measured the plasma KP level of 28 girls suffering from CPP and compared it to that of 13 years age-matched pre-pubertal controls. They found high KP level in patient with CPP and suggested that plasma KP levels can be used as a marker for diagnosis of CPP. Akıncı *et al*. (2012) reported higher plasma KP and prolactin levels in girls with premature thelarche. Their results indicated a positive correlation between plasma prolactin and KP levels and suggested that premature thelarche may result from the temporary activation of central stimulants.

Role of Kisspeptin-GPR54 signaling in pregnancy

KP plays very important role in pregnancy. Trophoblast invasion in the uterine extra cellular matrix is under the control of KP. In human, developing placenta shows significantly higher expression level of *KISS1* and *KISS1R* than in mature placenta (Bilban *et al*., 2004). Pregnant females have significantly higher plasma KP level as compared to male and non-pregnant female (Horikoshi *et al*., 2003). KP inhibits the trophoblast cells migration and invasion during pregnancy (Reynolds *et al*., 2009). Bilban *et al*. (2004) identified KP-10 in conditioned medium of first trimester human trophoblasts and reported that KP-10 increases the intracellular Ca^{2+} level in isolated first trimester trophoblasts and inhibits trophoblasts migration in an explant as well as transwell assay without affecting proliferation. They identified Kp-10 as a novel paracrine/endocrine regulator in fine-tuning trophoblast invasion generated by the trophoblast itself. This indicates that during human pregnancy, there occurs a controlled invasiveness of trophoblasts due to significantly higher level of KP. There appear striking similarity between trophoblasts invasion and cancer cells invasion (Murray and Lessey, 1999; Bilban *et al*., 2000; Bischof and Campana, 2000). KP regulates trophoblasts invasion by mechanism which is operating in suppression of cancer. For example, KP regulates trophoblasts invasion during pregnancy through induction of focal adhesion, formation of stress fiber (Kotani *et al.,* 2001, Ohtaki *et al.,* 2001), and phosphorylation of two important proteins i.e., focal adhesion kinase and paxillin that associate with integrin proteins to prevent migration of cells (Ohtaki *et al*., 2001). Kisspeptin is also involved in the inhibition of matrix metaloprotease-9 gene expressions by binding reduced nuclear factor kappa-light-chain-enhancer of activated B cells to the promotor of MMP9 gene that is the basis of inhibition of cell migration (Yan *et al*., 2001). During pregnancy, the LH concentration in circulation is not raised, though the level of KP in circulation is higher. The cause may be the decreased sensitivity of GPR54 receptor on GnRH neurons to KP (Tony and Ramaswamy 2009), or higher level of progesterone because of which GnRH neurons cannot respond to KP (Reynolds *et al*., 2009**).** In rats, LH response to higher KP level during pregnancy is preserved as evident from significantly higher LH and FSH levels (Roa *et al.,* 2006). Gaytan *et al.* (2007) identified *Kiss1* gene expression in the oviduct of rat and suggested the role of KP in the prevention of ectopic pregnancy.

