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Review

Genetic causes of central precocious puberty

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Highlights

- The review clarifies new genetic etiologies of CPP.
- The genetic etiologies are useful for a better understanding of the timing of puberty.
- The genetic imprinting plays an important role in the regulation of puberty.

Abstract. Central precocious puberty (CPP) is a condition in which the hypothalamus–pituitary–gonadal system is activated earlier than the normal developmental stage. The etiology includes organic lesions in the brain; however, in the case of idiopathic diseases, environmental and/or genetic factors are involved in the development of CPP. A genetic abnormality in *KISS1R*, that encodes the kisspeptin receptor, was first reported in 2008 as a cause of idiopathic CPP. Furthermore, genetic alterations in *KISS1*, *MKRN3*, *DLK1*, and *PROKR2* have been reported in idiopathic and/or familial CPP. Of these, *MKRN3* has the highest frequency of pathological variants associated with CPP worldwide; but, abnormalities in *MKRN3* are rare in patients in East Asia, including Japan. *MKRN3* and *DLK1* are maternal imprinting genes; thus, CPP develops when a pathological variant is inherited from the father. The mechanism of CPP due to defects in *MKRN3* and *DLK1* has not been completely clarified, but it is suggested that both may negatively control the progression of puberty. CPP due to such a single gene abnormality is extremely rare, but it is important to understand the mechanisms of puberty and reproduction. A further development in the genetics of CPP is expected in the future.

Key words: precocious puberty, genetic factor, *MKRN3, DLK1*

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Introduction

Precocious puberty (PP) is a condition in which secondary sexual characteristics appear earlier than normal, leading to difficulties in physical and psychosocial development (1, 2). The PP is categorized into gonadotropin-releasing hormone (GnRH)-dependent (central PP, CPP) and GnRH-independent types. Idiopathic CPP without organic disease occurs most often in females (1) and may be caused by genetic and/or environmental factors (1, 2).

In humans, pulsatile secretion of GnRH is observed during the fetal and neonatal periods (3–5). In childhood, pulsatile secretion of GnRH is suppressed; but, with age, pulsatile secretion of GnRH resumes, leading to the onset of puberty (3–5). The GnRH pulse generator may be located in the mediobasal hypothalamus (MBH) (5–7). Neurons expressing kisspeptin exist in the arcuate nucleus (ARC) of the MBH, and these neurons co-express neurokinin B and dynorphin A; thus, they are called kisspeptin, neurokinin B, and dynorphin (KNDy) neurons (5, 7, 8) (**Fig. 1**). These neurons act as pulse generators of GnRH. Kisspeptin is a 54 amino acid peptide that is cleaved from a 145 amino acid prepropeptide in humans (9) and stimulates gonadotropin secretion through GnRH (7). In KNDy neurons, neurokinin B functions as an autocrine stimulatory signal, whereas dynorphin A acts as an inhibitory signal (5, 7, 8). This synchronization produces pulsatile kisspeptin release, leading to pulsatile GnRH release (5, 7, 8).

In 2003, loss-of-function variants of *KISS1R* were identified as a cause of congenital hypogonadotropic hypogonadism (CHH) (10, 11). Further, a loss-of-function variant of *KISS1* was reported in patients with CHH (12). Furthermore, *TAC3*, that encodes neurokinin B, and *TAC3R*, that encodes the receptor for neurokinin B, have also been shown to cause CHH (13). These findings indicate that disruption of factors involving the GnRH pulse generator can cause human disease. Therefore, it is hypothesized that the enhanced function of the GnRH pulse generator or disruption of a repressor for the GnRH pulse generator may cause CPP. In 2008, PP due to a gain-of-function variant of *KISS1R* was first reported (14) and a gain-of-function variant of *KISS1* was also identified (15). Additionally, in 2013, genetic abnormalities in the maternal-imprinted makorin RING

