Genetic and epigenetic aspects of the KISS1 and KISS1R genes in pubertal development and central precocious puberty
A review

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ABSTRACT

The onset of puberty is a pivotal developmental milestone, and the release of gonadotropin-releasing hormone (GnRH) and luteinizing hormone is a key factor in the initiation of puberty. Both kisspeptin and its receptor (KISS1) and KISS1 receptor (KISS1R) play significant roles in regulating GnRH release, and consequently, the initiation of puberty. Central precocious puberty (CPP) is a condition in which the development of puberty is driven by the premature activation of the hypothalamic-pituitary-gonadal axis. In girls, CPP is primarily idiopathic, and genetic and epigenetic aspects of KISS1 and KISS1R have been implicated in its etiology. This review aimed to provide an overview of the current knowledge regarding mutations and polymorphisms in KISS1 and KISS1R associated with CPP. Additionally, this study provides a comprehensive review of the epigenetic regulation of the KISS1 gene in the context of puberty onset and CPP.

Keywords: Epigenesis; KISS1; KISS1R; Precocious puberty

INTRODUCTION

Puberty is a biological maturation process that signifies the physical, hormonal, and psychological shifts from childhood to adulthood, culminating in the development of secondary sexual characteristics and the attainment of reproductive capability [1]. Precocious puberty is delineated by the initiation of puberty occurring 2 to 2.5 standard deviations ahead of the mean, and is defined as the manifestation of secondary sexual characteristics, such as breast development before the age of 8 in girls and testicular enlargement before the age of 9 in boys [2-4]. The precocious puberty can be categorized as central precocious puberty (CPP) and peripheral precocious puberty. The CPP occurs because of the premature reactivation of pulsatile hypothalamic gonadotropin-releasing hormone (GnRH) secretion, while peripheral precocious puberty is caused by excessive sex hormone secretion originates from a tumor or exogenous source inde-
CPP etiologies can be broadly categorized into two groups: those associated with central nervous system (CNS) lesions and those without CNS lesions. In cases with CNS lesions, the causes can include tumors such as hypothalamic hamartomas, congenital malformations like arachnoid cysts or hydrocephalus, and acquired lesions such as encephalitis or radiation exposure [1,6,20-22]. However, cases without CNS lesions are more common. CPP can also be secondary to chronic exposure to sex steroid hormones or endocrine disruptors [1,6]. Moreover, though still contentious, air pollution is also being considered a potential cause of precocious puberty [23]. The nutritional status and elevated serum leptin levels in overweight are believed to contribute, at least in part, to the earlier onset of puberty in overweight children [24]. While environmental and nutritional influences play a role in the development of CPP, it's equally crucial to consider the underlying genetic factors that might predispose certain individuals to this condition [6]. This is supported by the fact that CPP occurs approximately five to 15 times more frequently in girls than in boys, suggesting that genetic differences between males and females play a significant role in the occurrence of CPP [6,14]. In addition, syndromic CPP combined with multiple anomalies, such as Temple syndrome, Xp11.23–p.11.22 duplication syndrome, and Williams-Beuren syndrome, also suggests a genetic etiology [2,6]. The fact that approximately one-third of idiopathic central precocious puberty (ICPP) cases are familial CPP also implies the significance of genetic factors in CPP occurrence [25].

The elucidation of neuromodulators such as kisspeptin has contributed to the comprehension of pubertal developmental processes [26]. Mutations in makorin ring finger protein 3 (MKRN3) and delta like non-canonical Notch ligand 1 (DLK1) have been identified in individuals with familial CPP over the past decade [27]. These findings highlight the significant role of genetic factors in the underlying pathophysiology of CPP and stimulate ongoing research on the connections between genes associated with puberty and CPP. However, distinct variations in the timing of puberty are evident, even among genetically identical individuals [28], and research findings indicating the association of imprinted regions with menarche [29] suggest that genetic factors as well as epigenetic mechanisms influence the occurrence of CPP.

Understanding the genetic causes of CPP has had a significant impact, enabling more accurate and earlier diagnosis, facilitating familial counseling, and establishing potential avenues for future treatment targets. This review provides a comprehensive exploration of the three representative genetic causes of CPP and epigenetic dysregulation that contributes to CPP.

