Molecular basis of normal and pathological puberty: from basic mechanisms to clinical implications

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Puberty is a major maturational event; its mechanisms and timing are driven by genetic determinants, but also controlled by endogenous and environmental cues. Substantial progress towards elucidation of the neuroendocrine networks governing puberty has taken place. However, key aspects of the mechanisms responsible for the precise timing of puberty and its alterations have only recently begun to be deciphered, propelled by epidemiological data suggesting that pubertal timing is changing in humans, via mechanisms that are not yet understood. By integrating basic and clinical data, we provide a comprehensive overview of current advances on the physiological basis of puberty, with a particular focus on the roles of kisspeptins and other central transmitters, the underlying molecular and endocrine mechanisms, and the pathways involved in pubertal modulation by nutritional and metabolic cues. Additionally, we have summarised molecular features of precocious and delayed puberty in both sexes, as revealed by clinical and genetic studies. This Review is a synoptic up-to-date view of how puberty is controlled and of the pathogenesis of major pubertal alterations, from both a clinical and translational perspective. We also highlight unsolved challenges that will seemingly concentrate future research efforts in this active domain of endocrinology.

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Introduction

Puberty is a fundamental period when sexual maturity and reproductive capacity are acquired,[1] and key somatic, behavioural, and psychological changes occur, leading to an adult phenotype. Its neuroendocrine substrate, defined by full activation of the hypothalamic–pituitary–gonadal (HPG) axis, includes: (1) hypothalamic gonadotropin-releasing hormone (GnRH); (2) pituitary gonadotropins, luteinising hormone and follicle-stimulating hormone, driven by GnRH; and (3) gonadal steroids and peptides, stimulated by gonadotropins,[2] all intertwined via feedback loops.

With its origin in early developmental events commencing in utero, normal pubertal timing in humans has a wide interindividual variation (occurring at age 8–13 years in girls and 9–14 years in boys) that is determined by genetic, nutritional, and environmental elements,[2] reflecting the complex genetic architecture and regulatory pathways underlying this developmental period. Pubertal onset is indicated by breast development in girls (Tanner stage 2),[3] and testicular enlargement (ie, testicular volume >4 mL or testicular length >25 mm) in boys (Tanner stage 2).[4] Although this definition remains arbitrary internationally, these indicators are required clinically to orient the diagnosis of pubertal pathology. Precocious puberty is defined as the onset of breast development before age 8 years in girls and increased testicular volume (>4 mL) in boys before age 9 years that is progressive and accompanied by acceleration of bone age and linear growth.[5] Delayed puberty is defined as the absence of somatic signs of pubertal development at an age 2 SD higher than the mean (approximately age 14 years for boys and 13 years for girls). Although epidemiological data of precocious and delayed puberty are scarce, multiple aetiologies of pubertal pathology, either congenital or acquired, are known.[6,7] Furthermore, trends indicate an advancement in the normal age range of puberty onset at the population level, especially in girls.[8,9] Although the reason for earlier pubertal onset is unknown, it can pose risks for disease development later in life.[10] In fact, earlier or later puberty, even within the normal limits of maturation in humans, has been linked to increased risk of multiple adverse outcomes in both sexes, including not only gynecological, but also cardiometabolic (eg, hypertension and type 2 diabetes), musculoskeletal, gastrointestinal, and cognitive conditions, and some forms of cancer.[10]

Because of its importance in human pathophysiology, we have provided an overview of the current knowledge of the molecular basis of normal puberty, its main regulatory pathways, and major deviations.

Neuropeptide control of puberty: kisspeptins and Kiss1 neurons

Considerable efforts have been made to define the neuroendocrine mechanisms for pubertal activation of GnRH neurosecretion; multiple trans-synaptic and glial inputs, of excitatory or inhibitory nature, were shown to contribute to this process.[2] Collectively, these studies, conducted in laboratory animals and non-human primates, have revealed the role of different central transmitters in the control of mammalian puberty.[2,11,12] Kisspeptins, products of the *Kiss1* gene that operate via the surface receptor, Kiss1r (previously known as Gpr54), have emerged as master gatekeepers of puberty, mainly by activating GnRH neurons.[13] Indeed, due to their essential roles, Kiss1 neurons, producing kisspeptins in discrete hypothalamic and extra-hypothalamic areas, have drawn extraordinary attention as pivotal elements for the brain's control of puberty. However, whether kisspeptins are the actual trigger of puberty, or rather operate as an amplifier of the cascade of events leading to full activation of GnRH neurons during pubertal maturation, remains contentious.[14]

The role of kisspeptins in puberty was disclosed by genetic studies showing that inactivating mutations

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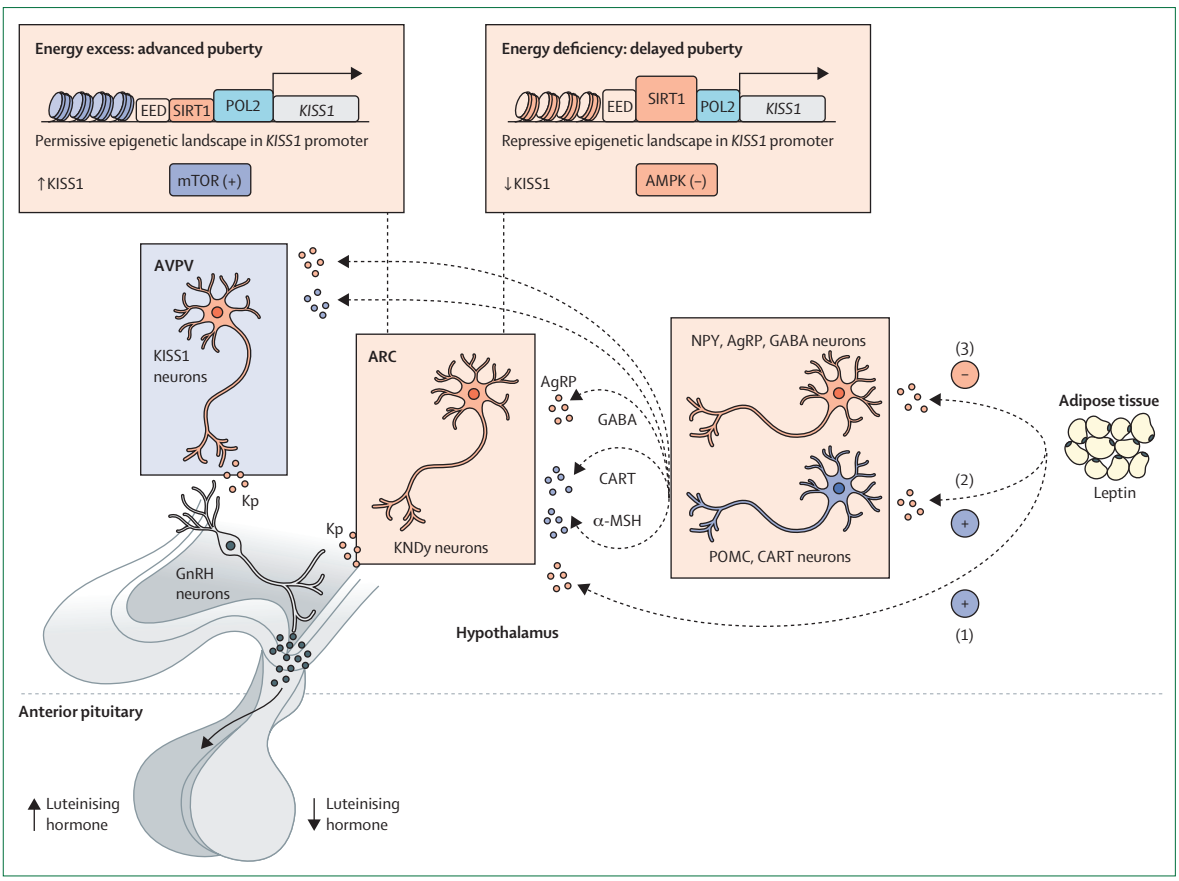

