Pathology or Normal Variant: What Constitutes a Delay in Puberty?

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Key Words

Hypogonadism · Puberty · Kallmann syndrome · Fertility treatment · Constitutional delay of puberty

Abstract

Puberty is a complex maturation process that begins during fetal life and persists until the acquisition of reproduction function. The fundamental event that activates puberty occurs in the hypothalamus. A complex neuron network stimulates GnRH secretion, which stimulates pituitary gonadotropin secretion and then gonadal steroid secretion. Pubertal delay is defined as the presentation of clinical signs of puberty 2–2.5 SD later than in the normal population. Three major groups of etiopathogeneses are described: (1) hypogonadotropic hypogonadism, (2) hypergonadotropic hypogonadism, and (3) constitutional delay of puberty (CDP) – the most common cause of delayed puberty in boys. The differential diagnosis between CDP and isolated hypogonadotropic hypogonadism remains difficult. Mechanisms of pubertal timing are now better understood and genetic or epigenetic causes can explain some pubertal delays. However, there are still unexplained mechanisms. Treatment of delayed puberty is necessary to ensure full pubertal development for the adolescent and in case of hypogonadism, to restore fertility. Finally, precocious diagnosis of hypogonadism is primordial but can be difficult during childhood and in

cases of partial hypogonadism. The study of genetic pubertal diseases or of different animal models could help to discover new diagnostic or therapeutic tools.

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Introduction

Puberty is a complex physical and psychological process that culminates in reproductive capacity. Pubertal onset requires activation of hypothalamic neurons to increase pulsatile GnRH secretion, with the gene network involved in its activation gradually coming to light. It is now accepted that increased excitatory and decreased inhibitory inputs, as well as glial secretory factors such as TGF- α and prostaglandin allow activation of the gonadotropic axis at pubertal onset [1–3]. Synthesis of GnRH starts early in fetal life, with this system being active during approximately the first 6 months of life in boys and 2 years in girls. This neonatal period is called ‘mini puberty’ and then the gonadotrope axis becomes quiescent. The timing of puberty is highly heritable with reactivation of hypothalamic GnRH secretion being determined by genetic, ethnic, nutritional and environmental influences [2].

Delayed puberty is defined as the absence of physical manifestations of sexual maturation at a chronological

Fig. 1. Gene network implicated in gonadotropic axis activation. Kisspeptin neurons located in the arcuate nucleus of the hypothalamus stimulate GnRH neurons. In yellow, the factors involved in GnRH neuron migration from the olfactory placodes; in red,

hypothalamic excitatory and inhibitory neurotransmitters and neuropeptides; in blue, hormones and other factors from the gonadotropic axis at the pituitary level, and in green, peripheral and environmental cues influencing GnRH secretion.

age 2–2.5 SD above the mean age of pubertal onset. Late puberty is classified into three major groups: (1) hypogonadotropic hypogonadism (HH), (2) hypergonadotropic hypogonadism, and (3) constitutional delay of puberty (CDP) [4]. The etiology of delayed puberty is congenital or acquired, with CDP being the most common cause of delayed puberty in boys, although it can only be diagnosed as a final diagnosis (fig. 1).

Diagnosis of Delayed Puberty

Delayed puberty is defined as the absence of testicular enlargement in boys or breast development in girls at an age 2–2.5 SD later than their population mean. In Europe, 13 years in girls and 14 years in boys serve as guidelines for determining the need for evaluation. Pubertal development should be evaluated clinically and biochemically.

Medical History

A full medical, family and lifestyle history (exercise level, nutritional status, developmental and psychological problems) should be evaluated. Details of birth and pregnancy (icterus, neonatal hypoglycemia), childhood growth patterns and surgical or medical treatments are required. Family history of pubertal delay, parental size and age at pubertal onset, infertility and anosmia [5] and personal history of chronic, autoimmune or endocrine diseases should be obtained. In case of possible acquired hypogonadism, signs of intracranial hypertension may exist.

Physical Examination

Weight and height should be measured and plotted. Tanner stage 2 marks the onset of pubertal development with breast development in girls and testicular volume >4 ml in boys. Dimorphic features (Turner or Klinefelter

syndrome), operative scars, cryptorchidism or undescended testes, micropenis, gynecomastia, sense of smell and signs of acquired disease should be analyzed.

