DLK1, Notch Signaling and the Timing of Puberty

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Semin Reprod Med 2019;37:174–181

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Abstract

The factors that trigger human puberty are among the central mysteries of reproductive biology. Several approaches, including mutational analysis of candidate genes, large-scale genome-wide association studies, whole exome sequencing, and whole genome sequencing have been performed in attempts to identify novel genetic factors that modulate the human hypothalamic–pituitary–gonadal axis to result in premature sexual development. Genetic abnormalities involving excitatory and inhibitory pathways regulating gonadotropin-releasing hormone secretion, represented by the kisspeptin (*KISS1* and *KISS1R*) and makorin ring finger 3 (*MKRN3*) systems, respectively, have been associated with sporadic and familial cases of central precocious puberty (CPP). More recently, paternally inherited genetic defects of *DLK1* were identified in four families with nonsyndromic CPP and a metabolic phenotype. *DLK1* encodes a transmembrane protein that is important for adipose tissue homeostasis and neurogenesis and is located in the imprinted chromosome 14q32 region associated with Temple syndrome. In this review, we highlight the clinical and genetic features of patients with CPP caused by *DLK1* mutations and explore the involvement of Notch signaling and DLK1 in the control of pubertal onset.

Keywords

- central precocious puberty
- *DLK1*
- genetics
- Notch signaling
- kisspeptin

Puberty is a complex developmental stage characterized by profound physical and psychosocial changes, ultimately resulting in the achievement of adult height, gonadal maturation, and reproductive capacity. In humans, puberty is initiated through activation of the hypothalamic–pituitary–gonadal (HPG) axis, marked by an increase in pulsatile gonadotropin-releasing hormone (GnRH) release, which in turn leads to pituitary secretion of luteinizing (LH) and follicle-stimulating hormone (FSH), and subsequent activation of the gonads.[1,2]

The HPG axis is active in the embryonic and early postnatal stages of life, and then becomes dormant during childhood. The reactivation of the HPG axis after this quiescent period culminates with the development of the clinical features of puberty. Early maturation of the HPG axis, resulting in pulsatile secretion of GnRH and subsequent activation of the gonads, causes GnRH dependent or central precocious puberty (CPP).[1,2] Clinically, CPP is characterized by breast development (thelarche) prior to age 8 years in girls or testicular enlargement prior to age 9 years in boys.

The exact mechanisms underlying the reinstatement of pulsatile GnRH secretion are not completely known, but it is thought that a conjunction of factors contribute to the initiation of puberty. While metabolic and environmental factors are important regulators of pubertal development, evidence has also supported a genetic influence in the determination of pubertal timing.[3,4] Understanding the causes of precocious puberty is important as early age at menarche has been linked with increased risk of cardiovascular disease, metabolic syndrome, and cancer.[5–7] Although mutations involving kisspeptin and its receptor have been identified in association with CPP, other rare variants in genes implicated in the regulatory control of GnRH secretion, such as *TAC3*, *TACR3*, *LIN28B*, *GABRA1*, and *NPY*, have been difficult to link with CPP, as unaffected family members may also carry the variant, or functional studies failed to show altered biological activity of the mutant proteins.[8–10]

In 2013, paternally inherited genetic defects in the makorin RING finger protein 3 (*MKRN3*) gene were

Issue Theme Kisspeptin, Neurokinin B and New Players in Reproduction: Part 3; Guest Editor, Waljit S. Dhillo, MD

DOI https://doi.org/10.1055/s-0039-3400963.
ISSN 1526-8004.

associated with familial CPP in 5 of 15 families studied by whole exome sequencing.[11] An increasing number of additional mutations in *MKRN3* have been successively described in association with familial and sporadic CPP, making *MKRN3* defects the most common genetic etiology of CPP identified to date, currently accounting for up to 46% of familial cases and approximately 4% of sporadic cases.[12–15]

More recently, loss-of-function mutations in the maternally imprinted Delta-like homolog 1 (*DLK1*) gene were identified as the cause of premature sexual development in four families with several members affected by CPP and metabolic changes.[16,17]

Interestingly, *MKRN3* and *DLK1* are both located in chromosomal regions associated with genetic syndromes such as Prader-Willi (chromosome 15) and Temple syndromes (TS: chromosome 14), respectively. Moreover, common single nucleotide polymorphisms (SNPs) in *MKRN3* and *DLK1* have been associated with the timing of menarche, when paternally inherited.[7,18]

In this review, we highlight clinical and genetic features of patients with CPP caused by *DLK1* mutations and explore the involvement of Notch signaling and DLK1 in the control of pubertal onset.

Loss-of-Function Mutations in *DLK1* as a Cause of Familial CPP

The first association between a defect in *DLK1* and CPP was reported in 2017, following linkage analysis and whole genome sequencing in a Brazilian family with five female patients with CPP.[16] Using this approach, Dauber et al[16] identified an approximately 14 kb heterozygous deletion, including the first exon of *DLK1* and its translational start site (►**Fig. 1a**). All affected patients demonstrated undetectable DLK1 serum