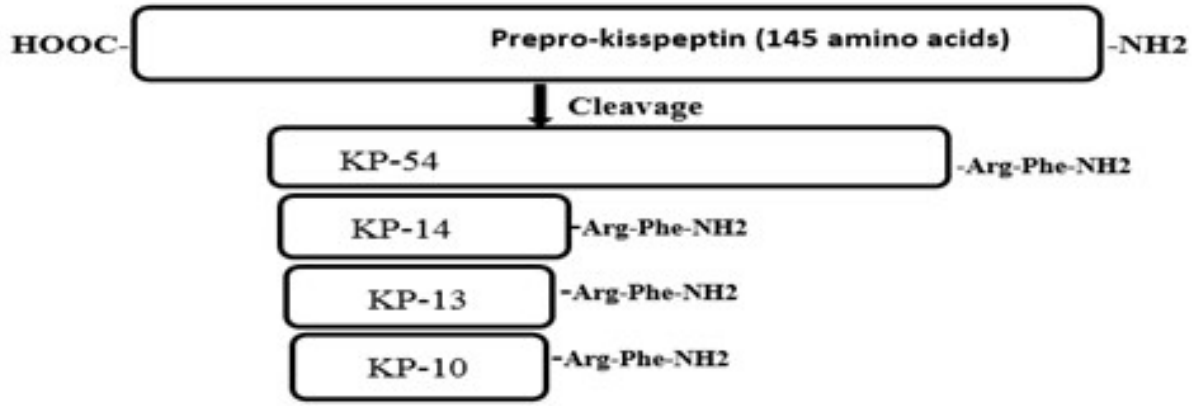

Fig. 1: Human prepro-kisspeptin and the cleaved fragments. Arg-Phe-NH2 represents RF-amide motif of kisspeptin. [fig. based on concept presented by Kirby et al. (2010) @Pharmacological Reviews].

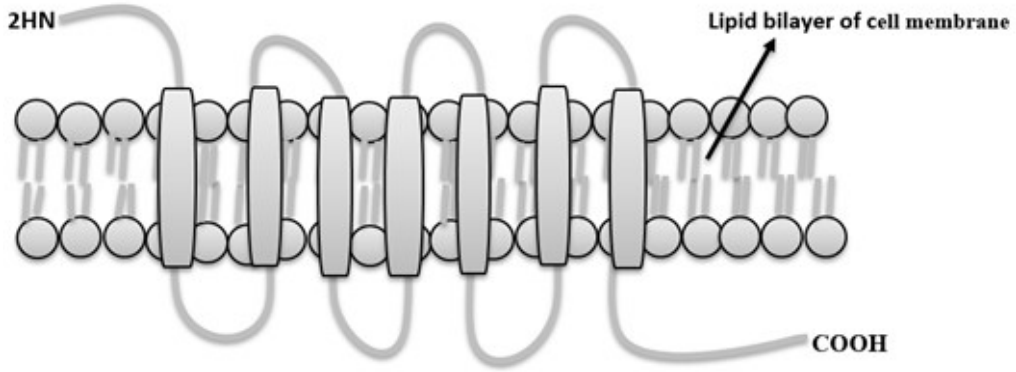

Fig. 2: Human kisspeptin receptor (GPR54), a polypeptide that spanning seven times through cell membrane lipid bilayer and has extracellular -NH_2 group and intracellular –COOH group. [Figure based on concept presented by Kirby *et al.* (2010) @Pharmacological Reviews].

CONCLUSION

Kisspeptin regulates reproductive functions at various levels of reproductive axis. Kisspeptin plays crucial role in the onset of puberty. Mutations in the kisspeptin gene (*KISS1*) or kisspeptin receptor (GPR54) gene (*KISS1R*) result in idiopathic hypogonadotropic hypogonadism, or central precocious puberty. Kisspeptin is also involved in regulation of trophoblastic invasion during pregnancy, ovulation and sperm hyperactivation.

REFERENCES

Akıncı A, Çetin D and İlhan N (2012). Plasma Kisspeptin Levels in Girls with Premature Thelarche. *J. Clin. Res. Pediatr. Endocrinol*., **4**(2): 61.

Bhagavath B and Layman LC (2007). The genetics of hypogonadotropic hypogonadism. *Semin. Reprod. Med.*, **25**(4): 272-286.

Bilban M, Ghaffari-Tabrizi N, Hintermann E, Bauer S, Molzer S, Zoratti C, Malli R, Sharabi A, Hiden U and Graier W (2004). Kisspeptin-10, a KiSS-1/metastin-derived decapeptide, is a physiological invasion inhibitor of primary human trophoblasts. *J. Cell Sci*., **117**(8): 1319-1328.

Bilban M, Head S, Desoye G and Quaranta V (2000). DNA microarrays: A novel approach to investigate genomics in trophoblast invasion a review. *Placenta*, **21**(Suppl. A): S99-S105.