Investigation

The diagnosis of hypogonadism and ascertain whether a primary or central pathology is involved should be made. Although a number of tests can be performed, in most cases it is very difficult to distinguish patients with delayed puberty from those with idiopathic hypogonadism. Even today, only the presence of pubertal development at age 16–18 years is the ‘gold standard’ for differentiating CDP from HH [5]. Although a family history of delayed puberty strongly suggests CDP, patients with CDP can be seen among pedigrees with isolated HH [6].

Hormone Measurements. Basal levels of FSH and LH and after GnRH injection are low in patients with HH or constitutionally delayed puberty and elevated in hypergonadotropic hypogonadism. When 0.1 mg of GnRH is injected, pubertal onset is characterized by LH/FSH >1. At pubertal onset, testosterone levels in boys are >0.05 ng/ml and estradiol in girls (<10 ng/ml before puberty) are >40 ng/ml. Inhibin B and anti-müllerian hormone (AMH) may be useful in differentiating CDP and hypogonadism as in prepubertal boys inhibin B >35 pg/ml and AMH >110 pmol/l are more frequent in CDP than in hypogonadism [7, 8]. Other pituitary deficits should be evaluated by measuring IGF-I, T₄, TSH and cortisol.

Bone Age. A bone age <11 years in girls or <13 years in boys with growth failure is encountered in constitutionally delayed puberty. Bone ages >11 years in girls or 13 years in boys require further investigation to eliminate hypogonadism.

Pelvic Ultrasonography. If ovarian volume is >2 ml and the uterus >35 mm, puberty is imminent [9]. In case of hypergonadotropic hypogonadism, gonads may be small or absent, with testes in males possibly located intra-abdominally.

Karyotype. A karyotype should be performed in cases of hypergonadotropic hypogonadism if the patient history cannot explain the gonadal pathology, whether or not dysmorphic features suggestive of Turner or Klinefelter are observed.

Brain Magnetic Resonance Imaging (MRI). In any gonadotropin deficiency MRI is the most efficient examination to eliminate organic pituitary or hypothalamic disease. Measurement of the pituitary and pituitary stalk is fundamental. Agenesis of the olfactory bulbs is found in Kallmann syndrome.

Molecular Studies. Patients with hypogonadism and a normal karyotype, and possibly other clinical features (syndromic hypogonadism), are potential candidates for genetic analysis. If the genes known to be implicated in pubertal diseases are normal, other genetic analyses forming part of ongoing research projects can be performed. Exome sequencing could identify mutations in genes representing new causes of hypogonadism [10]. If siblings are affected with similar hypogonadism phenotypes, genetic variants such as single nucleotide polymorphisms could be analyzed. If a single nucleotide polymorphism is more frequent in people with hypogonadism, it is said to be ‘associated’ with the disease [11] and to mark a region of the genome that influences the risk of disease.

Timing of Puberty

Males and females initiate and end puberty at different ages, with girls showing signs of puberty before boys [12]. Major advances in understanding pubertal onset have been achieved through the study of genetic determinants of normal puberty. In the hypothalamic arcuate nucleus diverse neurotransmitters and neuropeptides are important for reactivation of the gonadotropic axis. Loss of function mutations in genes encoding for neuropeptides such as kisspeptin (KISS1) or neurokinin B (TAC3) or their receptors (KISS1R and TACR3, respectively) cause hypogonadism [13, 14]. In sheep, neurokinin B is expressed by the same neurons that synthesize kisspeptin [15]. These neurons are located in the arcuate nucleus (in fundibular nucleus in man), which plays a role in pulsatile GnRH release [16]. Increased activity of a tumor-related gene network in the hypothalamus has been described at pubertal onset in female mice and is suggested to participate in reactivation of the gonadotropic axis [17]. Pubertal onset could also depend on epigenetic factors and complex regulation by LIN28 protein has been characterized [18].