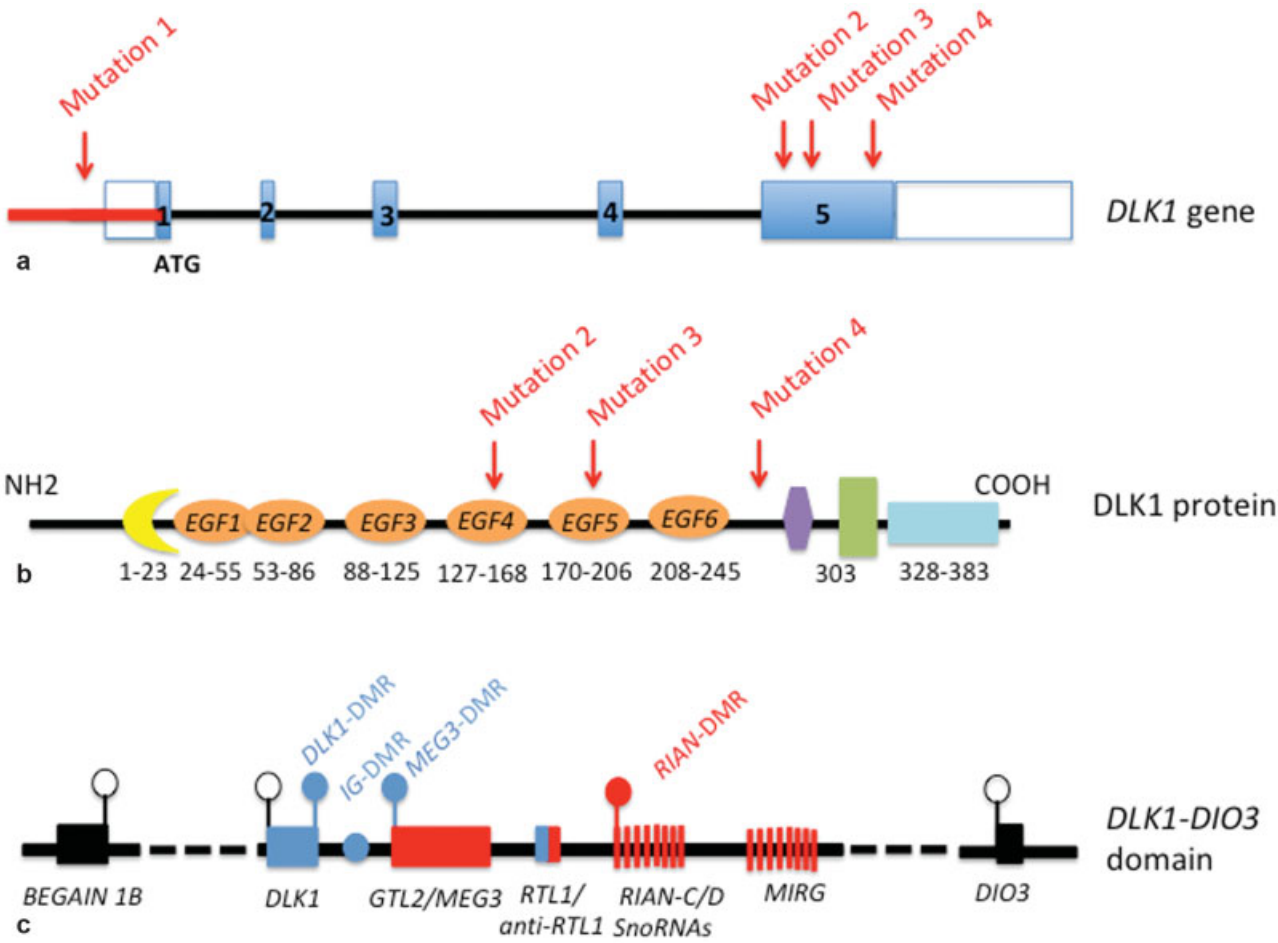

Fig. 1 Schematic representation of the human *DLK1* gene (**a**), the human DLK1 protein (**b**), and the *DLK1-DIO3* domain of human chromosome 14 (**c**). Mutation 1: 14 kb deletion; Mutation 2: c.479_479delC/p.Pro160Leufs*50; Mutation 3: c.594_594delC/p.Gly199Alafs*11; Mutation 4: c.810_810delT/p.Val271Cysfs*14. (**a**) Human *DLK1* gene (transcript length: 4657bp—ENST00000341267.9). Blue boxes indicate the coding sequences of the five exons of the human *DLK1* gene and open boxes represent 5′- and 3′-untranslated regions of exons 1 and 5, respectively. The locations of the four *DLK1* mutations identified in patients with familial CPP are indicated in red.[16,17] (**b**) Human DLK1 protein structure. The yellow arc indicates the signal peptide; orange ovals: 6 EGF-like repeats; purple hexagon: extracellular TACE—proteolytic cleavage domain; green rectangle: transmembrane domain; blue rectangle: intracellular domain. Locations of the mutations identified in DLK1 are indicated by red arrows. The numbers represent the amino acid positions of the indicated domains. (**c**) *DLK1-DIO3* domain of human chromosome 14: red rectangles represent maternally expressed genes, blue rectangles represent paternally expressed genes, and black rectangles represent genes in which imprinting status in humans has not been well established.[23] Open circles represent unmethylated DMRs: circles in red represent the maternally methylated DMRs and blue circles the paternally methylated DMRs. DMR, differentially methylated region.

levels, suggesting a complete lack of DLK1 protein in these individuals.[16]

More recently, Gomes et al[17] performed whole exome sequencing in two sisters with precocious menarche associated with polycystic ovarian syndrome (PCOS) and automated sequencing of the coding region of *DLK1* in 30 unrelated patients with familial CPP. In this study, three distinct loss-of-function mutations of *DLK1* (p.Gly199Alafs*11, p.Val271Cysfs*14, p.Pro160Leufs*50) were identified in five adult women from three unrelated families with CPP (►**Fig. 1a**). All of these frameshift mutations are located in the extracellular domain of DLK1, a region containing six epidermal growth factor (EGF)-like repeats that is key for inhibition of Notch activity (►**Fig. 1b**).[17,19] Moreover, since these are frameshift mutations, the proteins encoded by these mutant *DLK1* genes are likely not expressed due to increased RNA decay or increased protein degradation, as suggested by the absence of detectable serum DLK1 in these individuals.[17]

Segregation analysis in all four families demonstrated an inheritance pattern of a paternally expressed imprinted gene consistent with the known imprinting of *DLK1*.