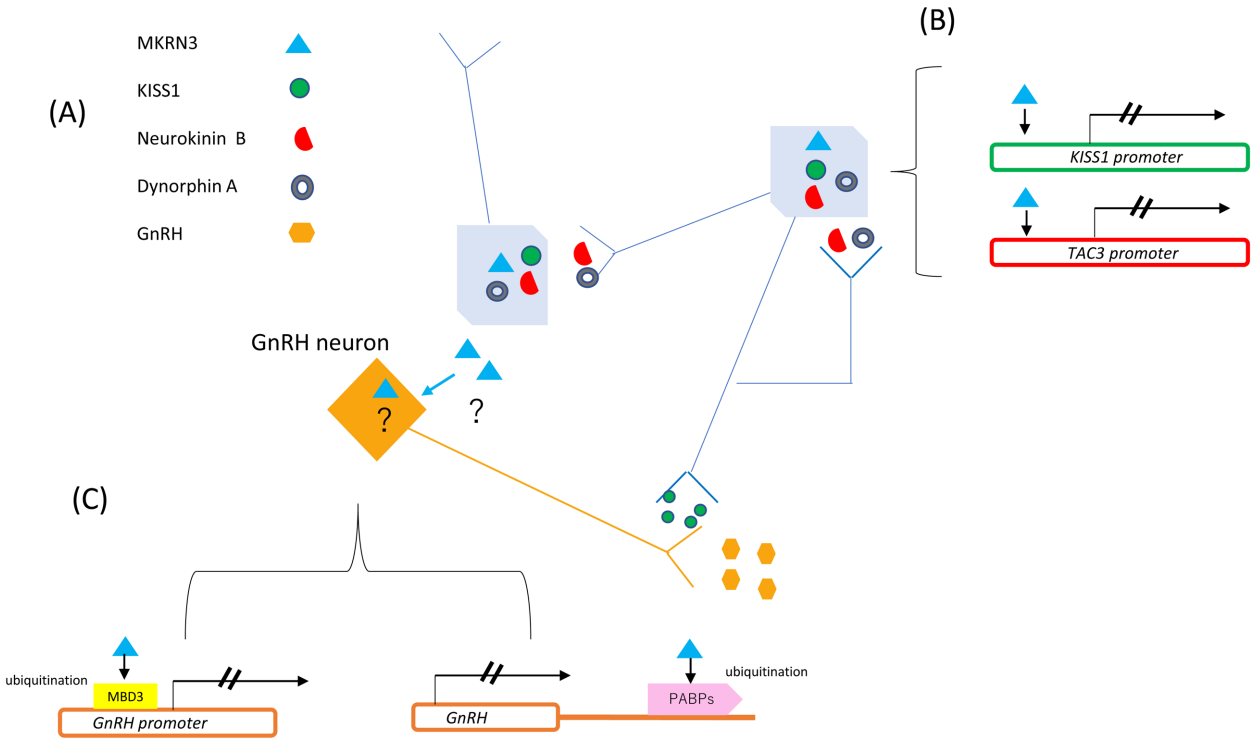

Fig. 1. Schema of KNDy and GnRH neurons. (A) The ARC comprises KNDy neurons that contain kisspeptin, neurokinin B, and dynorphin A. Kisspeptin is released from the KNDy neurons and stimulates GnRH release from GnRH neurons. Neurokinin B plays a dual role as a stimulator of kisspeptin release; and with a short delay, expression of dynorphin A upregulates, and the activity and release of kisspeptin decreases. MKRN3 is also expressed in the kisspeptin-expressing neurons. It remains uncertain whether MKRN3 is expressed in the GnRH neurons. (B) An *in vitro* study showed that MKRN3 represses *KISS1* and *TAC3* encoding neurokinin B promoter activity, suppressing the GnRH release. (C) MBD3 is known to bind gene promoters, exons, and enhancers, and actively regulates DNA transcription. In GnRH neurons, MKRN3 interacts with and ubiquinates MBD3. Ubiquitination of MBD3 promotes DNA methylation of the *GnRH* promoter and suppresses *GnRH* transcription. MKRN3 also ubiquitinates PABPs, destabilizing *GnRH* mRNA and decreasing transcription of *GnRH*. MBD, methyl-CpG-binding domain; PABPs, poly(A)-binding proteins.