The neurotransmitters GABA and glutamate directly control the excitability of GnRH neurons, with GABA exerting inhibition and glutamate stimulation [19]. The balance between GABAergic inhibitory and glutamatergic excitatory inputs to GnRH neurons is modified during puberty, swinging towards activation. Involvement of the opiate system is complex as different opioid peptides act on various receptor subtypes to inhibit GnRH secretion directly or indirectly [10, 20]. Neuropeptide RF amides, including RFRP1 and RFRP3, act on GnRH neurons via

Table 1. Etiologies of HH*Possible causes of acquired HH:*

Tumors: craniopharyngioma
 Infiltrating process: histiocytosis, granulomas, sarcoidosis, and hemochromatosis
 Head injury
 Infections of the central nervous system
 Stroke
 Cerebral surgery and radiotherapy
 Prolactinoma

Possible causes of congenital HH:

GnRH deficiency
 – With anosmia (Kallmann syndrome)
 – Without anosmia
 Isolated LH or FSH deficiency
 Leptin deficiency
 Panhypopituitarism
 – Complete or partial
 – Idiopathic or genetic
 – Associated with a lesion of the midline
 Associated with a syndrome
 – Prader-Willi
 – Laurence-Moon
 – Bardet-Biedl
 – CHARGE
 – Gordon Holmes
 – Boucher Neuhauser
 – Oliver-McFarlane
 – 4H
 – Warburg microsyndrome
 – Martsolf

the GPR147 receptor [21]. Peripheral hormones such as leptin are also involved in regulation of the GnRH network (table 1). Disruption of any of these factors can modify pubertal onset.

Etiopathogeny

Hypergonadotropic Hypogonadism

High FSH and LH levels indicate primary gonadal deficit, with hypergonadotropic hypogonadism being either congenital or acquired. Knowledge of previous surgery or disease affecting the gonads helps to focus the diagnosis, which is usually easy in delayed puberty associated with gonadal pathology. Klinefelter's syndrome (46,XXY) is the most frequent cause of hypergonadotropic hypogonadism in males. Hypergonadotropic hypogonadism is most often related to Turner syndrome (45,XO) in girls. Karyotype confirms diagnosis.

Hypogonadotropic hypogonadism

In boys the diagnosis is based on low plasma testosterone concentrations associated with normal or low LH and FSH (basal or after GnRH injection) and at 14 years of age a testicular volume <4 ml. In girls, HH is proposed when plasma gonadotropins are normal or low, with lack of pubertal signs at 13 years of age. Infiltrative or infectious lesions of the pituitary (histiocytosis or tumors), medication (GnRH analogs), brain trauma or radiation can cause acquired HH (table 1). To relate pubertal delay to a specific pathology, HH should reverse with correction of the pathology. This is the case of patients with hypercortisolism, renal failure, celiac disease and malnutrition, especially anorexia nervosa which is the main cause of HH among girls.

In isolated gonadotropin deficiency, congenital hypogonadism may or may not associate with anosmia. Normosmic HH is caused by congenital dysfunction of the hypothalamic-pituitary unit with a defect in synthesis, secretion or the ability of GnRH to induce the synthesis and release of gonadotropins (table 2). Kallmann syndrome is hypogonadism associated with hypoanosmia.

Segregation analyzes in informative families showed that normosmic HH is a monogenic mendelian disease, with six genes implicated to date: *GnRH1* [22, 23], GnRH receptor (GnRHR) [13, 24–30], *KISS1R* [31–37], *KISS1* [38], *TAC3 neurokinin B* and its receptor, *TACR3* [39, 40] (table 2). Studies in animal models have helped to better understand the role of these new factors in the gonadotropic axis [20, 39].

The clinical features of Kallmann syndrome are variable, with X-linked and autosomal-dominant and -recessive causes and variable penetrance described. Renal anomalies and syncynesia may exist. Its prevalence is 1/8,000 in men and five times less in women. MRI shows aplasia or hypoplasia of the olfactory bulbs, with defective migration of GnRH neurons through the cribriform plate of the ethmoid bone [41].