**GAIN-OF-FUNCTION MUTATIONS IN KISS1 AND KISS1R**

The kisspeptin system is primarily made up of the kisspeptin and its receptor (KISS1) gene, which encodes the neuropeptide kisspeptin, and its specific receptor, KISS1R, found on GnRH neurons [30,31]. KISS1 and KISS1R are widely distributed, with notable expression levels in various organs, including the placenta, ovaries, and specific regions of the hypothalamus, such as the arcuate nucleus (ARC) and anteroventral periventricular nucleus/periventricular nucleus continuum (AVPV) [32,33]. The discovery of KISS1R loss-of-function mutations and rare inactivation KISS1 mutations in patients with congenital hypogonadotropic hypogonadism emphasizes the significance of the kisspeptin system in human puberty and reproduction [30,31,34]. In addition, an elevation in serum kisspeptin levels has been observed in patients with CPP [35,36]. Moreover, the administration of kisspeptin triggers LH release in healthy individuals [37,38], whereas the LH increase...
following kisspeptin administration is diminished in men with congenital hypogonadotropic hypogonadism despite the preserved LH response to GnRH [39]. This suggests that the kisspeptin system is a critical regulator preceding GnRH release [40,41]. Specifically, kisspeptin neurons in the ARC contribute to pulsatile GnRH and LH secretion [42,43], whereas those in the AVPV/periventricular nucleus continuum participate in the positive feedback of sex steroids, ultimately triggering a pre-ovulatory LH surge [43,44].

The significant role of the kisspeptin system in GnRH regulation and the discovery of loss-of-function mutations in KISS1 and KISS1R associated with congenital hypogonadotropic hypogonadism suggest, conversely, that gain-of-function mutations could lead to the onset of precocious puberty. Nevertheless, only two rare mutations have been reported in KISS1 and one in KISS1R in individuals with CPP. Two novel KISS1 missense mutations, p.P74S and p.H90D, have been identified in patients with ICPP. Among them, the p.P74S variant exhibited increased resistance of kisspeptin to degradation compared to the wild-type, suggesting that this mutation could be a contributing factor to the development of precocious puberty [45]. In addition, an activating heterozygous mutation in KISS1R (p.R386P) was discovered in patients with CPP [46]. The p.R386P mutation induces prolonged activation of intracellular signaling pathways through kisspeptin owing to reduced degradation and internalization of KISS1R [46,47]. However, all three mutations are currently classified as either likely benign or variants of uncertain significance, according to the 2015 American College of Medical Genetics and Genomics Association for Molecular Pathology guidelines [48]. Therefore, the association between KISS1 and KISS1R gain-of-function mutations and CPP is still not well established. Further reinforcement in the form of additional patient data or results from functional studies is necessary to elucidate the association between these mutations and CPP.

Table 1. SNPs in KISS1 and KISS1R found to have association with CPP

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism position</th>
<th>dbSNP ID</th>
<th>Location</th>
<th>Major/Minor allele</th>
<th>Expression</th>
<th>Allele frequency</th>
<th>Risk of CPP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>KISS1</td>
<td>54650055</td>
<td>rs192636495</td>
<td>Exon 3</td>
<td>G/T</td>
<td>p.P110T</td>
<td>G: 0.961&lt;sup&gt;a&lt;/sup&gt;</td>
<td>G: 0.931&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Protect</td>
</tr>
<tr>
<td></td>
<td>55648176</td>
<td></td>
<td>Exon 3</td>
<td>T/G</td>
<td></td>
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<td>T: 0.069&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>C/G</td>
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</tr>
<tr>
<td></td>
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<td>rs35128240</td>
<td>Exon 3</td>
<td>-</td>
<td></td>
<td>T: 0.079</td>
<td>T: 0.941</td>
<td>Protect</td>
</tr>
<tr>
<td></td>
<td>204196482</td>
<td>rs5780218</td>
<td>5′ UTR</td>
<td>A/-</td>
<td></td>
<td>A: 0.539</td>
<td>A: 0.461</td>
<td>Increase</td>
</tr>
<tr>
<td>KISS1R</td>
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<td></td>
<td>Promoter region 5′ UTR</td>
<td>A/G</td>
<td>A: 0.963</td>
<td>A: 0.984</td>
<td>A: 0.984</td>
<td>Increase</td>
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<tr>
<td></td>
<td>c.738+64</td>
<td>rs350131</td>
<td>Intron 4</td>
<td>G/T</td>
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<td>G: 0.456</td>
<td>G: 0.359</td>
<td>Increase&lt;sup&gt;i&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td>Exon 5</td>
<td>T/A</td>
<td></td>
<td>T: 0.307</td>
<td>T: 0.222</td>
<td>Increase&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism; KISS1, kisspeptin and its receptor; KISS1R, KISS1 receptor; CPP, central precocious puberty; G, guanine; T, thymine; C, cytosine; UTR, untranslated region; A, adenine.