While these reports suggest a potential role of DLK1 in pubertal timing, the prevalence of *DLK1* mutations in patients with CPP is not completely known but is suggested to be low.[16–18] Grandone et al[12] did not find any rare variants in the coding region of *DLK1* in a cohort of 60 girls with idiopathic CPP (including 23 familial cases) screened by automated sequencing. It is possible that regulatory elements or large intragenic deletions in *DLK1*, which would not be identified by this study, could cause CPP[12]; however, these negative findings suggest at least that loss-of-function mutations in *DLK1* are not as common as *MKRN3* mutations as a cause of CPP.

Interestingly, missense variants in other Notch signaling pathway components (specifically, in *JAG1* and *NOTCH1*) have been identified in two patients with normosmic hypogonadotropic hypogonadism.[20] These variants were predicted to be deleterious by *in silico* programs, and the available pedigree analyses did not indicate that these were polymorphisms. In addition, Giannakopoulos et al[21] reported the occurrence of CPP in a boy diagnosed with a 22q13 deletion or Phelan-McDermid syndrome, who is also a carrier of a duplication of the 9q34.3 chromosomal region that included the *NOTCH1* gene. Although pubertal disorders were originally thought to be associated with Phelan-McDermid syndrome,[22] many patients do not manifest an abnormal pubertal phenotype, and recent genome association studies do not support an association between timing of menarche and the 22q13 chromosomal region.[21] Thus, it is possible that the *NOTCH1* gene duplication might have contributed to early pubertal initiation in this patient. Variants in the Notch signaling components that have been linked to pubertal disorders to date are summarized in ►**Table 1**.

Clinical Phenotypes Associated with *DLK1* Mutations

All patients with CPP due to loss-of-function mutations in *DLK1* presented with clinical and hormonal features consistent with the diagnosis of CPP, including early pubertal signs, accelerated linear growth, advanced bone age, and elevated basal or stimulated LH levels. The median age of pubertal onset was 5.5 years in girls (ranging from 4.6 to 7.0 years).[16,17] Short stature was reported in untreated or undertreated patients with familial early menarche or CPP due to *DLK1* mutations; however, the impact of absent or inadequate treatment on their final height appeared to be more severe than usually reported in untreated patients with idiopathic CPP from both sexes.[17] Metabolic alterations such as early-onset glucose intolerance or type 2 diabetes mellitus, overweight or obesity, hypercholesterolemia, and/or PCOS were frequently observed in the affected patients.[17]

Although alterations in pubertal timing have not been reported for *Dlk1* deletion or overexpression mouse models,[23–25] it is not clear if they were carefully phenotyped for age of puberty onset. *Dlk1* global knockout mice displayed growth retardation, obesity, blepharophimosis, skeletal malformation, and increased serum lipid metabolites, overlapping with the phenotype associated with human maternal uniparental disomy of chromosome 14 or TS.[25]

Since its first description in 1991,[26] more than 50 cases of TS have been published, most of which have maternal uniparental disomy, but loss of methylation of the intergenic differentially methylated region (IG-DMR), copy number changes, and mutation of expressed coding genes have also been reported.[27] Interestingly, the study of rare TS patients with copy number changes confined the region of interest to a 108-kb paternal deletion involving *DLK1* and *GTL2/MEG3*, and the IG-DMR (►**Fig. 1c**). The two patients with this deletion had many features of TS, but stature was more severely affected in a third reported case with a larger deletion (411 kb), which included *RTL1* (but not *DIO3*).[27] TS is characterized by intrauterine growth restriction, commonly followed by hypotonia, reduced skeletal length with relative macrocephaly, and perinatal failure to thrive.[26,27] Adults with TS have reduced final height, but frequently display high body mass index with onset in late childhood.[23] Metabolic diseases such as type 2 diabetes and dyslipidemias are present in 11 and 23% of the patients, respectively. The majority (76–90%) of TS cases in both males and females manifest early puberty.[28,29] Additional dysmorphic characteristics are also associated with TS, including a characteristic face with a broad nose and high forehead, and small hands and feet.[23] Except for precocious puberty, short stature, and metabolic changes, no other clinical features of TS were reported in patients with CPP and *DLK1* mutations.

DLK1 Gene, Protein Structure, and Expression

The *DLK1* gene was described simultaneously by three independent groups in 1993,[30–32] and, consequently, it is also referred to as fetal antigen 1 and preadipocyte factor 1.[33] *DLK1* is located on human chromosome 14q32.2 (chromosome 12qF1 in mouse) in a region containing a cluster of imprinted genes (*DLK1-DIO3* domain; ►**Fig. 1c**) associated

Table 1 Variants in the Components of Notch Signaling Associated with Pubertal Disorders