Eight genes involved in olfactory bulb development are reported to cause Kallmann syndrome. Inactivating mutations were first described in the *KAL1* gene (coding for anosmin-1) located on the X chromosome, then on autosomic genes including *FGFR1/Kal2* (fibroblast growth factor 1 receptor), *PROK2/Kal4* (prokineticin 2), *PROKR2/Kal 3* (prokineticin 2 receptor), *FGF8* (fibroblast growth factor 8), *NELF* (nasal embryonic LHRH factor), *WDR11* (WD repeat-containing protein 11) and more recently *SEMA3A* (semaphorin 3A) [42–48].

Miraoui et al. [49] reported five new genes of the FGF8-FGFR1 network with mutations in patients suffering

Table 2. Genetic causes of normosmic HH

Gene responsible of hypogonadism	Frequency	Heredity	Phenotype	Ref.
<i>GnRH1</i>	Europe: 1/150 USA: 1/310	recessive	complete HH	22, 23
<i>GnRH-R</i>	40% of cases of familial normosmic HH Sporadic mutation: 6–17% of cases of hypogonadism	recessive	variable HH	13, 24–28
<i>KISS1</i>	rare, no sporadic mutations described	recessive	severe gonadotropic deficiency, absence of puberty	
<i>KISS1-R</i>	rare, sporadic or familial idiopathic HH: 26 cases from 9 different families described	recessive	severe gonadotropic deficiency, absence of puberty	31–37
<i>TAC3 neurokinin B</i>	rare, no sporadic mutations described	recessive	2 of the 4 sisters with TAC3 mutations had spontaneous pregnancies, another has regular cycles and the fourth had an early miscarriage	39
<i>TAC3-R</i>	rare, no sporadic mutation but rare variant described in sporadic cases	recessive	6 of the 7 males and 4 of the 5 females demonstrated evidence for reversibility of their hypogonadotropism	14, 39, 40, 63

from HH. *FGFR1* mutations account for approximately 10% of IHH patients. Patients with the same *FGFR1* mutations can have severe hypogonadism or reversible phenotypes [50, 51].

Some hypogonadisms can be syndromic (table 1) and are also part of the HH entities associated with developmental anomalies of GnRH neurons. HH can be associated with other pituitary deficits and in cases of multiple deficits, tumoral pathology and infiltrative diseases must be eliminated (table 1). When several deficits are diagnosed in the neonatal period or infancy, it is classified as congenital panhypopituitarism. Combined or multiple pituitary hormone deficiencies can be acquired during childhood. Genetic testing for known monogenic or digenic causes of hypogonadism are performed in the second phase of evaluation. However, genetic overlap between Kallmann syndrome, combined pituitary deficiency and septo-optic dysplasia has been described; thus pituitary function should be re-evaluated in case of any doubt [52].

Patients with Constitutional Delay of Puberty

The first cause of pubertal delay in boys is CDP (idiopathic), which is difficult to distinguish from other congenital or acquired types of HH. It remains a diagnosis of exclusion and can only be confirmed when puberty occurs spontaneously. These healthy individuals spontaneously enter puberty after 13 years (girls) and 14 years

(boys) of age. Puberty, bone maturation and growth are all delayed. For this diagnosis, slow growth for age, but within the prepubertal range, with other siblings with constitutional delay of growth and puberty and a ‘normal’ physical examination with normal olfaction are required [5, 53, 54]. A family history of delayed puberty is seen in half of the CDP cases and strongly suggestive of CDP [53, 54], but patients with CDP can also be seen in pedigrees of families with isolated HH. In most cases the first signs of sexual maturation occur within 1 year after LH rises >2 UI/l in third-generation assays after administration of LHRH or within 1 year after gonadotropin and testosterone or estradiol concentrations begin to increase spontaneously [55]. Patients with CDP most frequently consult the first time for short stature and not delayed puberty. This condition is much more common in boys than in girls as pubertal growth does not occur and they remain small compared to other children of the same age. They have delayed epiphyseal maturation. An additional useful physical sign is a relatively short upper body segment, which is observed after 9 years of age with growth delay [56]. It is always important to eliminate a chronic illness or intense exercise, which can cause growth and pubertal delay.

In the absence of criteria of any suspected diseases, it is legitimate to exercise careful clinical monitoring and perform a brain MRI in teenagers with gonadotropin deficiency.