<sup>a</sup>Allele frequency among Chinese subjects only; <sup>b</sup>The participants included individuals with CPP and early puberty; <sup>i</sup>The authors reported an increased risk of CPP; however, the allele frequency results are contradictory. The table presents the data reported in this study.
SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH CPP IN KISS1 AND KISS1R

Several single nucleotide polymorphisms (SNPs) in KISS1 and KISS1R are associated with CPP (Table 1) [49-55]. The 54650055 G/T polymorphism (p.P110T, rs192636495) [49,50] and the 55648176 T/G polymorphism [51] in the KISS1 are suggested to have a protective effect against CPP. In addition, the haplotype GGGC-ACCC, comprising the G allele of SNP 55648176 T/G and the wild-type alleles of SNP 55648184 and SNP 55648186, along with the GGA haplotype, consisting of all the wild-type alleles of rs1132506 G/C, rs4889 G/C, and rs5780218 A/-, are suggested to have a protective effect against CPP [51,52]. On the contrary, three SNPs (rs1132506, rs35128240, and rs5780218) in the untranslated region of KISS1 have been linked to an increased risk of CPP [51,52].

Several SNPs have been reported in the KISS1R as well, but to date, only three have been reported to be associated with the risk of CPP (Table 1) [53,54]. Among them, rs350131 G>T and rs350132 T>A have been reported to increase the risk of CPP [54]; however, the allele frequency reported in the study showed that the minor allele frequency was lower in patients with CPP compared than in controls [54]. Considering that minor SNPs have also been reported in other studies among patients with CPP [55,56], reanalysis of the effect of this polymorphism on CPP is necessary.

EPIGENETIC MECHANISMS OF THE KISS1 GENE IN PUBERTAL DEVELOPMENT AND PRECOCIOUS PUBERTY

Mechanisms of epigenetic control

Epigenetic modifications, which entail alterations in gene expression without modifying the DNA sequence, are widely acknowledged for their crucial role in the proper development and differentiation of various cell lineages within an organism. Presently, three acknowledged epigenetic mechanisms include: (1) chemical changes in DNA through DNA methylation and hydroxymethylation; (2) alterations in chromatin structure via post-translational modifications (PTMs) of histones, the protein components of nucleosomes; and (3) provision of epigenetic information by noncoding RNAs (ncRNAs), which can be microRNAs (miRNAs) or long intergenic noncoding RNAs (lincRNAs) [57].

Primary epigenetic modification of DNA involves the addition of a methyl group to cytosine residues, specifically at 5′-cytosine-phosphate-guanine-3′ (CpG) dinucleotide sequences [58,59]. DNA methylation is performed by DNA methyltransferases (DNMTs) and leads to 5-methylcytosine (5-mC) formation. Conversely, enzymes from the ten-eleven translocation (TET) family oxidize 5-mC to 5-hydroxymethylcytosine (5-hmC) [60,61]. Generally, increased levels of 5-mC are associated with transcriptional repression, whereas hypomethylation, characterized by reduced 5-mC and 5-hmC, is linked to the activation of gene transcription [62,63]. Both 5-mC and 5-hmC coexist throughout the genome. The 5-mC is more prevalent in silenced genes and in compacted chromosomal regions associated with heterochromatin (closed or condensed chromatin). In contrast, 5-hmC is found in more accessible regions or euchromatin (open or exposed chromatin), and is enriched in the promoter and enhancer regions of active genes [62].