Variant								Reference
Gene (chromosome locus)	Nucleotide change	Protein change	Protein domain	Phenotype	Segregation	*In silico* prediction	No. of affected patients (index)/no. of total probands	
DLK1 (14q32)	~14 kb deletion, including exon 1	Truncated protein	–	CPP, overweight/obesity, glucose intolerance/type 2 DM, hypercholesterolemia	Sister and 2 paternal cousins HET, affected; Father HET, unaffected; Paternal grandmother HET, affected	–	–	Dauber et al[16]
	479_479delC	Pro160Leufs*50	EGF-like 4	CPP, glucose intolerance, hypercholesterolemia	Paternal aunt HET, affected; Father affected, DNA not available	–	1/31	Gomes et al[17]
	594_594delC	Gly199Alafs*11	EGF-like 5	CPP, PCOS, infertility, early-onset type 2 DM, hepatic steatosis	Father HET, affected; Mother, paternal aunt and younger brother WT, unaffected; Sister HET, affected	–	1/31	Gomes et al[17]
	810_810delT	Val271Cysfs*14	Juxta membrane	CPP, severe short stature	Father, and two paternal aunts affected, DNA not available; Son HET, unaffected (4 y old); Brother HET, affected	–	1/31	Gomes et al[17]
NOTCH1 (9q34)	2333C > T	Thr778Ile	EGF-like 20	nHH	Mother WT, unaffected	Deleterious ≥ 2 prediction programs	1/48	Quaynor et al[20]
	25 kb duplication	–	–	CPP, hypotonia, developmental delay in motor, cognitive and communication skills, fleshy hands, minor dysmorphic features	N/A	–	–	Giannakopoulos et al[21]
JAG1 (20p12)	323A > T	Asn108Ile	Near DSL domain (EC)	nHH	Father WT, unaffected; Sister WT, unaffected; Sister HET, nHH	Deleterious ≥ 2 prediction programs	1/48	Quaynor et al[20]

Abbreviations: CPP, central precocious puberty; DM, diabetes mellitus; DSL, Delta–Serrate–Lag2 domain; EC, extracellular; HET, heterozygous; N/A, not available; nHH, normosmic hypogonadotropic hypogonadism; PCOS, polycystic ovarian syndrome; WT, wild type.

with two different developmental disorders.[23] Mutations or loss of expression of the maternal chromosomal domain leads to Kagami-Ogata syndrome, while TS results from mutations or loss of expression of the paternally expressed genes, including *DLK1*.[23] *DLK1* parental origin-specific expression is conferred through silencing of the *DLK1* maternal allele, mainly due to DNA methylation.[34] Important epigenetically marked regulatory regions have been mapped in the *DLK1-DIO3* domain, including the promoter CpG islands of *BEGAIN 1B*, *DLK1*, and *DIO3*, which remain unmethylated in most cells, and four differentially methylated CpG regions (DMR; ►**Fig. 1c**).[23] Among these cytosine-rich DNA regions are an intragenic DMR within *DLK1* whose regulatory function is unknown, an IG-DMR that is located approximately 15 kb upstream of the *MEG3* promoter, a DMR in the *MEG3* promoter, and another within the *RIAN*-C/D SnoRNA cluster. Except for the *RIAN* C/D cluster that is maternally methylated, the other three DMRs are methylated on the paternally inherited chromosome (►**Fig. 1c**).[23,35,36] Several lines of evidence in both mice and humans have suggested that the IG-DMR is the imprinting center region at the *DLK1-DIO3* cluster, which is normally methylated only on the paternal allele, and acts to direct the methylation status of the *MEG3* promoter DMR, which then controls gene expression across the entire cluster.[23,37,38]

The *DLK1* gene has five exons (transcript length: 4657bp—ENST00000341267.9; ►**Fig. 1a**) in humans (six exons in mouse) and encodes for a transmembrane (TM) protein belonging to the EGF-like family of proteins, which also includes Notch receptors and Delta and Serrate ligands (discussed further later). Like other members of this family, DLK1 is involved in regulating differentiation and cell fate determination, playing a role in many differentiation processes, notably in osteogenesis and adipogenesis, but also in hematopoiesis and differentiation of neuroendocrine cells and hepatocytes.[39]

The DLK1 protein structure is composed of an extracellular region (EC) with six EGF-like repeats and a tumor necrosis factor α–converting enzyme (TACE) protease-sensitive target sequence, a TM domain, and a short intracellular region (►**Fig. 1b**).[39] TACE-mediated cleavage generates a soluble form of DLK1.[40] Alternative DLK1 splicing can also result in membrane-tethered isoforms that have been described in several mammalian species and whose biological activity is not fully understood.[41,42]

DLK1 expression is widely distributed during mouse embryonic development, but in adults, its expression becomes restricted to (neuro) endocrine tissues including the pituitary gland, adrenal glands, pancreas, monoaminergic neurons in the central nervous system, testes, prostate, and ovaries.[31] Additionally, it has been shown that DLK1 is also expressed postnatally in several hypothalamic nuclei, including the mediobasal hypothalamus, a key site for the control of GnRH secretion through the kisspeptin neurons.[43] Moreover, DLK1 expression has been shown in tumors with neuroendocrine features, such as neuroblastoma, pheochromocytoma, and a subset of small cell lung cancer cell lines.[30]

DLK1 and the Notch Signaling Pathway

The Notch signaling pathway is one of the most evolutionarily conserved signaling cascades across species that operates in a context-dependent way, promoting cell proliferation and apoptosis and activating specific differentiation programs during embryonic development.[44,45] In adult tissues, Notch-mediated signals are important regulators of tissue homeostasis and stem cell maintenance.[45] Given its widespread use in multiple cellular processes and tissues, aberrations resulting in gain or loss of Notch signaling components and functions have been linked to a variety of disorders, where Notch can act either as an activator or as a repressor.[46]