Bischof P and Campana A (2000). Molecular mediators of implantation. *Baillieres Best. Pract. Res. Clin. Obstet. Gynaecol*., **14**(5): 801-814.

Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombe`s M, Millar RP, Guiochon-Mantel A and Young J (2009). Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N. Engl. J. Med*., **360**(26): 2742-2748.

Bouloux PM, Hu Y and MacColl G (2002). Recent advances in the pathogenesis of Kallmann's syndrome. *Prog. Brain Res*., **141**: 79-83.

Caraty A, Smith J, Lomet D, Said SB, Morrissey A, Cognie J, Doughton B, Baril G, Briant C and Clarke I (2007). Kisspeptin synchronizes preovulatory surges in

cyclical ewes and causes ovulation in seasonally acyclic ewes. *Endocrinology*, **148**(11): 5258-5267.

Castellano J, Gaytan M, Roa J, Vigo E, Navarro V, Bellido C, Dieguez C, Aguilar E, Sanchez-Criado J and Pellicer A (2006). Expression of KiSS-1 in rat ovary: Putative local regulator of ovulation? *Endocrinology*, **147**(10): 4852-4862.

Clements MK, McDonald TP, Wang R, Xie G, O'Dowd BF, George SR, Austin CP and Liu Q (2001). FMRFamide-related neuropeptides are agonists of the orphan G-protein-coupled receptor GPR54. *Biochem Biophys. Res. Commun.,* **284**(5): 1189-1193.

Constantin S, Caligioni CS, Stojilkovic S and Wray S (2009). Kisspeptin-10 facilitates a plasma membrane-driven calcium oscillator in gonadotropin-releasing hormone-1 neurons. *Endocrinology*, **150**(3):1400-1412.

De Bond JA, Li Q, Millar RP, Clarke IJ and Smith JT (2013). Kisspeptin signaling is required for the luteinizing hormone response in anestrous ewes following the introduction of males. *Plos One,* **8**(2): e57972.

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL and Milgrom E (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc. Natl. Acad. Sci*., **100**(19: 10972-10976.

de Tassigny XDA, Fagg LA, Dixon JP, Day K, Leitch HG, Hendrick AG, Zahn D, Franceschini I, Caraty A, Carlton MB (2007). Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. *Proc. Natl. Acad. Sci*., **104**(25): 10714-10719.

Demirbilek H, Gonc EN, Ozon A, Alikasifoglu A and Kandemir N (2012). Evaluation of serum kisspeptin levels in girls in the diagnosis of central precocious puberty and in the assessment of pubertal suppression. *J. Pediatr. Endocrinol. Metab.,* **25**(3-4): 313-316.

Dhillo WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, McGowan BM, Amber V, Patel S and Ghatei MA (2005). Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J. Clin. Endocrinol. Metab*., **90**(12): 6609-6615.

Dhillo WS, Chaudhri OB, Thompson EL, Murphy KG, Patterson M, Ramachandran R, Nijher GK, Amber V, Kokkinos A and Donaldson M (2007). Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. *J. Clin. Endocrinol. Metab*., **92**(10): 3958-3966.

Gaytán M, Castellano JM, Roa J, Sánchez-Criado JE, Tena-Sempere M and Gaytán F (2007). Expression of KiSS-1 in rat oviduct: possible involvement in prevention of ectopic implantation? *Cell Tissue Res*., **329**(3): 571-579.

George J, Veldhuis J, Roseweir A, Newton CL, Faccenda E, Millar RP and Anderson R (2011). Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. *J. Clin. Endocrinol. Metab*., **96**(8): 1228-1236.

Gershengorn MC and Osman R (2001). Minireview: Insights into G protein-coupled receptor function using molecular models. *Endocrinology*, **142**(1): 2-10.

Gether U (2000). Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr. Re.,* **21**(1): 90-113.

Gottsch M, Cunningham M, Smith J, Popa S, Acohido B, Crowley W, Seminara S, Clifton D and Steiner R (2004). A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*, **145**(9): 4073-4077.