Figure 1: **Neuropeptide control of puberty and its metabolic regulation**
Schematic presentation of populations of Kiss1 neurons at the two main hypothalamic areas, namely the ARC and AVPV, and related hypothalamic neuronal pathways and cellular energy sensors involved in the physiological control of puberty. Related neuronal pathways seemingly include neurons producing NPY and AgRP, and neurons expressing POMC and CART neurons. These pathways participate in transmitting the modulatory actions of key metabolic hormones, such as leptin. Three possible modes of action of leptin are presented: (1) direct excitatory actions on Kiss1 neurons; (2) indirect excitatory actions via POMC neurons; and (3) indirect inhibitory actions on NPY/AgRP neurons. In addition, these circuits convey the influence of conditions of energy deficit, which result in delayed puberty (denoted by low luteinising hormone levels), and situations of energy excess (obesity), that advance pubertal onset (denoted by high luteinising hormone levels). Cellular energy sensors and metabolic mediators involved in this process include mTOR, AMPK and SIRT1. AgRP=agouti-related protein. AMPK=adenosine monophosphate-activated protein kinase. ARC=arcuate nucleus. AVPV=anteroventral periventricular nucleus. CART=cocaine-regulated and amphetamine-regulated transcript. EED=embryonic ectoderm development (component of polycomb repressive complex 2). GABA=γ-aminobutyric acid. GnRH=gonadotropin-releasing hormone. KNDy=kisspeptin, neurokinin B, and dynorphin. Kp=kisspeptin. MSH=melanocyte-stimulating hormone. mTOR=mammalian target of rapamycin. NPY=neuropeptide Y. POL2=RNA polymerase 2. POMC=proopiomelanocortin. SIRT1=sirtuin 1.

affecting their receptor (or kisspeptins themselves) caused central hypogonadism and absence of puberty in humans and rodents.[15–17] Subsequently, experimental studies showed a complex developmental programme of the hypothalamic Kiss1 system during pubertal maturation, involving not only increased expression of *Kiss1* during puberty, but also an increase in the number of Kiss1 neurons and their projections to GnRH neurons in rodents.[18,19] Although such changes are likely to affect both Kiss1 neuronal populations located in the arcuate nucleus and the rostral hypothalamus, arcuate nucleus Kiss1 neurons are thought to play a more prominent role because of the conservation of this population across species, including humans,[17] and findings on their function as a core component of the GnRH pulse generator.[20] A notable amount of arcuate nucleus Kiss1 neurons have been shown to co-express tachykinin, neurokinin B (NKB), and dynorphin (Dyn), and hence are named KNDy (for Kisspeptin, NKB, and Dyn expression) neurons. NKB and Dyn operate in a reciprocal manner to stimulate (NKB) or inhibit (Dyn) kisspeptin output to GnRH neurons (figure 1).[21] In addition, fragmentary evidence suggests that a third population of Kiss1 neurons located in the amygdala might also participate in the modulation of puberty in rodents.[22]

The underlying mechanisms for activation of the Kiss1 system at puberty are yet to be unfolded but involve epigenetic pathways. Of note, a key component of this process is probably the decline in repressive signals, by the concerted action of miRNAs at the level of Kiss1 neurons, as shown by studies in mice engineered to lack miRNA biosynthesis in Kiss1 cells.[23] This

phenomenon resembles similar miRNA-based regulatory pathways documented in GnRH neurons, indispensable also for pubertal maturation.[24]

Multiple neuropeptide pathways cooperate with kisspeptins in the central control of puberty. These include not only NKB, whose genetic inactivation prevents puberty in humans,[25] and expression rises in the rodent hypothalamus preceding puberty,[26] but involve also other tachykinins such as substance P and neurokinin A, both encoded by *Tac1*, because *Tac1* knock-out mice display delayed puberty.[27] Yet, the redundancy between different members of the tachykinin family suggests that some degree of compensation occurs among them. Other central transmitters cooperate with kisspeptin pathways in the control of the reproductive axis at puberty, including prominently melanocortin signalling,[28] as supported by human studies published in 2021.[29] However, most of these regulatory pathways are not exclusive to puberty and also participate in controlling the adult reproductive axis.

Molecular mechanisms for the control of puberty: emerging roles of epigenetics

Epigenetic processes are enzymatically driven alterations of gene expression induced by post-translational modifications of the chromatin structure or variations in mRNA turnover. These mechanisms are commonly divided into three modes of operation: DNA methylation and hydroxymethylation at the carbon-5 position of cytosines in CpG dinucleotides; histone post-translational modifications that affect chromatin packaging; and non-coding RNAs involved in mRNA degradation and half-life, chromatin condensation, and transcription factor binding.

Although epigenetic mechanisms also operate at other levels of the HPG axis,[30] during the past decade, a substantial number of studies have identified epigenetic processes that affect the reactivation of the hypothalamic GnRH pulse generator around puberty in rodents and primates. In the rat arcuate nucleus, the promoter region of the *Kiss1* gene has both repressing and activating epigenetic signatures, allowing fast activation or repression of gene expression. Before puberty, arcuate nucleus *Kiss1* expression is low, partly due to the presence of the Polycomb Group (PcG) of epigenetic silencers that induce chromatin compaction by trimethylating histone H3 at lysine 27 (H3K27me3) at its 5□ regulatory region,[31] and by repressing lysine demethylase 6b (Kdm6b) expression, the enzyme in charge of removing the H3K27me3 repressive mark from gene promoters.[32] As puberty progresses, arcuate nucleus *Kiss1* expression is enhanced by recruitment of the trithorax group (TrxG) of epigenetic activators to the *Kiss1* promoter and enhancer regions, imposing activational H3K4me3 and H3K27Ac post-translational modifications, promoting an open chromatin conformation that enhances access of the transcriptional machinery.[33] Similar mechanisms of epigenetic control have been identified in rhesus macaques. During pubertal transition, the chromatin landscape changes at both the *Kiss1* and the *Tac3* loci in the hypothalamus by removal of the PcG and GATA Zinc Finger Domain Containing 1 (GATAD1) and lysine-specific demethylase 1A (KDM1A) repressors of their promoter sites, after which there is increased binding of the TrxG of transcriptional activators, and heightening of *Kiss1* and *Tac3* expression due to increased H3K4me3 and recruitment of p300 and CBP acetyltransferases at enhancer sites.[34]