Environmental Influences

The secular trend in puberty indicates that pubertal development is plastic. The current tendency for earlier puberty reflects the quality of the modern environment. Global warming is one of the main reasons. Endocrine disruptors play a role, but the family context, and psychological development during pre-adult life are important factors that influence the transition between childhood and puberty, possibly via epigenetic events [57].

Principles of Treatment

The two objectives of treatment are to ensure full pubertal development and to achieve reproductive capacity.

Treatment of Lack of Pubertal Development

The cause of hypogonadism is treated whenever possible and pubertal development will resume a normal/better course after treatment of the underlying disease. Treatment of pituitary tumors is performed before hormone replacement to correct the delayed puberty. In other cases, the goal is to first ensure full pubertal development, with acceleration of growth, development of sexual features, achievement of optimal bone mass and normal sexual activity.

Before replacement therapy, to differentiate hypogonadism from CDGP, a short-term test with low sex steroid doses will induce the growth spurt, which will be sustained in CDGP. These low doses do not have an effect on final height. In boys, treatment with low doses of testosterone (50 mg intramuscularly every 4 weeks) for 6 months or classic protocols with low doses of anabolic steroid such as oxandrolone (1.25–2.5 mg/day for 6 months) have been used. In girls, low doses of estrogen (2–5 µg/day of ethinyl estradiol, or equivalent transcutaneous estrogen doses, or 5 µg/kg body weight of 17β-estradiol (E₂) for 6 months [5, 56]. During replacement therapy, testosterone in males and estrogens and estrogen-progestogens in girls are given in gradually increasing doses. In girls, E₂ is the most used replacement therapy via oral or cutaneous administration. Patches have fewer secondary effects, as the estrogens do not pass to the liver. In constitutional delay of growth and puberty, treatment should not begin before 13 years of age or a bone age of 12 years. There is no international consensus, but usually E₂ is given at the dose of 2–6 µg (1/12–1/4 of patch of 25 µg) per day (6 months to 1 year) [1]. In cases of hypogonadism, low doses are first given: 0.3 mg of estrogen or 5 µg/kg body weight daily of E₂ or one eighth of

transdermal patch of 25 µg, that is increased progressively between 0.3 and 0.6 mg or 1/8–1/4 patch every 6 months for 2–3 years until a dose of 2 mg E₂/day or 10 µg/kg/day is reached [5]. After 2 years of treatment, progestogens are given to induce cycles: 2 mg/day of E₂ from day 1 to day 21 and progestogens from day 10 to day 21. Estrogen-progestin pills can also be used. Bone age, ultrasonography and monitoring of the evolution of pubertal clinical features, growth and estrogen tolerance should be performed every 6 months. Lipid levels, glycemia, and liver enzymes levels are analyzed before beginning treatment.

In boys, constitutional delay of growth and puberty is treated when the delay has psychological consequences. Replacement therapy begins when bone age is 12–13 years. Treatment includes an intramuscular injection of an ester of testosterone (enanthate, cypionate or propionate) every 4 weeks beginning at 50 mg and increasing to 100 mg during 6 months to 1 year. Testosterone patches avoid an abrupt increase of testosterone at treatment onset. Clinical monitoring is necessary every 6 months. In both cases, if no response is observed after 1 year, hypogonadism should be considered.

Treatment with gonadotropins (subcutaneous weekly multi-LH or hCG and FSH or recombinant GnRH or pump) is usually used in adulthood for specific treatment of infertility in HH [58], but they can be used to induce puberty [59]. In this case, an increased testicular volume is observed. Bouvattier et al. [60] recently reviewed the possible benefits of neonatal gonadotropin treatment in males with congenital HH. Pulsatile GnRH could be effective to facilitate orchidopexy, as surgery on a small testis would be more difficult. However, these treatments are more complex and expensive, with compliance problems.