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finger protein 3 gene (*MKRN3)* were reported in patients with familial CPP (16). Finally, a deletion in the delta-like homolog 1 gene (*DLK1*), a maternal-imprinted gene, was reported in familial CPP (17).

The discovery of CPP due to monogenic abnormalities has provided new insights into the mechanism of pubertal onset. This review outlines recent findings on CPP due to monogenic abnormalities.

KISS1R

GPR54, a transmembrane receptor coupled with G-protein, was initially believed to be an orphan receptor; however, a few years after the discovery of the KISS1 peptide, GPR54 was found to bind KISS1. This receptor is known as KISS1R (10, 11).

In 2008, a gain-of-function variant of *KISS1R* was reported in a female patient with idiopathic CPP (14). In this patient, the thelarche began early after birth and progressed slowly reaching Tanner stage 4 breasts, along with Tanner stage 2 pubic hair at 8 yr of age. Estradiol (E2) levels had increased to adolescent levels at this time, but the basal and peak levels of LH after GnRH stimulation were borderline.

The pathogenic variant of *KISS1R* identified in this patient was p.Arg386Pro, located in the C-terminal tail. *In vitro* transfection study using COS-7 cells showed that the ability of this mutant receptor to bind kisspeptin was normal. Additionally, there was no difference in the dose-dependent curve of inositol phosphate production upon stimulation with kisspeptin. However, an examination of the time for decrease in inositol phosphate after stimulation with kisspeptin suggested that the mutant maintained a high level of inositol phosphate 18 h after stimulation than wild-type. Moreover, the mutant receptor was found to be expressed on the cell membrane surface for a longer period than wild-type after stimulation with kisspeptin (14). This finding indicated that the mutation is gain-of-function, leading to increased GnRH secretion.

KISS1

Two rare variants, p.Pro74Ser and p.His90Asp, have been reported in two patients with CPP (15). *In vitro* studies showed that the variant p.Pro74Ser had a similar binding ability to KISS1R and degree of signal transduction as wild-type. However, enhanced signal transduction was observed when it was pre-incubated with human serum *in vitro* (15). Thus, the variant may be resistant to degradation and show prolonged signal transduction activity than wild-type. A male child with this mutation showed an increase in the penis and testicular sizes beginning at one year of age. At 3 yr of age, the basal LH levels increased. However, this variant was also identified in the patient's mother and grandmother, who did not have CPP.

Regarding p.His90Asp, there was no functional difference between the mutant and wild-type *in vitro*, and it was unclear whether the variant was the true cause of CPP. A single-base substitution has been reported in the promoter region; but, whether it is also the cause of CPP cannot be concluded in the absence of functional analysis (18).

Moreover, as only these cases have been reported till date, CPP due to abnormalities in *KISS1R* and *KISS1* may be extremely rare. Clinically, both patients developed PP at an early stage, and GnRH neuron activation may have occurred after birth due to abnormalities in *KISS1R* and *KISS1*.

Makorin RING Finger Protein 3 (MKRN3)

MKRN3

In 2013, Abreu *et al*. (16) reported four pathogenic variants of *MKRN*3 in familial CPP. Variants, including whole gene deletions, have been identified since then in > 100 patients with familial and/or idiopathic CPP, the most frequently identified CPP worldwide (19–47).

MKRN3 was cloned in 1999 and identified as a maternally imprinted gene located at chromosome 15q11-13, that is responsible for the Prader–Willi syndrome (48). Human and mouse studies have shown that maternal *MKRN3* is methylated in the central nervous system (49, 50). *MKRN3* is a member of the makorin RING family, together with *MKRN1* and *MKRN2* (49). *MKRN1* and *MKRN2* are widely conserved in vertebrates and invertebrates, while *MKRN3* homologs are found in dogs and mice, but not in birds and fishes (49, 50).