Messina and colleagues[24] showed that GnRH-neuron specific Dicer knock-out produces hypogonadotropic hypogonadism and infertility in mice. With this model, the authors showed that the increase in GnRH expression during the infantile to juvenile transition depends on the integrity of miR-200 and miR-155.[24] Similarly, miRNA-mediated suppression of *Kiss1* transcriptional repressors, such as Mkrn3, Cbx7, and Eap1, seemingly plays a major role in full pubertal activation and attainment of reproductive capacity in mice, especially in females.[23] Some of these epigenetic mechanisms are involved in transmitting metabolic information to the hypothalamic gene networks that control pubertal maturation. Arcuate nucleus Kiss1 neurons react to metabolic challenges with changes in SIRT1 expression. Association of SIRT1 with the PcG alters the chromatin structure of the *Kiss1* gene promoter region with changes in H3K27me3 and H3K9-14Ac, thereby modulating pubertal timing.[35]

Several studies[36–40] aimed to identify patterns in DNA methylation or miRNA expression in blood or buccal samples across human puberty. Overall, methylation patterns in peripheral blood accurately predict pubertal development and are enriched in biological pathways related to growth and development of the reproductive system.[36–38] The number of differentially methylated CpG islands is higher in females than males, probably related to a more pronounced pubertal transition and hormonal variations due to the initiation of the ovarian cycle.[38] DNA methylation changes detected in buccal samples form cohesive networks that correlate with salivary testosterone levels independent of sex.[39] Conversely, CpG–miRNA pairs were identified in the imprinted region of 14q32 that are associated with age at menarche, suggesting a causal relationship.[40] Methylome profiling in peripheral blood identified 48 zinc finger (ZNF) genes as having hypermethylated CpGs in patients with central precocious puberty and zinc finger protein 57 (ZFP57) was hypomethylated during normal puberty. ZFP57 is a transcriptional repressor involved in maintenance of imprinting regions.[41] More research needs to be done to improve our knowledge of peripheral epigenetic markers and their connection with central mechanisms for the control of puberty.

Nutritional and metabolic control of puberty: basic mechanisms and clinical implications

Nutritional and metabolic cues are prominent among the multiple modulators of pubertal timing in humans and

other species.[42] The concept that body energy reserves have a permissive role in pubertal maturation was scientifically formulated by Frisch and colleagues[43] in 1973, proposing the critical weight (fat) mass hypothesis. In humans, the interaction of these physiological axes is observed at two extremes of body energy status: (1)chronic energy deficiency, malnutrition, or anorexia, which can be accompanied by delayed puberty or absence of pubertal progression, and (2) energy excess, or obesity, which can be associated with advanced pubertal onset.[42,44] This nutritional effect on pubertal development has been shown in girls,[8,45] but might also occur in boys.[46]

Clinical and experimental studies have identified some key hormonal signals responsible for the coupling of puberty and metabolism. Although extensive recapitulation of these findings is beyond the scope of this Review, the adipose hormone, leptin, can be singled out as the most prominent metabolic modulator of puberty, by signalling sufficient body energy reserves.[42] Although initial studies proposed that leptin triggered puberty, subsequent evidence showed that leptin acts rather as a permissive factor for pubertal maturation, especially in girls.[42] Hence, threshold leptin levels are needed, but not sufficient per se, to progress through puberty. Other metabolic hormones cooperate with leptin to modulate puberty, including the gut hormone, ghrelin, and the pancreatic hormone, insulin. Elevated ghrelin levels, an index of energy deficit, have been shown to inhibit puberty in rodents.[47] By contrast, insulin's actions are mandatory for puberty to proceed normally, as indicated by human and rodent studies.[48] Women with uncontrolled type 1 diabetes can have delayed puberty, and neuronal ablation of the insulin receptor in rodents led to impaired ovarian maturation.[48]

The interaction between these peripheral hormones and the brain centres controlling puberty takes place primarily in the hypothalamus and probably involves prominent modulation of neuronal pathways upstream of GnRH neurons. Among these pathways, Kiss1 neurons are clearly sensitive to leptin and other metabolic factors, although some of these regulatory actions probably occur indirectly (ie, on afferents of Kiss1 neurons rather than Kiss1 eurons themselves).[42] Conditions of energy deficit and leptin deficiency can suppress the hypothalamic Kiss1 system, whereas early-onset obesity in rodent models is linked to premature *Kiss1* activation, compatible with partly accelerated puberty onset.[42] Cellular energy or metabolic sensors, acting in Kiss1 neurons and their afferents to transduce metabolic information, include the mammalian target of rapamycin (mTOR) and the AMP-activated kinase (AMPK), which operate in a reciprocal manner to centrally modulate energy homoeostasis.[49] Using preclinical models, we have documented that brain mTOR signalling is needed for transmission of the permissive effects of leptin on female puberty,[50] whereas AMPK activity in Kiss1 neurons participates in the inhibitory actions of subnutrition on pubertal timing (figure 1).[51]

AMPK probably cooperates with SIRT1, a member of the sirtuin family, in the modulation of pubertal timing. In arcuate nucleus Kiss1 neurons, SIRT1 content increases in conditions of subnutrition and operates as a repressor of the *Kiss1* promoter, thereby contributing to pubertal delay. By contrast, conditions of early obesity in female rats result in premature eviction of SIRT1 from the *Kiss1* promoter, thus contributing to activation of *Kiss1* expression and advanced puberty onset (figure 1).[35] However, it should be taken into consideration that changes in *Kiss1* RNA expression could be a proxy marker, but might not directly translate into equivalent changes in kisspeptin neurosecretion. Although this mechanism is compatible with enhanced kisspeptin output to GnRH neurons in conditions of obesity, leading to heightened gonadotropin drive to the ovary, we also documented a complementary pathway for advanced puberty due to obesity, involving kisspeptin projections to the paraventricular nucleus, and de novo ceramide synthesis in this nucleus, as putative origin of enhanced sympathetic input to the ovary.[52] Thus, obesity might contribute to pubertal acceleration via gonadotropin-dependent and gonadotropin-independent pathways, involving Kiss1 neurons.