Treatment of Fertility

When hypogonadism is diagnosed during adulthood, most young men and women want to be fertile and this requires hormone therapy. Men with hypergonadotropic hypogonadism do not respond to hormonal treatment for fertility because the disease is caused by testicular dysfunction. Enanthate of testosterone is given to reverse symptoms and signs of hypogonadism. GnRH and gonadotropin therapies are the best option for men who wish to have children. Treatment with hCG alone, 1,000–2,500 IU twice a week for 8–12 weeks, increases testosterone and sometimes induces spermatogenesis, or combined with recombinant FSH (75–150 IU three times per week) to stimulate sperm production and testosterone levels. Subcutaneous GnRH administration with a pump (100–400 ng/kg of GnRH every 2 h in the abdominal sub-

cutaneous tissue) during 4 months can also restore fertility in HH. Sperm concentration often remains below the normal range. Treatment is applied between 6 and 12 months, which is necessary to restore spermatogenesis. This treatment is expensive and may interfere with the patient's everyday life [61, 62].

Final Remarks

Precocious diagnosis of hypogonadism is primordial, but the appearance of clinical characteristics depends on when hypogonadism begins. In congenital delayed puberty, the degree to which the child is affected is influenced by when during fetal development the gonadotropin axis is affected [4]. Affection early in utero courses with more severe defects, with these possibly explaining differences between severe, moderate or even 'reversible', delayed puberty [4]. Cryptorchidism and micropenis are found when GnRH deficiency occurs during fetal life. When GnRH deficiency begins in infancy, before puberty or after, infertility, lack of libido, gynecomastia and low bone density are common features, but testis and penis sizes can be normal and secondary sexual characteristics existent.

Diagnosis of hypogonadism is difficult in children if no features are observed in the newborn. In adults, hypogonadism can be postpubertal or partial with hormone levels during a GnRH test, AMH and inhibin B confirming diagnosis. MRI is important in the diagnosis of secondary hypogonadism and Kallmann syndrome. Specific

medical treatment is necessary in hypogonadism and depends on when to treat. In constitutional delay of growth and puberty, the decision of whether to treat or not requires consideration.

Important advances in understanding the initiation of puberty have been obtained through the study of genetic diseases, populations with normal puberty and animal studies. The initiation of puberty is due to postnatal maturation of the hypothalamus resulting in increased secretion by hypothalamic GnRH neurons. A complex gene network is implicated. New monogenic diseases have helped to identify new members of this network. Genotyping and epigenetics could be important for determining the status of the complex neuronal hypothalamic network. Hence, these methods could help to explain potential abnormalities in the gonadotropic axis even before puberty should start. These questions need further investigation to be answered.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

References

- 1 Ankarberg-Lindgren C, Elfving M, Wikland KA, Norjavaara E: Nocturnal application of transdermal estradiol patches produces levels of estradiol that mimic those seen at the onset of spontaneous puberty in girls. *J Clin Endocrinol Metab* 2001;86:3039–3044.
- 2 Palmert MR, Boepple PA: Variation in the timing of puberty: clinical spectrum and genetic investigation. *J Clin Endocrinol Metab* 2001;86:2364–2368.
- 3 Ojeda SR, Lomniczi A, Sandau US: Glial-gonadotrophin hormone (GnRH) neurone interactions in the median eminence and the control of GnRH secretion. *J Neuroendocrinol* 2008;20:732–742.
- 4 Rey RA, Grinspon RP, Gottlieb S, Pasqualini T, Knoblovits P, Aszpis S, Pacenza N, Stewart Usher J, Bergada I, Campo SM: Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. *Andrology* 2013;1:3–16.
- 5 Palmert MR, Dunkel L: Clinical practice. Delayed puberty. *N Engl J Med* 2012;366:443–453.
- 6 Bhagavath B, Podolsky RH, Ozata M, Bolu E, Bick DP, Kulharya A, Sherins RJ, Layman LC: Clinical and molecular characterization of a large sample of patients with hypogonadotropic hypogonadism. *Fertil Steril* 2006;85:706–713.
- 7 Coutant R, Biette-Demeneix E, Bouvattier C, Bouhours-Nouet N, Gatelais F, Dufresne S, Rouleau S, Lahlou N: Baseline inhibin B and anti-müllerian hormone measurements for diagnosis of hypogonadotropic hypogonadism in boys with delayed puberty. *J Clin Endocrinol Metab* 2010;95:5225–5232.
- 8 Hero M, Tommiska J, Vaaralahti K, Laitinen EM, Sipilä I, Puhakka L, Dunkel L, Raivio T: Circulating anti-müllerian hormone levels in boys decline during early puberty and correlate with inhibin B. *Fertil Steril* 2012;97:1242–1247.
- 9 Stanhope R, Adams J, Jacobs HS, Brook CG: Ovarian ultrasound assessment in normal children, idiopathic precocious puberty, and during low-dose pulsatile gonadotrophin-releasing hormone treatment of hypogonadotropic hypogonadism. *Arch Dis Child* 1985;60:116–119.
- 10 Margolin DH, Kousi M, Chan YM, Lim ET, Schmähmann JD, Hadjivassiliou M, Hall JE, Adam I, Dwyer A, Plummer L, Aldrin SV, O'Rourke J, Kirby A, Lage K, Milunsky A, Milunsky JM, Chan J, Hedley-Whyte ET, Daly MJ, Katsanis N, Seminara SB: Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. *N Engl J Med* 2013;368:1992–2003.
- 11 Wehkalampi K, Widen E, Laine T, Palotie A, Dunkel L: Association of the timing of puberty with a chromosome 2 locus. *J Clin Endocrinol Metab* 2008;93:4833–4839.