Secondly, histones undergo various PTMs to reshape their chromatin structure, primarily on the N-terminal tails of core histones (H2A, H2B, H3, and H4) [64,65]. These tails are the most accessible regions for PTMs, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation [64]. Acetylation and methylation of lysine residues on histone tails are the most common PTMs and exhibit distinct patterns in heterochromatin and euchromatin [66]. Generally, acetylation by histone acetyltransferase enzymes (HATs) activates gene transcription, while deacetylation by histone deacetylases (HDACs) represses it [64,67,68]. Acetylation reduces the positive charge of lysine residues, weakening–histone interactions, and allows easier access to the transcription machinery [67]. In contrast, histone methylation can either activate or repress transcription depending on the specific lysine residue and degree of methylation [66]. In particular, the polycomb group (PcG) and trithorax group (TrxG) are associated with alterations in chromatin structure through PTMs of histones and are pivotal regulators of numerous developmental genes. They operate in an antagonistic manner: PcG proteins induce repression by introducing repressor marks, such as H3K27Me3 and H2AK119Ub, whereas TrxG proteins activate gene expression by depositing activating marks, such as H3K4Me, H3K4Me2, and H3K4Me3, in the regulatory regions of genes [69,70].

ncRNAs also play a role in modulating epigenetic mechanisms. Contrary to previous beliefs, recent research has revealed that most of the human genome is transcribed into ncRNAs rather than protein-encoding mRNAs [71,72]. These ncRNAs have diverse biological roles, including regulation of gene expression at the transcriptional, RNA processing, and...
KISS1 gene is included among the puberty-activating genes. Among these groups, the KISS1 promoter is a bivalent region in which both repressive and activating marks coexist, enabling the promoter to be in a poised state of activation in response to various incoming signals [84]. During the prepubertal period, the KISS1 gene is repressed by a series of epigenetic modifications in its promoter region. Specifically, CpG islands in the promoter region undergo methylation and a repressive histone mark, H3K27me3, is added. These modifications are catalyzed by PcG enzymes, specifically embryonic ectoderm development (EED) and chromobox protein homolog 7 (CBX7) [57,64,77]. In a study involving female rats, the presence of two critical PcG members, Eed and Cbx7, within ARC kisspeptin neurons and their protein products were observed to interact with the Kiss1 promoter during prepubertal development [77]. Transcriptional repressors of the PcG prevent early onset of puberty by suppressing KISS1 transcription in kisspeptin, neurokinin B, and dynorphin (KNDy) neurons located within the ARC [77]. Reinforcing the repressive effect on KISS1, the sirtuin type 1 (SIRT1) enzyme interacts with PcG proteins and removes histone acetylation [85]. Furthermore, KISS1 expression undergoes additional repression through the action of enzymes such as GATA zinc finger domain containing 1 (GATAD1) and KDM1A, which function as histone demethylases. These enzymes have distinct roles: GATAD1 serves as a chromatin reader that recruits the histone eraser KDM1A [57, 86]. In vitro studies demonstrated that KDM1A recruitment increases with the overexpression of GATAD1, leading to a significant reduction in the loss of activating H3K4me3/2 marks in the regulatory regions of the KISS1 gene [87]. These findings support the notion that GATAD1 contributes to the attenuation of KISS1 activity partly by facilitating the removal of the H3K4me2 mark from the promoter of the gene through the recruitment of KDM1A [57].

In contrast, to initiate the transition to puberty, KISS1 undergoes changes in its regulatory regions, shifting from a repressed to an activated state. This transformation begins with the removal of the repressor enzymes, EED, CBX7, and SIRT1, from the KISS1 promoter. As puberty nears completion, there is a simultaneous increase in methylation within the promoter regions of Eed and Cbx7 in the ARC, along with a significant reduction in the expression of both genes, independent of estrogen influence [57,77]. Crucially, the removal of PcG components, EED and CBX7, from the promoter is accompanied by reorganization of the chromatin state, marked by increased levels of epigenetic modifications, such as H3K9ac, H3K14ac, and H3K4me3, which are associated with gene activation. This activation is likely mediated by members of the TrxG complex because of their well-established antagonistic activi-
ty against PcG [88]. Mixed lineage leukemia 1 (MLL1) and MLL3, two components of the TrxG complex, exert their trans-activational influence on the promoter and enhancer regions of the Kiss1 gene, respectively, during a period when the inhibitory effects of the PcG complex diminish [89]. Additionally, the TrxG member ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX) may aid in PcG removal by demethylating the repressive histone H3K27me3 mark, thereby allowing for an increase in H3K27ac, a characteristic feature of an active enhancer [89,90]. Additionally, other activating enzymes such as HAT and p300/CBP participate in this activation process, catalyzing the addition of acetylations, such as H3K9ac, H3K14ac, and H3K27ac, in both the promoter and enhancer regions of KISS1 to promote its expression [57,77]. Furthermore, KISS1 mRNA expression increased, whereas GATAD1 expression decreased in the medial basal hypothalamus (MBH) of ovariectomy-intact females during the transition from juvenility to puberty. The decline in the association of GATAD1 and KDM1A with KISS1 promoters, along with the simultaneous increase in H3K4me2 levels observed in monkey MBH at the onset of puberty, strongly supports the presence of an epigenetic repression mechanism that is alleviated during the re-establishment of GnRH pulsatility during the transition from infancy to juvenility in monkeys [57]. Consequently, in line with these crucial histone PTMs, the epigenetic regulation of Kiss1 shifts from a repressive to an active state around the time of puberty, and there is an upregulation of Kiss1 mRNA expression in the ARC [57,77].