In mammals, four Notch receptors (Notch 1–4) and five different activating canonical ligands (Dll1, Dll3, Dll4, Jagged1, and Jagged2) characterized by the presence of a DSL (Delta, Serrate and Lag2) domain and two noncanonical ligands (Dlk1 and 2) have been described.[39,40,46,47] Notch receptors are TM proteins showing high structure homology (especially Notch1 and Notch2) and displaying both common and unique functions.[46] During maturation in the Golgi and trafficking to the cell surface, the Notch receptors are processed by a furin-like protease to produce a heterodimeric molecule composed of an N-terminal EC, a TM domain, and a C-terminal intracellular (IC) domain.[45] The EC portion of Notch receptors contains a series (29–36) of EGF-like repeats, some of which are crucial in mediating ligand interactions and responses.[48] Within the EC domain, the EGF-like repeats are followed by three cysteine-rich LIN12 repeats that prevent ligand-independent activation.[46] The IC portion of the Notch receptors is composed of a protein-binding RBPJk-associated molecule, seven ankyrin repeats, and less conserved regions including a C-terminal region rich in proline, glutamate, serine, and threonine (PEST domain), which regulates protein stability and degradation as it contains the substrate site that is recognized by E3 ubiquitin ligases.[44,46] Among family members, Notch1 and Notch2 are the most widely expressed receptors, being present in many tissues during the developmental stage, as well as in adults, while Notch3 is found mainly in vascular smooth muscle and pericytes, and Notch4 is most highly expressed in endothelium.[46]

Canonical Notch ligands are cell surface proteins that share a common structural arrangement in their extracellular domains comprising an N-terminal DSL domain, a specialized tandem EGF-repeat domain called the DOS domain, and a variable number of EGF-like repeats (both calcium binding and non–calcium binding).[45] The mammalian DSL ligands are classified as either delta-like (Dll1, Dll3, and Dll4) or Serrate (Jagged)-like (Jagged1 and Jagged2), based on homology to their *Drosophila* prototypes Delta and Serrate.[49]

While the Delta and Serrate family members represent the classical Notch ligands for activating Notch signaling through cell–cell interactions, a growing repertoire of noncanonical ligands, including Dlk1 and Dlk2, has been reported to interact with Notch receptors.[19,39,45] Dlk1 and Dlk2 are both TM proteins, whose structure and expression pattern are closely related to those of the canonical DLL ligands, except that the DSL domain is absent.[50] The high degree of homology between the proteins Dlk1 and Dlk2, particularly in their EGF-like ECs,

suggests that Dlk2 might compensate for the absence of Dlk1.[39] Dlk2 expression is detected in several mouse embryonic and adult tissues and cell lines, but shows a different pattern of expression from Dlk1.[39] Dlk2 expression is absent from liver during the first days of life, when Dlk1 expression is elevated, but increases around the 16th day of life, when Dlk1 expression starts to decline.[39]

The most extensively characterized Notch signaling pathway triggered in response to Notch ligands is known as canonical Notch signaling. In this cascade, a Notch receptor on a receiving cell interacts extracellularly with a canonical Notch ligand, leading to a conformational change of the receptor and subsequently initiating a series of proteolytic cleavages of the receptor. ADAM-mediated extracellular cleavage releases the TM-IC regions from the EC portion of the receptor, generating a short-lived membrane-bound form of Notch that is rapidly further cleaved in the membrane by the γ-secretase complex, releasing Notch IC domain (NICD) from the membrane and allowing its translocation to the nucleus (►**Fig. 2a**).[44,45,47,51] Once in the nucleus, the NICD functions as a transcriptional activator, interacting with RBPJk and other coactivators of the Mastermind-like family, as well as the histone acetyl transferase p300, to initiate transcription of Notch target genes (►**Fig. 2a**).[47,52] Major Notch target genes include the basic helix-loop-helix class of transcription factors, such as *HES1* and *HEY1*, which act as repressors of transcription, playing critical roles in developmental processes.[46,47] Even though functional differences have been ascribed to the four Notch receptors, activation with either DLL or Jagged family ligands leads to the activation of the same canonical signaling pathway.[49]

Noncanonical Notch signaling differs from canonical signaling in that it can be initiated by a noncanonical ligand, or may not require cleavage of the Notch receptor.[53] Alternatively, in some forms of noncanonical signaling, there is no involvement of RBPJk, which may reflect interactions with other signaling pathways upstream of the Notch ICD–RBPJk interaction.[53] Although the function of noncanonical ligands is still poorly understood, it has been shown that noncanonical ligands like Dlk1 can bind to Notch receptors, inhibiting Notch signaling by acting as a dominant negative protein.[40] Notch signaling can be regulated in different ways, including competition between noncanonical and canonical ligands for receptor binding sites[39] and through effects of posttranslational processing on Notch receptor activity during its synthesis and secretion, ligand-dependent activation at the surface, endocytic trafficking, and degradation.[39,52]