Whereas Kiss1 neurons are a major integratory hub for the metabolic control of puberty, other neuronal pathways also play a prominent role, including neurons producing glutamate or pituitary adenylate-cyclase activating polypeptide from the ventral premammillary nucleus,[53,54] and leptin-sensitive melanocortin producing neurons from the arcuate nucleus;[28] relevance of the second pathway is reinforced by recent human data supporting a fundamental role of melanocortin signalling via MC3-receptors in the integration of nutritional state, rate of growth, and puberty in humans.[29] Variants in this receptor were associated with a delay in sexual maturation, similar to that found in mice lacking Mc3r.[29] Indeed, pathways from the ventral premammillary nucleus and melanocortin pathways are known to transmit leptin actions and interact with Kiss1 neurons (figure 1). For instance, MC3-receptors are expressed in Kiss1 neurons, supporting the relevance of this pathway involving leptin, hypothalamic anorexigenic proopiomelanocortin (POMC) and Kiss1 neurons, operating via MC3R signalling. Our studies show that GnRH neurons are also equipped with energy-sensing mechanisms, involving AMPK and the G-protein-coupled receptor kinase, GRK2, that probably contribute to suppression of puberty onset in conditions of energy insufficiency.[55,56]

Central precocious puberty

Precocious puberty is defined as the development of secondary sexual characteristics before the age of 8 years in girls and 9 years in boys, and it has a clear female predominance.[7] The premature activation of pulsatile hypothalamic GnRH secretion leads to central precocious puberty, the most common form of premature sexual

development. In the past decade, genetic and epigenetic causes of central precocious puberty have been documented by the identification of rare pathogenic gene variants, causing perturbation of specific hypothalamic pathways.[7,57] We have schematically presented typical examples in a neuroendocrine context in this Review (figure 2).

In 2008, a rare heterozygous activating mutation of KISS1R (p.Arg386Pro) was identified in a girl with central precocious puberty.[58] This mutation, located in the C-terminal tail of the receptor, led to prolonged activation of intracellular signalling pathways in response to kisspeptin in mammalian cells.[59] A rare kisspeptin variant, p.Pro74Ser, was subsequently identified in the heterozygous state in a boy who developed sporadic central precocious puberty at the age of 1 year.[60] The capacity to stimulate signal transduction was prolonged for p.Pro74Ser mutant kisspeptin compared with wild type, suggesting that this variant might be more resistant to degradation.

MKRN3 is an important neuroendocrine player in the control of pubertal timing, acting as an upstream inhibitor of GnRH secretion.[61–63] The gene encoding MKRN3 (*MKRN3*) is located within the maternally imprinted Prader-Willi syndrome critical region on chromosome 15q11.2. The role of MKRN3 in the pathogenesis of central precocious puberty was first shown in 2013, when whole exome sequence analysis was done in several families with central precocious puberty.[61] 15 individuals (eight girls and seven boys) from five unrelated families carried loss-of-function *MKRN3* mutations, characterising a monogenic familial central precocious puberty with autosomal dominant inheritance and exclusively paternal transmission.[61] To date, approximately 59 different mutations in the *MKRN3* gene have been identified in children with central precocious puberty.

A systematic review and meta-analysis evaluating 857 patients with sporadic or familial central precocious puberty from 14 studies showed a pooled overall *MKRN3* mutation prevalence of 9·0% in quantitative analyses.[62] Interestingly, higher prevalence was found in males, familial cases, and in non-Asian countries.[62] In 2021, a multi-ethnic cohort of 716 children with central precocious puberty revealed 71 cases with different types of loss-of-function *MKRN3* mutations.[63] Patients with more severe *MKRN3* mutations (ie, frameshift, nonsense, or promoter mutations) had greater bone age advancement and higher basal luteinising hormone levels at the time of presentation compared with patients with missense mutations. Central precocious puberty due to loss-of-function mutations of *MKRN3* is clinically indistinct from idiopathic central precocious puberty; however, the type of genetic defect might affect the severity of the phenotype.[63]

In mice, *Mkrn3* mRNA levels in the mediobasal hypothalamus are high before puberty, suggesting a potential action as a suppressor of GnRH secretion.[61] Hypothalamic *Mkrn3* expression declines before puberty, mediated by miR-30 microRNA, which binds to a highly conserved region of the *Mkrn3* mRNA 3□-UTR.[64] Abreu and colleagues[65] showed that *Mkrn3* is expressed in Kiss1 neurons of the mouse hypothalamic arcuate nucleus, whereas cell reporter assays documented that MKRN3 represses human *KISS1* and *TAC3* gene promoter activities, in a manner dependent upon its ubiquitin ligase activity. This activity is reduced by pathogenic mutations affecting the RING finger domain of the protein.[65] In addition, interaction of MKRN3 with several proteins, including some implicated in pubertal timing, has been identified in vitro.[66–68] Loss of *Mkrn3* in mice can cause central precocious puberty, with early pubertal onset, as shown by advanced timing of preputial separation and vaginal opening in male and female Mkrn3-deficient mice, respectively.[67] By contrast, persistence of peripubertal

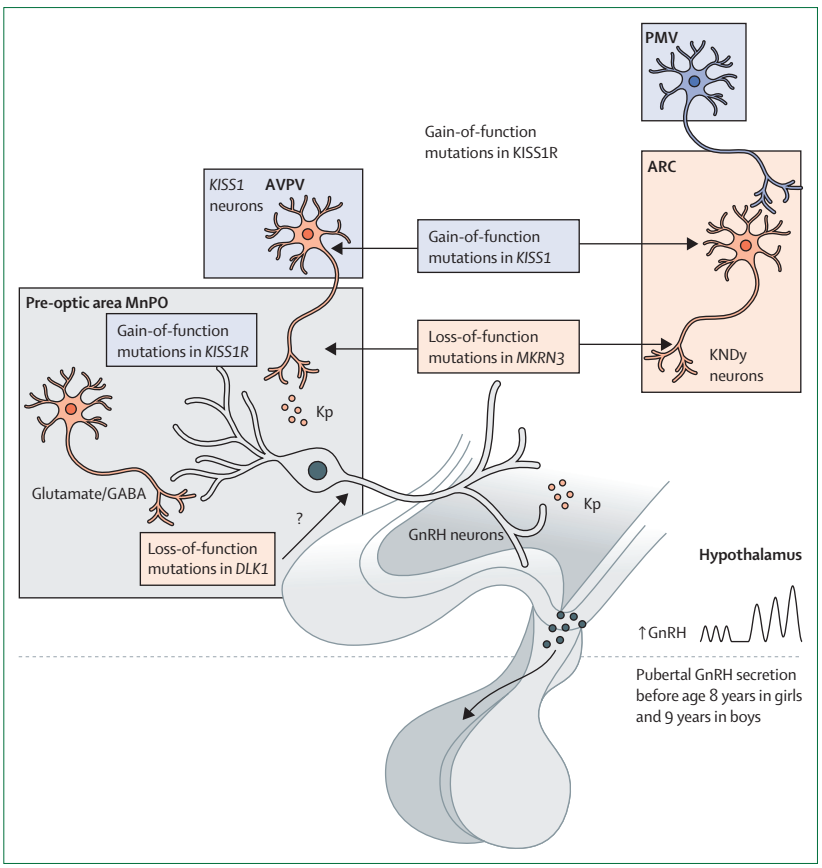

Figure 2: **Monogenic causes of central precocious puberty**
Central precocious puberty is a hypothalamic disorder with multiple aetiologies, including congenital and acquired conditions. Schematic representation of the hypothalamic regions and paradigmatic monogenic causes of central precocious puberty in humans are shown. These monogenic forms include gain-of-function mutations in *KISS1*, gain-of-function mutations of *KISS1R*, loss-of-function mutations in *MKRN3*, and loss-of-function mutations in *DLK1*. Schematic presentation of the main sites of alteration of hypothalamic circuits due to the above mutations is provided. ARC=arcuate nucleus. AVPV=anteroventral periventricular nucleus. GABA=γ-aminobutyric acid. GnRH=gonadotropin-releasing hormone. KNDy=kisspeptin, neurokinin B, and dynorphin. Kp=kisspeptin. ME=median eminence. MnPO=median preoptic nucleus. PMV=ventral pre-mammillary nucleus.