The regulatory response of kisspeptin neurons to estradiol (E2) differs depending on their location, with distinct reactions observed in the ARC versus the AVPV. E2 inhibits Kiss1 expression in ARC KNDy neurons, but enhances it in AVPV kisspeptin neurons; however, the specific mechanisms underlying this difference remain unknown. In AVPV, E2 plays an epigenetic role by promoting the acetylation of H3 in the Kiss1 promoter region, leading to its increased expression [91]. E2 also induces estradiol receptor alpha (ERα) binding to the Kiss1 promoter exclusively in the AVPV. Conversely, H3 acetylation is reduced in the ARC, resulting in decreased Kiss1 expression [91]. Furthermore, an estrogen-responsive enhancer region in the intergenic 3’ region of the Kiss1 gene was identified in AVPV kisspeptin neurons but not in ARC [91]. Therefore, these findings have unveiled an epigenetic role in E2 positive feedback within the AVPV; however, it is still unclear whether a similar epigenetic mechanism is involved in the inhibitory effect of estrogen on ARC Kiss1 expression.

Epigenetic mechanisms of the KISS1 gene in precocious puberty

Despite numerous studies indicating the importance of the kisspeptin system in pubertal development and the initiation of puberty, which depends on the epigenetic control of the KISS1 gene’s repression or expression, there is still insufficient research on the association between precocious puberty and the epigenetic mechanisms of KISS1. Current research has predominantly focused on correlations with delayed puberty or hypogonadism. For instance, downregulation of Mll1 expression in the ARC using siRNA results in inhibited Kiss1 expression, thereby delaying puberty [89], and clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) system epigenetic remodeling approach that hinders Mll3 action on the Kiss1 enhancer region also postpones the peripubertal increase in Kiss1 expression and the onset of puberty [89]. Additionally, inactive mutations in chromodomain helicase DNA binding protein 7 (CHD7), which normally antagonizes PcG activity by binding to activating marks H3K4me2/me3 via its chromodomain, lead to hypothalamic hypogonadism in humans, suggesting a translational perspective on the role of TrxG in puberty control [92]. Furthermore, GATAD1 overexpression in the ARC of immature rats significantly delays the onset of puberty and disrupts estrous cyclicity [93]. On the contrary, it has been observed that the elimination of EED and SIRT1 repressor enzymes from the KISS1 promoter to initiate puberty can be accelerated depending on nutritional status, potentially causing either precocious puberty or delayed puberty [77].

CONCLUSION

The genetic basis of CPP has been widely discussed, with particular emphasis on the kisspeptin system because of its central role in pubertal onset. Studies have examined multiple genetic mutations and polymorphisms in KISS1 and KISS1R, some of which have been correlated with CPP. Additionally, given the importance of epigenetic regulation in determining the onset of puberty through the expression/repression of the KISS1 gene, active research on the epigenetic alterations of the KISS1 gene and its relationship with puberty onset is ongoing. However, studies on the epigenetic alteration of the KISS1 gene as a cause of CPP remain limited. Instead, epigenetic alterations have been identified in other genes such as MKRN3, DLK1, tachykinin precursor 3 (TAC3), GNRH, and more. It is crucial to consider the epigenetic regulation of various genes related to pubertal onset as a potential cause of CPP. Under-
standing the genetic causes of CPP has significant implications, allowing for a more precise and earlier diagnosis, supporting familial counseling, and paving the way for potential future treatment targets.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Conception or design: AK.  
Acquisition, analysis, or interpretation of data: AK.  
Drafting the work or revising: AK.  
Final approval of the manuscript: AK.

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