Gottsch ML, Clifton DK, Steiner RA (2009). From KISS1 to Kisspeptins: An Historical Perspective and Suggested Nomenclature. *Peptides*, **30**(1): 4-9.

Han SK, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, Clifton DK, Steiner RA and Herbison AE (2005). Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J. Neurosci*., **25**(49): 11349-11356.

Horikoshi Y, Matsumoto H, Takatsu Y, Ohtaki T, Kitada C, Usuki S and Fujino M (2003). Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans. *J. Clin. Endocrinol. Metab*., **88**(2): 914-919.

Inoue N, Sasagawa K, Ikai K, Sasaki Y, Tomikawa J, Oishi S, Fujii N, Uenoyama Y, Ohmori Y and Yamamoto N (2011). Kisspeptin neurons mediate reflex ovulation in the musk shrew (Suncus murinus). *Proc. Natl. Acad. Sci*., **108**(42): 17527-17532.

Iovane A, Aumas C and de Roux N (2004). New insights in the genetics of isolated hypogonadotropic hypogonadism. *European J. Endocrinol.*, **151**(Suppl 3): U83-U88.

Jayasena C, Nijher G, Comninos A, Abbara A, Januszewski A, Vaal M, Sriskandarajah L, Murphy K, Farzad Z and Ghatei M (2012). Contrasting effects of kisspeptin-10 between men and women reveal sexual dimorphism in the hypothalamic regulation of human reproduction. *Endocrine Abstracts*, **28**: OC2.1.

Keen KL, Wegner FH, Bloom SR, Ghatei MA and Terasawa E (2008). An increase in kisspeptin-54 release occurs with the pubertal increase in luteinizing hormone-releasing hormone-1 release in the stalk-median eminence of female rhesus monkeys in vivo. *Endocrinology*, **149**(8): 4151-4157.

Kirby HR, Maguire JJ, Colledge WH, Davenport AP (2010). International Union of Basic and Clinical Pharmacology. LXXVII. Kisspeptin receptor nomenclature, distribution and function. *Pharmacol. Rev*., **62**(4): 565-578.

Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brézillon S, Tyldesley R, Suarez-Huerta N and Vandeput F (2001). The metastasis suppressor gene KiSS-1 encodes

kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J. Biol. Chem*., **276**(37): 34631-34636.

Lanfranco F, Gromoll J, von Eckardstein S, Herding EM, Nieschlag E, Simoni M (2005). Role of sequence variations of the GnRH receptor and G protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism. *European J. Endocrinol*., **153**(6): 845-852.

Lee DK, Nguyen T, O'Neill GP, Cheng R, Liu Y, Howard AD, Coulombe N, Tan CP, Tang-Nguyen ATand George SR (1999). Discovery of a receptor related to the galanin receptors. *FEBS letters*, **446**(1): 103-107.

Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE and Welch DR (1996). KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *J. Natl. Cancer Inst.,* **88**(3): 1731-1737.

Li C, Chen P, Smith MS (1999). Morphological evidence for direct interaction between arcuate nucleus neuropeptide Y (NPY) neurons and gonadotrophin-releasing hormone neurons and possible involvement of NPYY1 receptors. *Endocrinology*, **140**(11): 5382-5390.

Liu X and Herbison AE (2008). Small-conductance calcium-activated potassium channels control excitability and firing dynamics in gonadotropin-releasing hormone (GnRH) neurons. *Endocrinology*, **149**(7): 3598-3604.

Magee C, Foradori CD, Bruemmer JE, Arreguin-Arevalo JA, McCue PM, Handa RJ, Squires EL and Clay CM (2009). Biological and anatomical evidence for kisspeptin regulation of the hypothalamic-pituitary-gonadal axis of estrous horse mares. *Endocrinology*, **150**(6): 2813-2821.