The structure of MKRN3 is shown in **Fig. 2**. It comprises C3H and RING zinc finger domains (49, 50). The C3H zinc finger domains, that are rich in cysteine and histidine, are presumed to function in RNA binding; thus, MKRN3 may be involved in RNA splicing, post-transcriptional modification, and nuclear export of mRNA (49, 50). The RING zinc finger domain is present in a majority of the E3 ubiquitin ligases (49, 50). E3 ubiquitin ligases transfer ubiquitin to a specific substrate of a protein and may be responsible for proteolysis, modification of protein function, structural changes, and localization (49, 50).

Variants of *MKRN3*

Fifty-six variants have been reported till date and are summarized in **Fig. 2**. These variants include a gene deletion, six promoter region abnormalities, and five nonsense, 13 frameshift, and 31 missense variants (19–47). *In vitro* studies have shown that the promoter activity of *MKRN3* is reduced in variants with promoter region abnormalities than that in wild-type (37, 40, 42). Five variants (p.Ile100Phe, p.Ile204Thr, p.Gln226Pro, p.Lys233Asn, and p.Ser396Arg) reported in patients from South Korea were considered benign amino acid changes by *in silico* analysis (26).

The frequency of pathogenic variants of *MKRN3* in CPP is reported to be 19% in familial and 2% in

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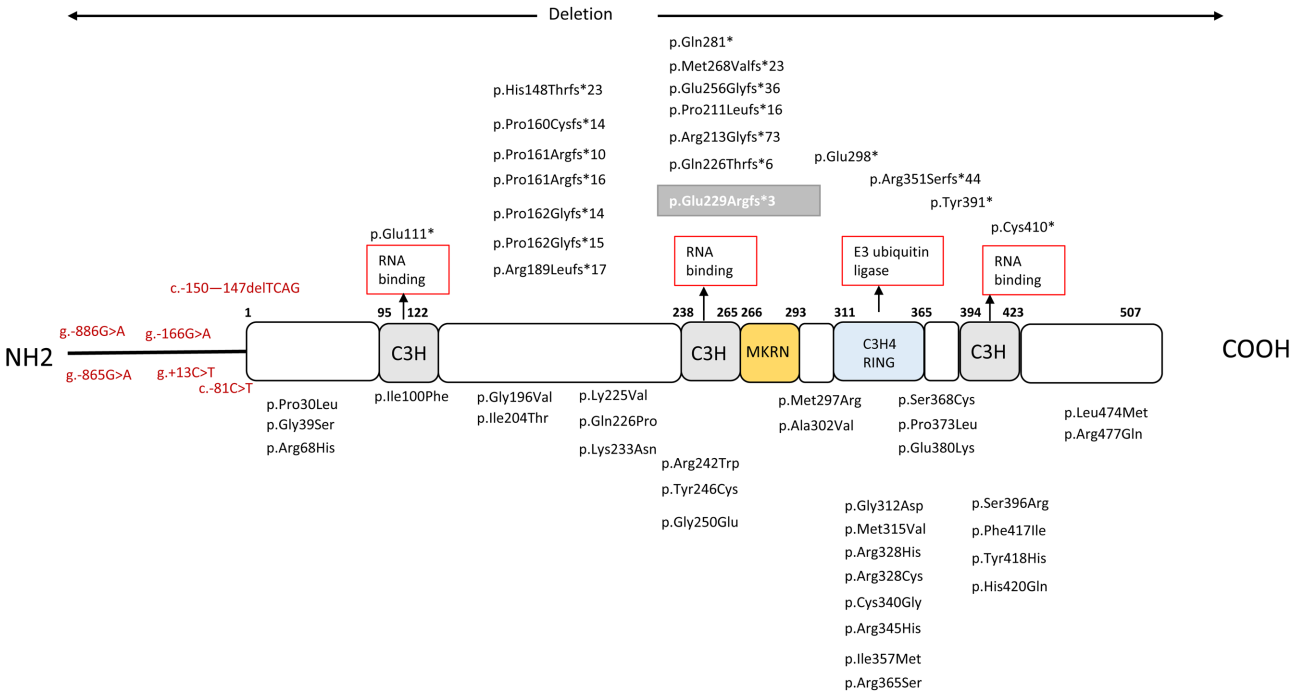