- 12 Sisk CL, Foster DL: The neural basis of puberty and adolescence. *Nat Neurosci* 2004;7: 1040–1047.
- 13 Karges B, Karges W, de Roux N: Clinical and molecular genetics of the human GnRH receptor. *Hum Reprod Update* 2003;9:523–530.
- 14 Guran T, Tolhurst G, Bereket A, Rocha N, Porter K, Turan S, Gribble FM, Kotan LD, Akcay T, Atay Z, Canan H, Serin A, O'Rahilly S, Reimann F, Semple RK, Topaloglu AK: Hypogonadotropic hypogonadism due to a novel missense mutation in the first extracellular loop of the neurokinin b receptor. *J Clin Endocrinol Metab* 2009;94:3633–3639.
- 15 Goodman RL, Lehman MN, Smith JT, Coolen LM, de Oliveira CV, Jafarzadehshirazi MR, Pereira A, Iqbal J, Caraty A, Ciofi P, Clarke IJ: Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. *Endocrinology* 2007;148: 5752–5760.
- 16 Plant TM, Moossy J, Hess DL, Nakai Y, McCormack JT, Knobil E: Further studies on the effects of lesions in the rostral hypothalamus on gonadotropin secretion in the female rhesus monkey (*Macaca mulatta*). *Endocrinology* 1979;105:465–473.
- 17 Roth CL, Mastronardi C, Lomniczi A, Wright H, Cabrera R, Mungenast AE, Heger S, Jung H, Dubay C, Ojeda SR: Expression of a tumor-related gene network increases in the mammalian hypothalamus at the time of female puberty. *Endocrinology* 2007;148:5147–5161.
- 18 Sangiao-Alvarellos S, Manfredi-Lozano M, Ruiz-Pino F, Navarro VM, Sanchez-Garrido MA, Leon S, Dieguez C, Cordido F, Matagne V, Dissen GA, Ojeda SR, Pinilla L, Tena-Sempere M: Changes in hypothalamic expression of the Lin28/let-7 system and related microRNAs during postnatal maturation and after experimental manipulations of puberty. *Endocrinology* 2013;154:942–955.
- 19 Ojeda SR UH: Puberty in the Rat. New York, Raven Press, 2007.
- 20 Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA: Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *J Neurosci* 2009;29:11859–11866.
- 21 Ducret E, Anderson GM, Herbison AE: Rfamide-related peptide-3, a mammalian gonadotropin-inhibitory hormone ortholog, regulates gonadotropin-releasing hormone neuron firing in the mouse. *Endocrinology* 2009;150:2799–2804.
- 22 Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombes M, Millar RP, Guiochon-Mantel A, Young J: Isolated familial hypogonadotropic hypogonadism and a GnRH1 mutation. *N Engl J Med* 2009;360:2742–2748.
- 23 Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, Gaspert A, Lavoie HB, Wu CH, Crowley WF Jr, Amory JK, Pitteloud N, Seminara SB: GnRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA* 2009;106:11703–11708.
- 24 De Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E: A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med* 1997;337:1597–1602.
- 25 Layman LC, Cohen DP, Jin M, Xie J, Li Z, Reindollar RH, Bolbolan S, Bick DP, Sherins RR, Duck LW, Musgrove LC, Sellers JC, Neill JD: Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. *Nat Genet* 1998;18:14–15.
- 26 Beranova M, Oliveira LM, Bedecarrats GY, Schipani E, Vallejo M, Ammini AC, Quintos JB, Hall JE, Martin KA, Hayes FJ, Pitteloud N, Kaiser UB, Crowley WF Jr, Seminara SB: Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2001;86:1580–1588.