Millar RP, Lu ZL, Pawson AJ, Flanagan CA, Morgan F and Maudsley SR (2004). Gonadotropin-releasing hormone receptors. *Endocr. Rev.,* **25**(2): 235-275.

Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A, Szekeres PG, Sarau HM, Chambers JK and Murdock P (2001). AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-*1*. *J. Biol. Chem*., **276**(31): 28969-28975.

Murray MJ and Lessey BA (1999). Embryo implantation and tumor metastasis: Common pathways of invasion and angiogenesis. *Semin. Reprod. Endocrinol*., **17**(3): 275-290.

Navarro V, Castellano J, Fernandez-Fernandez R, Tovar S, Roa J, Mayen A, Nogueiras R, Vazquez M, Barreiro M and Magni P (2005). Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. *Endocrinology*, **146**(1): 156-163.

Nimri R, Lebenthal Y, Lazar L, Chevrier L, Phillip M, Bar M, Hernandez-Mora E, de Roux N and Gat-Yablonski G (2011). A novel loss-of-function mutation in GPR54/KISS1R leads to hypogonadotropic hypogonadism in a highly consanguineous family. *J. Clin. Endocrinol. Metab*., **96**(3): 536-545.

Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y and Masuda Y (2001). Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature.*, **411(**6837): 613-617.

Papathanasiou A and Hadjiathanasiou C (2006). Precocious puberty. Pediatr. Endocrinol. Rev 3, 182-187. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F (1995). "Effects of the obese gene product on body weight regulation in ob/ob mice". *Science,* **269**(5223): 540-543.

Pinto F, Cejudo-Román A, Ravina C, Fernández-Sanchez M, Martín-Lozano D, Illanes M, Tena-Sempere M and Candenas M (2012). Characterization of the kisspeptin system in human spermatozoa. *Int. J. Androl*., **35**(1): 63-73.

Pitteloud N, Acierno Jr JS, Meysing A, Eliseenkova AV, Ma J, Ibrahimi OA, Metzger DL, Hayes FJ, Dwyer AA, Hughes VA, Yialamas M, Hall JE, Grant E, Mohammadi M and Crowley Jr WF (2006). Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc. Natl. Acad. Sci. USA.*, **103 (16)**: 6281-6286.

Pompolo S, Pereira A, Estrada K and Clarke I (2006). Colocalization of kisspeptin and gonadotropin-releasing hormone in the ovine brain. Endocrinology, **147**(2): 804-810.

Porzionato A, Fenu G, Rucinski M, Macchi V, Montella A, Malendowicz LK and De Caro R (2011). *KISS1* and KISS1R expression in the human and rat carotid body and superior cervical ganglion. *Eur. J. Histochem.*, **55**(2): e14.

Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek J, Kanarek R and Maratos-Flier E (1996). A role for melanin-concentrating hormone in the central regulation of feeding behavior. *Nature*, **380(**6571): 243-247.

Ramaswamy S, Gibbs R and Plant T (2009). Studies of the Localization of Kisspeptin within the Pituitary of the Rhesus Monkey (Macaca mulatta) and the Effect of Kisspeptin on the Release of Non-Gonadotropic Pituitary Hormones. *J. Neuroendocrinol*., **21**(10): 795-804.

Ramaswamy S, Weinbauer GF (2014). Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. *Spermatogenesis*, **4**(2): e996025.

Redmond JS, Macedo GG, Velez IC, Caraty A, Williams GL and Amstalden M (2011). Kisspeptin activates the hypothalamic-adenohypophyseal-gonadal axis in prepubertal ewe lambs. *Reproduction*, **41**(4): 541-548.

Revel FG, Ansel L, Klosen P, Saboureau M, Pévet P, Mikkelsen JD and Simonneaux V (2007). Kisspeptin:

A key link to seasonal breeding. *Rev. Endocr. Metab. Disord.*, **8**(1): 57-65.

Reynolds RM, Logie JJ, Roseweir AK, McKnight AJ and Millar RP (2009). A role for kisspeptins in pregnancy: Facts and speculations. *Reproduction,* **138**(1): 1-7.