Fig. 2. The structure of MKRN3 and known variants. MKRN3 is a zinc-finger protein comprising 507 amino acids. MKRN3 has a unique composition of several C3H zinc-finger motifs, including a motif that is rich in Cys and His residues. C3H zinc-finger domains are characteristic of ribonucleoproteins and function in RNA-binding. A RING zinc-finger domain is found in E3 ubiquitin ligase enzymes that mediates the transfer of ubiquitin from E2 ubiquitin-conjugating enzymes to target proteins. Frameshift and nonsense variants are shown above the MKRN3 structure. Missense variants are shown below the MKRN3 structure. In Japan, only p.Glu229Argfs*3 has been reported (shaded). Six variants in the 5'-upstream region have also been reported.

sporadic cases (51). In Japan, only one patient harboring c.683_684insA (p.Glu229fsArg3*) has been reported (33). Suzuki *et al*. (52) analyzed *MKRN3* in 22 Japanese and two Chinese patients with CPP, including methylation defects and copy number variations, but failed to identify any abnormalities. Lee *et al*. (26) analyzed 260 patients with CPP from South Korea and identified only one pathogenic variant (p. Glu281*). Chen *et al*. (41) also identified two pathogenic variants (p. Glu380Lys and p. Ile357Met) in two subjects from a cohort of 173 patients with CPP. Thus, as indicated by Suzuki *et al*. (52), the frequency of *MKRN3* abnormalities in individuals in East Asia is low; whereas, the high frequency of abnormalities in individuals in Brazil, the United States, and Europe is considered the founder effect of some variants. Some variants may have been caused by the founder effect.

Genotype–phenotype relation

Valadares *et al*. (51) reported that the median age for the development of pubertal signs in girls was 6.0 (range, 3.0–7.8) yr and that in boys was 8.5 (range, 5.9–9.0) yr in patients with defects in MKRN3. Regarding the genotype–phenotype correlation of *MKRN3*, the median age at diagnosis was 6.75 and 7.72 yr in patients with stop and frameshift variants and in those with missense and promoter region abnormalities, respectively (51). The age at diagnosis was slightly younger in patients with severe genotypes than in those with low or moderate genotypes, but the levels of LH, FSH, and bone age were similar in both genotype groups (51).

A recent study from Brazil examined the phenotypic differences between 71 patients with *MKRN3* mutations and 156 with idiopathic CPPs (47). The study suggested that patients with stop and frameshift variants had a significantly advanced bone age (2.3 ± 1.6 yr [means ± standard deviation]) and high basal levels of LH (2.2 ± 1.8 IU/L [means ± standard deviation]) than patients with missense variants (1.6 ± 1.4 yr [means ± standard deviation] in advanced bone age) and (1.1 ± 1.1 IU/L [means ± standard deviation] in LH levels). The study also compared cases with *MKRN3* abnormalities with those of idiopathic CPPs without *MKRN3* abnormalities. The median duration between puberty onset and the first medical evaluation was 0.8 ± 0.8 yr for patients with *MKRN3* defects and 2.4 ± 2.1 yr for patients without *MKRN3* defects; but, there was no difference in the age at onset between the thelarche and pubarche. The shorter interval between the initial signs of puberty and first evaluation in patients with *MKRN3* variants may be due to a family history of CPP; this finding highlights the difficulty in confirming the presence or absence of *MKRN3* abnormalities in daily practice.