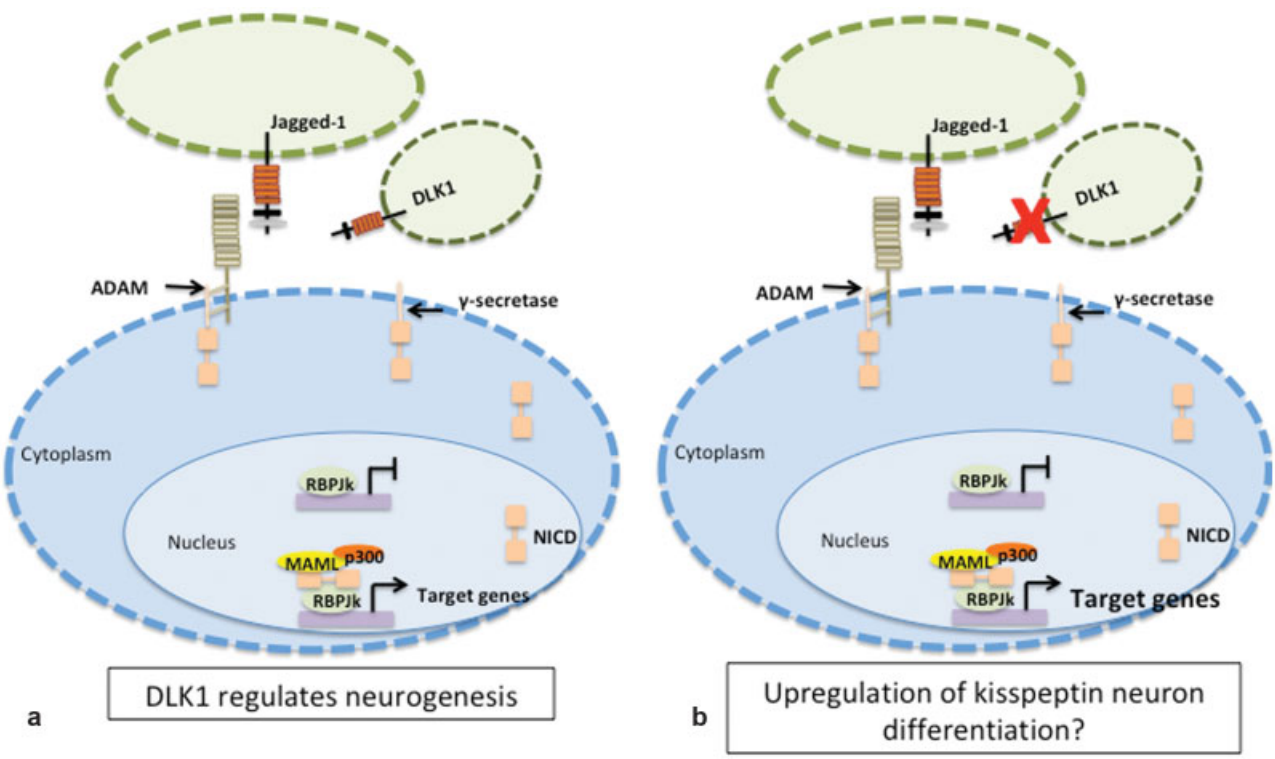

Fig. 2 Notch signaling. (**a**) Canonical Notch signaling pathway and its regulation by DLK1. This schematic shows a simplified overview of the main components of Notch signaling. Upon Notch ligand (e.g., Jagged-1) binding, a two-step proteolytic cleavage process (dashed arrows) within the juxta-membrane region and transmembrane domain of the Notch receptor is catalyzed by ADAM family *proteases* and *γ-secretase*, releasing the Notch intracellular domain (NICD). The NICD translocates to the nucleus where it forms a transcriptional activation complex with RBPJk and coactivators (e.g., Mastermind-like family [MAML], p300), thereby inducing the transcription of target genes. DLK1, a noncanonical Notch ligand, also binds to specific sites in the Notch receptor and acts to regulate the Notch signaling cascade, possibly by competition with Jagged-1 or another canonical ligand. (**b**) In the absence of DLK1, Notch signaling is expected to be upregulated, leading to an increase in the expression of some target gene(s). We hypothesize that the lack of DLK1 would lead to an upregulation of kisspeptin neuron formation, maturation, and/or secretion contributing to the CPP phenotype.

Kisspeptin as a Potential Link between *DLK1* and HPG Axis

It is known that the HPG axis is the most critical modulator of reproductive function. Effects of genetic or environmental insults to the HPG axis during developmental windows can persist throughout childhood and into adulthood, affecting timing of puberty and fertility.[54] In the hypothalamus, multiple regions develop at different times and are under the control of several signaling pathways and transcription factors required for patterning and maturation.[54] Two different hypothalamic nuclei, the arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV), are of particular interest in regard to reproductive function due to the presence of kisspeptin neurons. Kisspeptin is an excitatory neuropeptide that was identified as a permissive factor in puberty onset by the discovery of patients with hypogonadotropic hypogonadism and loss-of-function mutations in the KISS1 receptor (*KISS1R*).[55,56] It has been shown that an appropriate level of active RBPJk-dependent Notch signaling is required for progenitor cell maintenance and is critical for the formation of kisspeptin neurons in both ARC and AVPV.[57] Interestingly, the importance of the Notch signaling in the development of kisspeptin neurons is not restricted to early neurogenesis but is also required in adulthood.[57] As a negative regulator of Notch signaling, *Dlk1* has been involved in the inhibition of several differentiation processes, including adipogenesis and osteogenesis.[30,32,39] The exact mechanism by which DLK1 regulates pubertal timing is not yet understood; however, it is quite feasible that DLK1 could play a role in the regulation of neurogenesis within the hypothalamus, indirectly interfering with kisspeptin neuron formation, maturation, and/or secretion of kisspeptin through the activation or inhibition of Notch target genes (►**Fig. 2b**). Indeed, considering the importance of the Notch signaling pathway for neurogenesis and development of the hypothalamus, particularly the "reproductive center," it would be reasonable to speculate that this pathway could potentially represent a link between the three most relevant genes associated with CPP: *KISS1*, *MKRN3*, and *DLK1*. In this scenario, DLK1 would titrate the appropriate level of Notch signaling essential for the differentiation of *KISS1*-expressing neurons by competing with the canonical ligands for Notch receptor binding, and MKRN3, acting as an E3 ubiquitin ligase, might be speculated to regulate the amount of Notch ligands and receptors available at the cell surface.