Rhie JY, Lee KH, Eun SH, Choi BM, Chae HW, Kwon AR Lee WJ, Kim JH and Kim HS (2011). Serum Kisspeptin Levels in Korean Girls with Central Precocious Puberty. *J. Korean Med. Sci*., **26 (7)**: 927-931.

Roa J, Vigo E, Castellano J, Navarro V, Fernandez-Fernandez R, Casanueva F, Dieguez C, Aguilar E, Pinilla L and Tena-Sempere M (2006). Hypothalamic expression of KiSS-1 system and gonadotropin-releasing effects of kisspeptin in different reproductive states of the female Rat. *Endocrinology*, **147**(6): 2864-2878.

Seminara SB, Hayes FJ and Crowley WF (1998). Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): Pathophysiological and genetic considerations. *Endocr. Rev*., **19**(5): 521-539.

Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM and Hendrick AG (2003). The GPR54 gene as a regulator of puberty. *N. Engl. J. Med*., **349 (17)**: 1614-1627.

Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, O'Rahilly S and Aparicio SA (2005). Two novel missense mutations in G protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J. Clin. Endocrinol. Metab.*, **90**(3): 1849-1855.

Silveira LG, Noel SD, Silveira-Neto AT, Abreu AP, Brito VN, Santos MG, Bianco SDC, Kuohung W, Xu S, Gryngarten M, Escobar ME, Arnhold IJP, Mendonca BB, Kaiser UB and Latronico AC (2010). Mutations of the KISS1 gene in disorders of puberty. *J. Clin. Endocrinol. Metab.*, **95**(5): 2276-228.

Smith JT, Acohido BV, Clifton DK and Steiner RA (2006a). KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *J Neuroendocrinol*., **18**(4): 298-303.

Smith JT, Coolen LM, Kriegsfeld LJ, Sari IP, Jaafarzadehshirazi MR, Maltby M, Bateman K, Goodman RL, Tilbrook AJ and Ubuka T (2008). Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurons in the brain: A novel medium for seasonal breeding in the sheep. *Endocrinology,* **149**(11): 5770-5782.

Smith JT, Shahab M, Pereira A, Pau KYF and Clarke IJ (2010). Hypothalamic expression of KISS1 and gonadotropin inhibitory hormone genes during the menstrual cycle of a non-human primate. *Biol. Reprod*., **83**(4): 568-577.

Suzuki S, Kadokawa H and Hashizume T (2008). Direct kisspeptin-10 stimulation on luteinizing hormone secretion from bovine and porcine anterior pituitary cells. *Anim. Reprod. Sci.,* **103**(3-4): 360-365.

Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB and Latronico AC (2008). A GPR54-activating mutation in a patient with central precocious puberty. *N. Engl. J. Med.,* **358**(7): 709-715.

Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP and Yuksel B (2012). Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *N. Engl. J. Med.*, **366**: 629-635.

Tony PM and Ramaswamy S (2009). Kisspeptin and the regulation of the hypothalamic-pituitary-gonadal axis in the rhesus monkey (*Macaca mulatta*). *Peptides*, **30**(1): 67-75.

Tsukamura H and Maeda KI (2011). GnRH pulse generation and its application to the manipulation of follicular development and ovulation. *Thai J. Vet. Med.,* **41**: 69-72.

West A, Vojta PJ, Welch DR and Weissman BE (1998) Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene (KISS1). *Genomics*, **54**(1): 145-148.

Yan C, Wang H and Boyd DD (2001). KiSS-1 represses 92-kDa type IV collagenase expression by down-regulating NF-kappa B binding to the promoter as a consequence of Ikappa Balpha -induced block of p65/p50 nuclear translocation. *J. Biol. Chem*., **276**(2): 1164-1172.

Zhang C, Roepke TA, Kelly MJ and Rønnekleiv OK (2008). Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. *J. Neurosci.,* **28**(17): 4423-4434.