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Mechanism of *MKRN3* defects in PP

MKRN3 is ubiquitously expressed, especially in eukaryotes and in the developing central nervous system (49, 50). Abreu *et al*. (53) clarified that the expression of Mkrn3 and MKRN3 in the MBH gradually decreased as mice and rats, and rhesus monkeys, respectively, reached the prepubertal stages. In a study on mice, co-expression of Mkrn3 was observed in the ARC kisspeptin-expressing neurons, and this co-expression was the highest immediately after birth (53). Furthermore, luciferase assays of the promoters of *KISS1* and *TAC3* encoding neurokinin B with co-transfection of MKRN3 were performed to investigate whether MKRN3 suppresses the secretion of kisspeptin and neurokinin B. Wild-type MKRN3 binds to the two gene promoter regions and suppresses the transcriptional activity of both genes. In contrast, analyses of the missense variants p.Cys340Arg, p.Arg365Ser, p.Phe417Ile, and p.His 420Gln in patients with CPP suggested that p.Cys340Arg and p.Arg365Ser in the RING finger domain attenuated the suppression activity than wild-type MKRN3. The p.Phe417Ile and p.His 420Gln, located downstream to the C-terminus of the RING finger domain, only slightly suppressed the promoter activity of *KISS1* than wild-type. Based on these findings, it is hypothesized that MKRN3 suppresses the gene expression of kisspeptin and neurokinin B during the prepuberty, and negatively regulates the GnRH pulse (53) (**Fig. 1**).

Further, two studies suggested that MKRN3 directly controls the expression of *GnRH* mRNA (54, 55). Li *et al*. (54) identified the methyl-CpG binding domain (MBD) 3 as the target protein of MKRN3 ubiquitination. The MBD3 is known to bind to gene promoters, enhancers, and exons, and regulate gene expression in various ways (56). Furthermore, the study showed that MKRN3 ubiquitinates and disrupts MBD3 binding, leading to the methylation of the promoter of *GnRH*. MKRN3 also silences the *GnRH* promoter (**Fig. 1**). The study also indicated that missense variants p.Cys340Arg, p.Arg365Ser, p.Phe417Ile, and p.His 420Gln reduced MBD3 ubiquitination and increased *GnRH* promoter activity. Moreover, MKRN3 may be involved in the ubiquitination of Poly (A) binding proteins that increase the instability of *GnRH* mRNA and negatively regulate its expression (55) (**Fig. 1**). However, to the best of our knowledge, there is no clear evidence for *MKRN3* expression in the GnRH neurons.

Delta-like Homolog 1 (DLK1)

In 2017, a deletion in *DLK1*, a maternally imprinted gene (similar to *MKRN3)*, was reported in a family with CPP (17). Pathogenic variants have since been reported in three Brazilian families and in one sporadic case (57, 58). Only family members who inherited the defect from their fathers had CPP, consistent with the known pattern of imprinting *DLK1*. Thus, genomic imprinting, including that of *MKRN3*, plays a pivotal role in the regulation of the pubertal timing in humans. The structure of the DLK1 and defects in *DLK1* are summarized in **Fig. 3**. *DLK1* encodes a transmembrane glycoprotein, has six extracellular epidermal growth factor (EFG)-like repeats, and is also known as preadipocyte factor 1, that mainly inhibits adipocyte differentiation (59–61). Additionally, DLK1 is expressed in many stem cells/progenitor cells and has various activities, including control of cell proliferation and differentiation through various mechanisms (61, 62). The genetic defects reported in *DLK1* include deletions containing the 5' upstream region and exon 1, frameshift variants, and deletions of eight bases of exon 4 and introns 4–5, that cause splicing abnormalities (16, 57, 58).

The median age at the thelarche was 5 years in girls with *DLK1* defects (17, 57, 58). Moreover, of the four girls with *DLK1* deletion from the first study, three had obesity and increased body fat (17). Obesity, dyslipidemia, and impaired glucose tolerance were also observed in three patients with frameshift variants (57). As mentioned above, DLK1 suppresses adipocyte differentiation (59), and *Dlk1* knockout mice are known to develop obesity and dyslipidemia (60). Therefore, metabolic abnormalities may be a characteristic of CPP caused by *DLK1* defects.