Panel 1: **Causes of central precocious puberty**

CNS lesions

Congenital

- Hypothalamic hamartoma
- Arachnoid cyst
- Hydrocephalus
- Septo-optic dysplasia
- Chiari malformations
- Myelomeningocele

Acquired

- Tumours: low grade gliomas, ependymoma, pinealoma, craniopharyngioma, germinoma
- Cranial irradiation
- Traumatic brain injury
- CNS infection
- Granulomatous disease
- Intracranial bleeding
- Cerebral palsy secondary to perinatal hypoxic–ischemic encephalopathy

No documented CNS lesions

Genetic

- *KISS1* gain-of-function mutations
- *KISS1R* gain-of-function mutations
- *MKRN3* loss-of-function mutations
- *DLK1* loss-of-function mutations
- Chromosomal abnormalities

Environmental

- International adoption (ie, adoption of a child from another country)
- Endocrine-disrupting chemicals
- Obesity

Idiopathic

- A diagnosis of exclusion

Secondary central precocious puberty

- Following treatment of peripheral precocious puberty, after withdrawal of chronic sex hormone exposure

hypothalamic *Mkrn3* expression resulted in delayed puberty in female mice.[69]

In 2017, a complex genetic defect (14 kb deletion associated with a 269 bp insertion) involving another maternally imprinted gene, Delta-like homologue 1 (*DLK1*, located on chromosome 14q), was identified in a family with central precocious puberty.[70] In the past 3 years, new, rare frameshift mutations of *DLK1* in girls with central precocious puberty or precocious menarche (age <9 years) were identified.[71,72] In all reported cases, familial segregation analysis was consistent with the known maternal imprinting of *DLK1*. To date, 7 distinct deleterious defects in *DLK1* have been identified in cases of central precocious puberty, all located in the extracellular region, which contains EGF-like domains. Notably, metabolic conditions, such as overweight or obesity and insulin resistance, were more prevalent in individuals with central precocious puberty associated with *DLK1* mutations than in people with idiopathic central precocious puberty, suggesting that DLK1 is a new factor linking reproduction and metabolism.[71] DLK1 is a non-canonical ligand of the Delta-Notch evolutionarily conserved signalling pathway, which controls a broad range of developmental processes, including cell fate determination, terminal differentiation, and proliferation; however, the potential mechanisms by which DLK1 deficiency leads to human central precocious puberty remains unknown (panel 1).

Abnormal genetic and epigenetic findings associated with human central precocious puberty have revealed that this paediatric endocrine condition is strongly influenced by epigenetic mechanisms, as shown by the identification of loss-of-function mutations in two imprinted genes (*MKRN3*, *DLK1*) and its potential association with other epigenetic syndromes, such as Prader-Willi syndrome, Temple syndrome, and Rett syndrome.[7]

Desensitisation therapies using GnRH analogues constitute the treatment of choice in patients with central precocious puberty.[73] Several analogues are available, including leuprorelin and triptorelin, which can be administered as intramuscular injections every 1 or 3 months (leuprorelin), or every semester (triptorelin). Another therapeutic option is the subcutaneous implantation of the long-acting GnRH analogue, histreline, which is replaced every 1–2 years.[73] There is no doubt regarding the therapeutic benefit of these treatments in girls younger than 6 years and boys younger than 9 years. However, this treatment needs to be individualised in girls aged between 6 years and 8 years.[74] Genetic and epigenetic discoveries regarding the aetiology of central precocious puberty could provide the basis for additional treatment targets in the future.

Peripheral precocious puberty

Peripheral precocious puberty defines clinical presentations of precocious puberty with an increase in sex steroids without activation of the HPG axis.[75] Although epidemiological information is scarce, peripheral precocious puberty is clearly a rare entity, much less frequent than central precocious puberty, with its prevalence (excluding congenital adrenal hyperplasia) estimated at 14 per million of the paediatric population at risk, with a female to male ratio of 4:1.[76]

The cause of peripheral precocious puberty can be congenital or acquired, with congenital adrenal hyperplasia being the most frequent congenital cause of peripheral precocious puberty in boys (panel 2). Both classic and non-classic congenital adrenal hyperplasia can debut as the appearance of pubarche, axillary hair, penis enlargement, and growth acceleration, with testicular size less than 4 mL. Because thelarche before the age of 8 years is the main indication of possible precocious puberty in girls, congenital adrenal

hyperplasia is not included as a possible cause of peripheral precocious puberty, since late-onset forms usually appear as the display of pubic or axillary hair without thelarche before the age of 8 years.[77,78]

Males and females with McCune-Albright syndrome have somatic gain-of-function mutations in *GNAS1* (20q13.2) inducing mosaic $G\alpha_s$ activation, which, in turn, induces an elevated output of intracellular cyclic adenosine monophosphate (cAMP).[79] Excessive production of cAMP and, therefore, the clinical spectrum of McCune-Albright syndrome (fibrous dysplasia of bone, café-au-lait skin macules, and hyper-functioning endocrinopathies) is mainly conditioned by the location and extent of tissues in which the *GNAS1* mutation is expressed.[80] In McCune-Albright syndrome endocrinopathies, gonadotropin-independent gonadal hyperfunction and peripheral precocious puberty are paradigmatic; the over-production of sex steroids in McCune-Albright syndrome, due to excessive cAMP synthesis in the gonads, is more common in girls, with about 85% of all girls with GNAS1 activation displaying oestrogen over-production and peripheral precocious puberty, compared with only 21% of boys with this condition showing excessive testosterone production.[80,81]

Familial male limited precocious puberty, or testotoxicosis, is an infrequent form of due to heterozygous constitutively activating mutations of the luteinising hormone receptor gene (*LHR*) that can occur de novo or be inherited in an autosomal dominant pattern. This condition is distinguished by signs of virilisation before the age of 4 years, with mild testicular enlargement usually being observed.[75,82] In boys, peripheral precocious puberty can also be due to mutations in *NR0B1,* which encodes *DAX1*. This is an X-linked recessive disorder that mainly presents as congenital adrenal hypoplasia.[83]

In males, acquired aetiology includes stromal cell tumours in the testes, mainly Leydig cell tumours, adrenal tumours, and extragonadal β-human chorionic gonadotropin-producing germ cell tumours mostly located in the liver, mediastinum, and brain. In girls, this acquired aetiology can include ovarian cysts and ovarian stromal cell tumours, predominantly granulosa cell tumours. Primary hypothyroidism and the exogenous administration of sex steroids are exceptional causes of peripheral precocious puberty in both sexes.[75,84,85]