Conclusion

Pubertal onset is thought to result from a decrease in factors that inhibit the release of GnRH combined with an increase in stimulatory factors. However, the main drivers that trigger the release of the puberty "brake" at the end of the juvenile period remains a mystery. Recently, studies of patients with idiopathic familial CPP uncovered the role of DLK1 in puberty initiation. DLK1 is known to be a negative regulator of Notch signaling, a well-conserved pathway involved in neurogenesis and other differentiation processes. Although animal studies have suggested that Notch signaling is relevant for the development of kisspeptin neurons in the hypothalamus, how DLK1 interacts with kisspeptin and other major players of puberty such as GnRH, MKRN3, and other excitatory and inhibitory neurotransmitters is still a subject for further study by scientists interested in the reproductive axis.

Conflict of Interest
The authors have nothing to disclose.

Acknowledgments
This work was supported by NIH R01 HD082314 (to U.B.K.) and Coordenação de Aperfeiçoamento de Ensino Superior-CAPES Grant 88881.170070/2018–01 (to D.B.M.).

References

1 Latronico AC, Brito VN, Carel JC. Causes, diagnosis, and treatment of central precocious puberty. Lancet Diabetes Endocrinol 2016;4(03):265–274
2 Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol 2016;4(03):254–264
3 de Vries L, Kauschansky A, Shohat M, Phillip M. Familial central precocious puberty suggests autosomal dominant inheritance. J Clin Endocrinol Metab 2004;89(04):1794–1800
4 Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. Pediatrics 1997;99(04):505–512
5 Prentice P, Viner RM. Pubertal timing and adult obesity and cardiometabolic risk in women and men: a systematic review and meta-analysis. Int J Obes 2013;37(08):1036–1043
6 Elks CE, Ong KK, Scott RA, et al; InterAct Consortium. Age at menarche and type 2 diabetes risk: the EPIC-InterAct study. Diabetes Care 2013;36(11):3526–3534
7 Day FR, Thompson DJ, Helgason H, et al; LifeLines Cohort Study; InterAct Consortium; kConFab/AOCS Investigators; Endometrial Cancer Association Consortium; Ovarian Cancer Association Consortium; PRACTICAL consortium. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. Nat Genet 2017;49(06):834–841
8 Teles MG, Bianco SD, Brito VN, et al. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008;358(07):709–715
9 Silveira LG, Noel SD, Silveira-Neto AP, et al. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab 2010;95(05):2276–2280
10 Durand A, Bashamboo A, McElreavey K, Brauner R. Familial early puberty: presentation and inheritance pattern in 139 families. BMC Endocr Disord 2016;16(01):50
11 Abreu AP, Dauber A, Macedo DB, et al. Central precocious puberty caused by mutations in the imprinted gene MKRN3. N Engl J Med 2013;368(26):2467–2475
12 Grandone A, Capristo C, Cirillo G, et al. Molecular screening of MKRN3, DLK1, and KCNK9 genes in girls with idiopathic central precocious puberty. Horm Res Paediatr 2017;88(3-4):194–200
13 Simon D, Ba I, Mekhail N, et al. Mutations in the maternally imprinted gene MKRN3 are common in familial central precocious puberty. Eur J Endocrinol 2016;174(01):1–8
14 Macedo DB, Abreu AP, Reis AC, et al. Central precocious puberty that appears to be sporadic caused by paternally inherited mutations in the imprinted gene makorin ring finger 3. J Clin Endocrinol Metab 2014;99(06):E1097–E1103
15 Valadares LP, Meireles CG, De Toledo IP, et al. *MKRN3* mutations in central precocious puberty: a systematic review and meta-analysis. J Endocr Soc 2019;3(05):979–995