Abnormalities at chromosome 14q32.2, including in *DLK1*, are known to cause the Temple syndrome (17, 63) that is characterized by intrauterine growth retardation, postnatal failure to thrive, and prominent forehead. Approximately 80% of genetically identified patients with the Temple syndrome present with early puberty or CPP (63). However, cases with *DLK1* defects had no features of the Temple syndrome other than CPP.

The mechanism by which *DLK1* regulates puberty

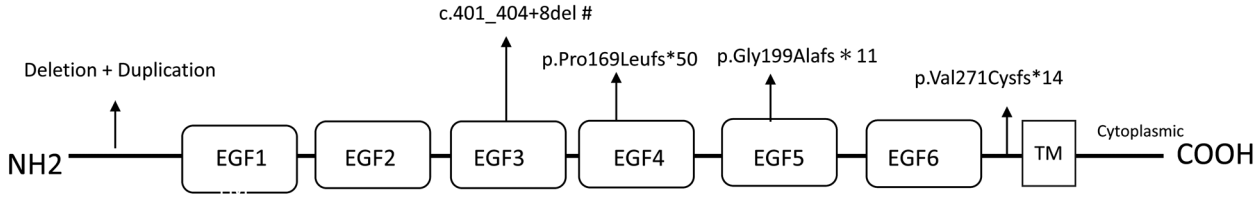

Fig. 3. The structure of DLK1 and reported variants. DLK1 contains 383 amino acids and consists of an extracellular region with six epidermal growth factor-like repeats, a transmembrane domain, and a short intracellular tail. Six abnormalities in DLK1 have also been reported.

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is poorly understood. The expression of *Dlk1* in the hypothalamus has been shown to increase postnatally in mice, as opposed to that of *Mkrn3* (17). Additionally, *Dlk1* is expressed in the MBH and kisspeptin-expressing cells that regulate GnRH pulses (16, 59). Moreover, DLK1 interacts with NOTCH1 receptor and competes for canonical activation by NOTCH ligands (64). Furthermore, Biehl *et al.* (65) showed that decreasing or enhancing Notch signaling in mice reduced the number of neurons expressing kisspeptin in the ARC, indicating the physiological role of the Notch system in regulating the proliferation and/or differentiation of kisspeptin neurons.

Based on these findings, it is hypothesized that defects in DLK1 may enhance NOTCH signaling, leading to abnormalities in neurons expressing kisspeptin, causing CPP (59) (**Fig. 4**). However, these issues warrant further research.

Prokineticin Receptor 2 (PROKR2)

Prokineticin receptor 2 (PROKR2) is a G protein-coupled receptor. The PROK2R signaling pathway regulates the olfactory bulb morphogenesis and plays a role in GnRH neuron development, but neither developing nor mature GnRH neurons express prokineticin receptors (66, 67). In humans, loss-of-function pathological variants of *PROK2* and *PROKR2* have been identified in the CHH and Kallmann syndrome (66, 68).

Fukami *et al.* (69) reported that a variant of *PROKR2* caused CPP. In this case, the thelarche was observed at 3 yr and 5 mo of age. The blood levels of gonadotropin and E2 had increased to those at puberty. Molecular analysis identified a heterozygous deletion of c.724_727delTGCT in *PROKR2*, leading to the introduction of a premature termination codon (p.Cys242fs × 305). This variant was also identified in the patient's mother who did not have CPP. *In vitro*, the variant was not subjected to nonsense-mediated decay of mRNA, and mRNA expression was similar to that in the wild-type. The mutant PROKR2 translated from this mRNA that lacks two transmembrane domains at the C-terminus. Furthermore, when only the mutant was expressed, ligand-dependent signal transduction was not observed; but, when it was co-expressed with the wild-type, an enhanced ligand-dependent signal transduction was observed than that of wild-type alone. These findings suggest that when co-expressed with the wild-type, the mutant and wild-type form a heterodimer that acts as a gain-of-function variant, leading to CPP. Moreover, Sposini *et al.* (70) demonstrated that PROKR2 lacking the 6th and 7th transmembrane domains showed enhanced ligand-dependent signal transduction. Thus, only certain special variants of *PROKR2* may develop CPP, although the exact mechanism of CPP development remains unknown. Additionally, Aiello *et al.* (71) analyzed *PROKR2* in 31 patients with CPP, but found no pathogenic variants.