The differential diagnosis between peripheral precocious puberty and central precocious puberty can be challenging; data on the emergence and progression of secondary sexual characteristics and the existence of family cases, together with the presence of additional pathology, can help to guide the diagnostic suspicion. The presence of café-au-lait spots and abdominal masses should be excluded. In boys, testicular palpation allows exclusion of testicular asymmetry and evaluation of testicular volume, which might be slightly larger than 4 mL only in familial male limited precocious puberty and some hCG-producing extragonadal tumours. The requirement of additional diagnostic tools that could include basal or stimulated hormonal studies, tumour markers, and radiological tests can be decided.[84–86]

Treatment of acquired peripheral precocious puberty should focus on the underlying pathology. Experience in treatment of congenital forms, such as McCune-Albright syndrome and familial male limited precocious puberty, is scarce due to the absence of randomised clinical trials, together with the impossibility of recruiting a relevant number of patients in the pilot studies that have been done.[75,80,82]

Panel 2: **Causes of peripheral precocious puberty**

Boys

Congenital

- Congenital adrenal hyperplasia
- Familial male limited precocious puberty
- McCune-Albright syndrome
- *NR0B1* mutations

Acquired

- Testicular tumour
- Adrenal tumour
- β-human chorionic gonadotropin secreting tumours (mediastinum, liver, brain)
- Exogenous exposure to sex steroids
- Primary hypothyroidism (Van Wyk-Grumbach syndrome)

Girls

Congenital

- McCune-Albright syndrome

Acquired

- Ovarian cyst
- Ovarian tumour
- Exogenous exposure to sex steroids
- Primary hypothyroidism (Van Wyk-Grumbach syndrome)

Delayed puberty

Delayed puberty is commonly defined as puberty commencing more than 2 SD later than the mean age for the population.[6] Cutoffs used in clinical practice are the absence of testicular enlargement (volume <4 mL) in boys by the age of 14 years and no breast development in girls by the age of 13 years.[87]

The differential diagnosis of delayed puberty can be divided into three main categories: (1) hypergonadotropic hypogonadism, characterised by elevated luteinising hormone and follicle-stimulating hormone concentrations, due to gonadal disorders; (2) transient hypogonadotropic hypogonadism (or functional hypogonadotropic hypogonadism), in which pubertal delay is due to delayed maturation of the HPG axis secondary to underlying adverse conditions; and (3) permanent hypogonadotropic hypogonadism, with constitutively low luteinising hormone and follicle-stimulating hormone concentrations (figure 3). Young people and

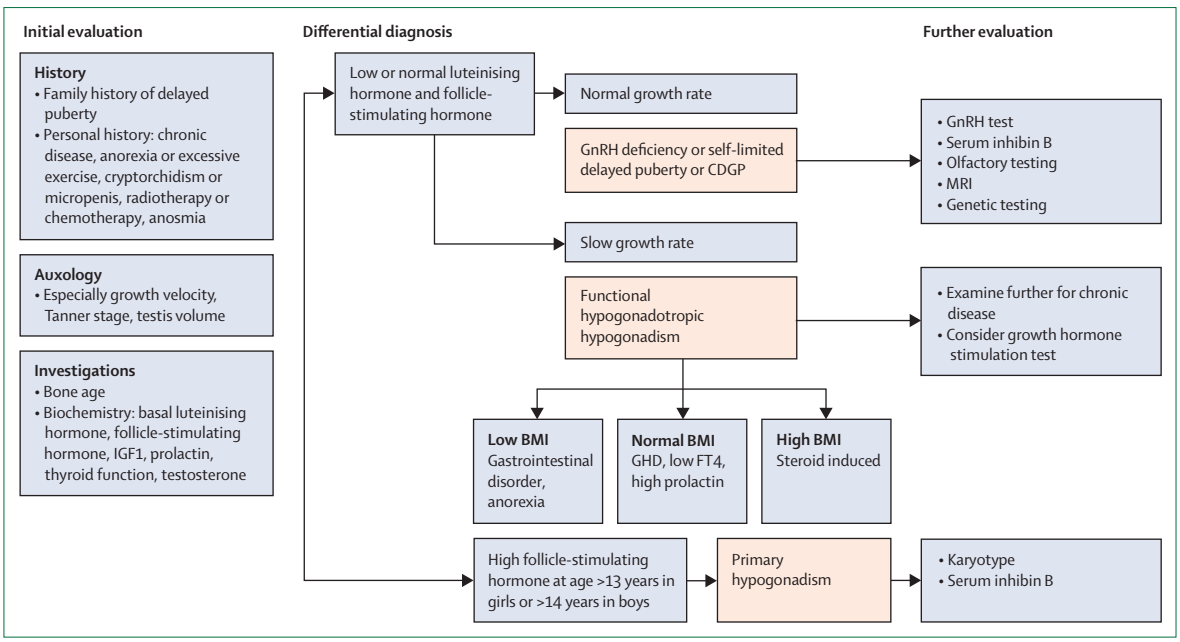

Figure 3: **Flowchart for the evaluation of a patient with delayed puberty**
CDGP=constitutional delay of growth and puberty. CHH=congenital hypogonadotropic hypogonadism. FT4=free thyroxine. GHD=growth hormone deficiency. GnRH=gonadotropin-releasing hormone. hCG=human chorionic gonadotropin.

their parents should be questioned about a history or symptoms of chronic disease, with emphasis on disorders (eg, anorexia, inflammatory bowel disease, celiac disease, or thyroid disease), that could cause temporary delay of puberty (ie, functional hypogonadotropic hypogonadism), as well as medication usage, nutritional status, and psychosocial habits, which could lead also to delayed puberty.

In this section, we aim to highlight the heterogeneity of genetic defects resulting in delayed and disordered puberty, with a special emphasis on congenital hypogonadotropic hypogonadism and the condition called self-limited delayed puberty, also known as constitutional delay.

Although congenital hypogonadotropic hypogonadism and self-limited delayed puberty present with delayed pubertal onset, these two conditions differ in clinical course and requirement for treatment.[88] In congenital hypogonadotropic hypogonadism, or Kallmann syndrome (ie, congenital hypogonadotropic hypogonadism with anosmia), hormone therapy is usually needed for the induction of secondary sexual characteristics, whereas in self-limited delayed puberty, puberty will ultimately commence spontaneously. If congenital hypogonadotropic hypogonadism is diagnosed, treatment methods to allow optimisation of future fertility (particularly for boys) can be used—in the form of gonadotropins rather than sex steroids—for induction of puberty, and commenced earlier than the puberty induction regimen used for patients with self-limited delayed puberty.[89] Moreover, precise diagnosis can be helpful to facilitate appropriate counselling on likelihood of inheritance of the condition within families.[90]

In congenital hypogonadotropic hypogonadism, different inheritance patterns, including autosomal dominant, autosomal recessive, and X-linked inheritance have been found. In the past few years, the traditional Mendelian view of congenital hypogonadotropic hypogonadism as a monogenic disorder has been revised after the identification of oligogenic forms in about 20% of patients.[91] Furthermore, reversal of congenital hypogonadotropic hypogonadism occurs in about 10–20% of patients with congenital hypogonadotropic hypogonadism, which challenges the view that the condition is lifelong.[92]

More than 60 genes are associated with congenital hypogonadotropic hypogonadism (table).[93] Causal genes have functions in (1) regulating GnRH neuronal development, migration, and maturation (eg, *ANOS1, FGFR1, FGF17, FGF8, IL17RD, PROK2, PROKR2, HS6ST1, FEZF1, DCC, NTN1, NDNF, SOX10, DUSP6, FLRT3, SPRY4, KLB, WDR11,* and *CHD7*), (2) GnRH neuronal activity (*KISS1, KISS1R, TAC3,* and *TACR3*), and (3) GnRH downstream function (*GNRH1, GNRHR, FSHB,* and *LHB*). Importantly, the majority of the known gene defects are associated with either syndromic presentation or other clinical features, which might be helpful for correct diagnosis (table).