16 Dauber A, Cunha-Silva M, Macedo DB, et al. Paternally inherited DLK1 deletion associated with familial central precocious puberty. J Clin Endocrinol Metab 2017;102(05):1557–1567
17 Gomes LG, Cunha-Silva M, Crespo RP, et al. DLK1 is a novel link between reproduction and metabolism. J Clin Endocrinol Metab 2019;104(06):2112–2120
18 Perry JR, Day F, Elks CE, et al; Australian Ovarian Cancer Study; GENICA Network; kConFab; LifeLines Cohort Study; InterAct Consortium; Early Growth Genetics (EGG) Consortium. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. Nature 2014;514(7520):92–97
19 Baladrón V, Ruiz-Hidalgo MJ, Nueda ML, et al. Dlk acts as a negative regulator of Notch1 activation through interactions with specific EGF-like repeats. Exp Cell Res 2005;303(02):343–359
20 Quaynor SD, Bosley ME, Duckworth CG, et al. Targeted next generation sequencing approach identifies eighteen new candidate genes in normosmic hypogonadotropic hypogonadism and Kallmann syndrome. Mol Cell Endocrinol 2016;437:86–96
21 Giannakopoulos A, Fryssira H, Tzetis M, Xaidara A, Kanaka-Gantenbein C. Central precocious puberty in a boy with 22q13 deletion syndrome and NOTCH-1 gene duplication. J Pediatr Endocrinol Metab 2016;29(11):1307–1311
22 Phelan K, McDermid HE. The 22q13.3 deletion syndrome (Phelan-McDermid syndrome). Mol Syndromol 2012;2(3-5):186–201
23 Howard M, Charalambous M. Molecular basis of imprinting disorders affecting chromosome 14: lessons from murine models. Reproduction 2015;149(05):R237–R249
24 Lee K, Villena JA, Moon YS, et al. Inhibition of adipogenesis and development of glucose intolerance by soluble preadipocyte factor-1 (Pref-1). J Clin Invest 2003;111(04):453–461
25 Moon YS, Smas CM, Lee K, et al. Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity. Mol Cell Biol 2002;22(15):5585–5592
26 Temple IK, Cockwell A, Hassold T, Pettay D, Jacobs P. Maternal uniparental disomy for chromosome 14. J Med Genet 1991;28(08):511–514
27 Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet 2014;51(08):495–501
28 Kagami M, Nagasaki K, Kosaki R, et al. Temple syndrome: comprehensive molecular and clinical findings in 32 Japanese patients. Genet Med 2017;19(12):1356–1366
29 Mitter D, Buiting K, von Eggeling F, et al. Is there a higher incidence of maternal uniparental disomy 14 [upd(14)mat]? Detection of 10 new patients by methylation-specific PCR. Am J Med Genet A 2006;140(19):2039–2049
30 Laborda J, Sausville EA, Hoffman T, Notario V. Dlk, a putative mammalian homeotic gene differentially expressed in small cell lung carcinoma and neuroendocrine tumor cell line. J Biol Chem 1993;268(06):3817–3820
31 Jensen CH, Meyer M, Schroder HD, et al. Neurons in the monoaminergic nuclei of the rat and human central nervous system express FA1/dlk. Neuroreport 2001;12(18):3959–3963
32 Smas CM, Sul HS. Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation. Cell 1993;73(04):725–734
33 Charalambous M, Da Rocha ST, Radford EJ, et al. DLK1/PREF1 regulates nutrient metabolism and protects from steatosis. Proc Natl Acad Sci U S A 2014;111(45):16088–16093
34 da Rocha ST, Edwards CA, Ito M, Ogata T, Ferguson-Smith AC. Genomic imprinting at the mammalian Dlk1-Dio3 domain. Trends Genet 2008;24(06):306–316
35 Edwards CA, Mungall AJ, Matthews L, et al; SAVOIR Consortium. The evolution of the DLK1-DIO3 imprinted domain in mammals. PLoS Biol 2008;6(06):e135
36 Hung KH, Wang Y, Zhao JC. Regulation of mammalian gene dosage by long noncoding RNAs. Biomolecules 2013;3(01):124–142
37 Lin SP, Youngson N, Takada S, et al. Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtl2 imprinted cluster on mouse chromosome 12. Nat Genet 2003;35(01):97–102
38 Kagami M, O'Sullivan MJ, Green AJ, et al. The IG-DMR and the MEG3-DMR at human chromosome 14q32.2: hierarchical interaction and distinct functional properties as imprinting control centers. PLoS Genet 2010;6(06):e1000992
39 Sánchez-Solana B, Nueda ML, Ruvira MD, et al. The EGF-like proteins DLK1 and DLK2 function as inhibitory non-canonical ligands of NOTCH1 receptor that modulate each other's activities. Biochim Biophys Acta 2011;1813(06):1153–1164
40 Falix FA, Aronson DC, Lamers WH, Gaemers IC. Possible roles of DLK1 in the Notch pathway during development and disease. Biochim Biophys Acta 2012;1822(06):988–995
41 Deiuliis JA, Li B, Lyvers-Peffer PA, Moeller SJ, Lee K. Alternative splicing of delta-like 1 homolog (DLK1) in the pig and human. Comp Biochem Physiol B Biochem Mol Biol 2006;145(01):50–59
42 Mei B, Zhao L, Chen L, Sul HS. Only the large soluble form of preadipocyte factor-1 (Pref-1), but not the small soluble and membrane forms, inhibits adipocyte differentiation: role of alternative splicing. Biochem J 2002;364(Pt 1):137–144
43 Villanueva C, Jacquier S, de Roux N. DLK1 is a somato-dendritic protein expressed in hypothalamic arginine-vasopressin and oxytocin neurons. PLoS One 2012;7(04):e36134
44 Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. Development 2011;138(17):3593–3612
45 D'Souza B, Meloty-Kapella L, Weinmaster G. Canonical and non-canonical Notch ligands. Curr Top Dev Biol 2010;92:73–129
46 Arruga F, Vaisitti T, Deaglio S. The NOTCH pathway and its mutations in mature B cell malignancies. Front Oncol 2018;8:550
47 Yuan X, Wu H, Xu H, et al. Notch signaling: an emerging therapeutic target for cancer treatment. Cancer Lett 2015;369(01):20–27
48 Rebay I, Fleming RJ, Fehon RG, Cherbas L, Cherbas P, Artavanis-Tsakonas S. Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. Cell 1991;67(04):687–699
49 Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell 2009;137(02):216–233
50 Rodríguez P, Higueras MA, González-Rajal A, et al. The non-canonical NOTCH ligand DLK1 exhibits a novel vascular role as a strong inhibitor of angiogenesis. Cardiovasc Res 2012;93(02):232–241
51 Sjöqvist M, Andersson ER. Do as I say, Not(ch) as I do: lateral control of cell fate. Dev Biol 2019;447(01):58–70
52 Fortini ME. Notch signaling: the core pathway and its post-translational regulation. Dev Cell 2009;16(05):633–647
53 Heitzler P. Biodiversity and noncanonical Notch signaling. Curr Top Dev Biol 2010;92:457–481
54 Biehl MJ, Raetzman LT. Developmental origins of hypothalamic cells controlling reproduction. Semin Reprod Med 2017;35(02):121–129
55 Seminara SB, Messager S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. N Engl J Med 2003;349(17):1614–1627
56 de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 2003;100(19):10972–10976
57 Biehl MJ, Raetzman LT. Rbpj-κ mediated Notch signaling plays a critical role in development of hypothalamic Kisspeptin neurons. Dev Biol 2015;406(02):235–246