Conclusion

This review summarizes the single genetic causes of CPP identified to date. Defects in *KISS1*, *KISS1R*, and *PROKR2* have been identified in CPP.

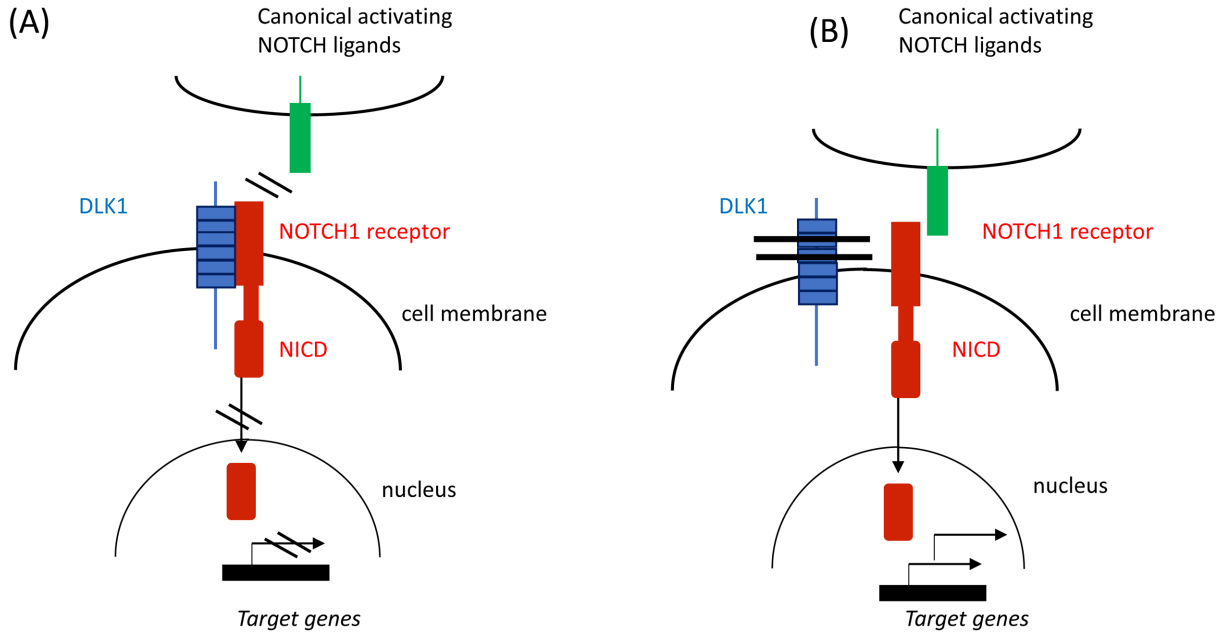

Fig. 4. The schema of DLK1 function in the MBH. (A) DLK1 is expressed in the cell membrane of kisspeptin-expressing neurons. DLK1 interacts with NOTCH1 receptor and competes with binding by canonical-activating NOTCH ligands, blocking the NOTCH signaling. (B) It is hypothesized that defects in DLK1 excessively activate NOTCH signaling; the enhanced signaling causes abnormal cell proliferation and/or differentiation of kisspeptin neurons and the development of CPP. NICD, Notch intracellular domain.

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The defects in *TAC3* and *TAC3R* have not yet been found in CPP, but more investigations are required to identify CPP with defects in these genes.

MKRN3 and *DLK1* are maternally imprinted genes, and the mechanism by which *MKRN3* and *DLK1* abnormalities disturb the physiological control of the hypothalamic kisspeptin neurons and induce CPP has been hypothesized; but, further studies are required. Various factors, such as genetic and/or environmental factors, are involved in the development of CPP, but elucidation of the single genetic abnormality causing CPP may aid in a better understanding of puberty and reproduction in humans.

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