The underlying genetic basis of self-limited delayed puberty remains less completely understood. Self-limited delayed puberty is often seen in multiple generations of the same family; 50–75% of patients with self-limited delayed puberty have a positive family history.[94]

	Inheritance	Contributes to oligogenity	Kallmann syndrome	CHH	Reversal	Self-limited delayed puberty	Other phenotypic or syndromic features	Phenotype MIM ID
AMH	AD	..	Yes	Yes	..	Yes	..	..
AMHR2	AD	..	Yes	Yes	..	Yes	..	..
ANOS1 (KAL1)	XLR	Yes	Yes	Yes	Yes	Yes	Bimanual synkinesis, aplasia or hypoplasia of olfactory bulbs, unilateral renal aplasia	308700
AXL	AD	..	Yes	Yes	..	Yes	..	..
BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12	AR, DR	Yes	..	Yes	..	..	Bardet-Biedl syndrome	209900
CCDC141	AD, AR	..	Yes	Yes	..	Yes	..	..
CHD7	AD	Yes	Yes	Yes	Yes	Yes	CHARGE syndrome	612370
CPE	AR	..	..	Yes	..	..	BDV syndrome	114855
DCAF17	AR	..	..	Yes	..	..	Woodhouse-Sakati syndrome, alopecia, diabetes mellitus, mental retardation, deafness	241080
DCC/NTN1	AD	Yes	Yes	Yes	..	Yes	Synkinesis, mid brain malformation, deafness	..
DMXL2	AD, AR	..	..	Yes	..	..	PEPNS (polyendocrine polyneuropathy)	616113
DUSP6	AD	..	Yes	Yes	..	..	Dental agenesis, syndactyly, color blindness	615269
EAP1 (IRF2BPL)	AD	..	..	..	..	Yes	..	611720
FEZF1	AR	..	Yes	Yes	..	Yes	..	616030
FGF17	AD	Yes	Yes	Yes	..	..	..	615270
FGF8	AD	Yes	Yes	Yes	..	..	Combined pituitary hormone deficiency	612702
FGFR1	AD	Yes	Yes	Yes	Yes	Yes	SOD, Hartsfield syndrome, split hand/foot malformation, combined pituitary hormone deficiency, bimanual synkinesis	147950
FLRT3	AD	Yes	Yes	Yes	..	..	..	615271
FSHB	AR	..	..	Yes	..	..	..	229070
FTO	AD	..	..		..	Yes	..	612938
GNRH1	AR	..	..	Yes	..	..	..	614841
GNRHR	AR	Yes	..	Yes	Yes	Yes	..	146110
HDAC8	XLR	..	..		..	..	Cornelia de Lange syndrome	300882
HESX1	AD, AR	..	Yes	Yes	..	..	Combined pituitary hormone deficiency, SOD	182230
HFE	AD, AR	..	..	..	..	..	Hereditary hemochromatosis	235200
HS6ST1	AD	Yes	Yes	Yes	Yes	Yes	..	614880
IGSF1	XLR	..	..	Yes	..	Yes	Central hypothyroidism, macroorchidism	300888
IGSF10	AD	..	..	..	..	Yes	..	617351
IL17RD (SEF)	AD, AR or digenic dominant	Yes	Yes	Yes	..	Yes	..	615267
KISS1	AR	..	..	Yes	..	..	..	614842
KISS1R	AR	Yes	..	Yes	..	..	..	614837
KLB	AD, complex	Yes	Yes	Yes	Yes	Yes	Obesity, insulin resistance	..
LEP	AR	..	..	Yes	..	..	Obesity, recurrent respiratory infections	614962
LEPR	AR	Yes	..	Yes	..		Obesity, recurrent respiratory infections	614963
LGR4	AD	..	..		..	Yes	..	619613
LHB	AR	..	..	Yes	..	..	..	228300
LHX	AR	..	..	Yes	..	..	Combined pituitary hormone deficiency, sensorineural deafness (variable)	221750
NDN, SNRPN	AD, deletion of the paternal copy	..	..	Yes	..	..	Prader-Willi syndrome	176270
NDNF	AD	..	Yes	Yes	..	Yes	..	616506
NR0B1 (DAX1)	XLR	..	..	Yes	..	..	Congenital adrenal hypoplasia	300200
NRP1	AD	Yes	Yes	Yes	..	..	..	..
NRP2	AD	Yes	Yes	Yes	..	..	..	..
NSMF (NELF)	AD	Yes	Yes	Yes	Yes	..	..	614838
OTUD4, RNF216	AR	..	..	Yes	..	..	Gordon Holmes syndrome	212840

(Table continues on next page)

	Inheritance	Contributes to oligogenity	Kallmann syndrome	CHH	Reversal	Self-limited delayed puberty	Other phenotypic or syndromic features	Phenotype MIM ID
(Continued from previous page)								
PCSK1	AR	..	..	Yes	..	..	Obesity, small-intestinal dysfunction, complex endocrinopathies	600955
PHF6	XLR	..	..	Yes	..	..	Börjeson-Forssman-Lehmann syndrome	301900
PLXNA1	AD	Yes	Yes	Yes	..	Yes		..
PLXNA3	Complex	Yes	Yes	Yes	..	..	..	..
PNPLA6	AR	..	..	Yes	..	..	Boucher-Neuhauser, Gordon Holmes, Oliver McFarlane, Lawrence Moon syndromes	215470
POLR3A	AR	..	..	Yes	..	..	Leukodystrophy, oligodontia, ataxia	607694
POLR3B	AR	..	..	Yes	..	..	Leukodystrophy, oligodontia, ataxia	614381
PROK2	AD	Yes	Yes	Yes	..	..	..	610628
PROKR2	AD	Yes	Yes	Yes	Yes	Yes	Combined pituitary hormone deficiency, synkinesis	244200
PROP1	AR	..	..	Yes	..	..	Combined pituitary hormone deficiency	262600
RAB18	AR	..	..	Yes	..	..	Warburg micro syndrome	614222
RAB3GAP1	AR	..	..	Yes	..	..	Warburg micro syndrome	600118
RAB3GAP2	AR	..	..	Yes	..	..	Martsolf syndrome	212720
REV3L/PLXND1	AD	..	Yes	..	Yes	..	Moebius syndrome	..
RMB28	AR	..	..	..	..	..	Alopecia, neurological defects, and endocrinopathy syndrome	612079
SEMA3A	AD	Yes	Yes	..	..	Yes	..	614897
SEMA3F	complex	Yes	Yes	Yes	..	..	..	..
SEMA3E	AD	..	Yes	Yes	..	..	CHARGE syndrome	608166
*SEMA7A**	DR	Yes	Yes	Yes	..	..	Pending full validation	..
SMCHD1	AD	..	..	Yes	..	..	Bosma arhinia microphthalmia syndrome	603457
SOX10	AD	..	Yes	Yes	..	..	Waardenburg syndrome type 2E	611584
SOX2	AD	..	..	Yes	..	..	Optic nerve hypoplasia, CNS abnormalities	206900
SOX3	XLR	Yes	..	Yes	..	..	Intellectual disability, craniofacial abnormalities, multiple pituitary hormone deficiency	..
SPRY4	AD	Yes	Yes	Yes	..	..	Hearing loss	615266
SRA1	AD	..	..	Yes	..	..	..	..
STUB1	AR	..	..	..	..	..	Spinocerebellar ataxia	615768
TAC3	AR	Yes	Yes	Yes	Yes	Yes	..	614839
TACR3	AR	Yes	..	Yes	Yes	Yes	..	614840
TBC1D20	AR	..	..	Yes	..	..	Warburg micro syndrome	615663
TCF12	AD	..	Yes	Yes	..	..	Craniosynostosis 3	615314
TUBB3	AD	..	Yes	Yes	..	..	Congenital fibrosis of the extraocular muscles	600638
WDR11	AD	Yes	Yes	Yes	Yes	..	Combined pituitary hormone deficiency	614858

AD=autosomal dominant. AR=autosomal recessive. CHARGE=coloboma of the eye, heart defects, atresia choanae, retardation of growth, genital abnormalities, and ear abnormalities. CHH=congenital hypogonadotropic hypogonadism. DR=digenic recessive. XLR=X-linked recessive. MIM=Mendelian Inheritance in Man. *Pending full validation.

Table: **Genes associated with congenital hypogonadotropic hypogonadism or self-limited delayed puberty**

Self-limited delayed puberty and congenital hypogonadotropic hypogonadism might sometimes have an overlapping genetic basis; for instance, homozygous mutations in genes such as *GNRHR*, *HS6ST1*, *TAC3*, and *TAC3R*, cause congenital hypogonadotropic hypogonadism, whereas heterozygous carriage of the same variants is associated with self-limited delayed puberty.[6]

To date, at least 24 genes have been identified as contributing to self-limited delayed puberty, including those found in relatives of congenital hypogonadotropic hypogonadism probands and others identified from large cohorts of families segregating exclusively with self-limited delayed puberty.[95] As with congenital hypogonadotropic hypogonadism, genes confined to self-limited delayed puberty have functions related to regulation of GnRH neuronal development and migration (eg, *IGSF10*, *LGR4*, *CCDC141*),[96] GnRH upstream control,[97] or GnRH downstream action and energy metabolism.[98]

Central to the evaluation process for diagnosing congenital hypogonadotropic hypogonadism or self-limited delayed puberty is the exclusion of differential

diagnoses (figure 3).[87] The diagnosis of functional hypogonadotropic hypogonadism should be guided by clinical signs and symptoms, because many underlying causes of delayed puberty can be found in this category. First-line biochemical screening tests can include basic blood biochemistry, kidney, liver, and thyroid function tests, as well as screening for coeliac disease. Further testing should be based on the suspected diagnosis by using an approach integrating the patient's family history, clinical signs and symptoms, auxological data, and biochemistry. At times, several rounds of analyses are needed until the definite underlying cause for delayed puberty is found. Of note, although a variety of clinical and biochemical parameters are available to assist with diagnosis of delayed puberty, none of these can reliably distinguish congenital hypogonadotropic hypogonadism from self-limited delayed puberty in adolescence. Both conditions might present with similar hormonal profiles, defined by low gonadotropin and sex steroid concentrations. Micropenis or the history of cryptorchidism in males are suggestive signs pointing to diagnosis of congenital hypogonadotropic hypogonadism.

As per genetic testing, the first step is to establish the inheritance pattern. Testing should also be guided by the presence of syndromic or other phenotypical features (table).[99] Syndromic features might suggest a contiguous gene syndrome for which a karyotype or comparative genomic hybridisation (CGH) array analysis are useful for identifying chromosomal abnormalities. Despite the rapid expansion of knowledge in the area, approximately only 50% of patients receive a precise genetic diagnosis. Although there is overlap in the genetic background of congenital hypogonadotropic hypogonadism and self-limited delayed puberty, the majority of mutations are distinct between these two conditions (table).[95,100]

Conclusions

The progress in our understanding of the mechanisms controlling puberty is a success story in modern neuroendocrinology. Discoveries on the genetic determinants of puberty are now being translated into clinical practice to allow a better comprehension of the basis of disordered pubertal development, which has a complex genetic landscape that is being revealed by the increasing genetic data linked to clinical information. In parallel, as exemplified by *KISS1* and *TAC3* mutations, clinical findings have fuelled basic mechanistic studies, using suitable models, which have been instrumental to further expose the neuroendocrine circuitry responsible for pubertal maturation, and its modulation by nutritional and other environmental cues. This bidirectional interaction between basic and clinical research will probably continue in coming years, thereby making it possible for us to have an integral understanding of puberty. Novel tools for sophisticated exploration of pubertal neuroendocrine circuits in vivo in suitable preclinical models, from fibre photometry to genetic editing of key loci in Kiss1 (and related) neurons, will help to deepen our knowledge of the physiological control of puberty. In turn, massive genetic studies in patients with pubertal disorders, coupled to big data (including clinical) analysis, will permit further definition of the pathogenic basis of pubertal alterations. Basic and clinical studies on the environmental determinants of puberty, from nutritional conditions to endocrine disruptors, are needed also and might allow novel insights into key modifiers of pubertal timing and potential effects in long-term health.

Search strategy and selection criteria

We did a non-systematic search on MEDLINE of original articles and reviews, published from Jan 1, 2006, to Sept 1, 2022. We searched multiple terms, including, "puberty", "kisspeptins", "neuroendocrine-control", "genetics", "epigenetics", "obesity", "metabolic homeostasis", "precocious puberty", "delayed puberty", "hypogonadism and mutations", variably connected by Boolean operators. We filtered searches using our previous knowledge, focusing on clinical and preclinical studies, and addressing mechanistic, neuroendocrine, and clinical aspects of pubertal maturation. We also considered studies on genetics determinants of normal and pathological puberty.

Contributors

JA and MT-S initially planned the contents and distribution of the sections of the Review, with the feedback and approval of the co-authors. All authors (JA, LD, UBK, ACL, AL, LSG, and MT-S) contributed to writing the different sections and preparation of the corresponding figures, critically reviewed the complete manuscript, and approved the final version. MT-S was responsible for integration, final editing, and submission of the manuscript.

Declaration of interests

UBK has received honoraria for lectures related to puberty from Novartis/Sandoz, National Institutes of Health (NIH), and the universities of Toronto, Vanderbilt, and Chicago. All other authors declare no competing interests.

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