- 27 Trarbach EB, Teles MG, Costa EM, Abreu AP, Garmes HM, Guerra G Jr, Baptista MT, de Castro M, Mendonca BB, Latronico AC: Screening of autosomal gene deletions in patients with hypogonadotropic hypogonadism using multiplex ligation-dependent probe amplification: detection of a hemizygosity for the fibroblast growth factor receptor 1. *Clin Endocrinol (Oxf)* 2010;72:371–376.
- 28 Chevrier L, Guimiot F, de Roux N: GnRH receptor mutations in isolated gonadotropic deficiency. *Mol Cell Endocrinol* 2011;346:21–28.
- 29 De Roux N, Young J, Brailly-Tabard S, Misrahi M, Milgrom E, Schaison G: The same molecular defects of the gonadotropin-releasing hormone receptor determine a variable degree of hypogonadism in affected kindred. *J Clin Endocrinol Metab* 1999;84:567–572.
- 30 Topaloglu AK, Lu ZL, Farooqi IS, Mungan NO, Yuksel B, O'Rahilly S, Millar RP: Molecular genetic analysis of normosmic hypogonadotropic hypogonadism in a Turkish population: identification and detailed functional characterization of a novel mutation in the gonadotropin-releasing hormone receptor gene. *Neuroendocrinology* 2006;84:301–308.
- 31 Seminara SB, Messenger S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwino KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH: The GPR54 gene as a regulator of puberty. *N Engl J Med* 2003;349:1614–1627.
- 32 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 USA* 2003;100:10972–10976.
- 33 Lanfranco F, Gromoll J, von Eckardstein S, Herding EM, Nieschlag E, Simoni M: Role of sequence variations of the GnRH receptor and G protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism. *Eur J Endocrinol* 2005;153:845–852.
- 34 Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, O'Rahilly S, Aparicio SA: Two novel missense mutations in G protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2005;90:1849–1855.
- 35 Tenenbaum-Rakover Y, Commenges-Ducos M, Iovane A, Aumas C, Admoni O, de Roux N: Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54. *J Clin Endocrinol Metab* 2007;92:1137–1144.
- 36 Teles MG, Trarbach EB, Noel SD, Guerra-Junior G, Jorge A, Beneduzzi D, Bianco SD, Mukherjee A, Baptista MT, Costa EM, De Castro M, Mendonca BB, Kaiser UB, Latronico AC: A novel homozygous splice acceptor site mutation of KiSS1R in two siblings with normosmic isolated hypogonadotropic hypogonadism. *Eur J Endocrinol* 2010;163: 29–34.
- 37 Nimri R, Lebenthal Y, Lazar L, Chevrier L, Phillip M, Bar M, Hernandez-Mora E, de Roux N, Gat-Yablonski G: A novel loss-of-function mutation in GPR54/KiSS1R leads to hypogonadotropic hypogonadism in a highly consanguineous family. *J Clin Endocrinol Metab* 2011;96:E536–E545.
- 38 Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B: Inactivating KiSS1 mutation and hypogonadotropic hypogonadism. *N Engl J Med* 2012;366:629–635.
- 39 Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK: TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in the central control of reproduction. *Nat Genet* 2009;41:354–358.
- 40 Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, Abreu AP, Carroll J, Trarbach E, Silveira LF, Costa EM, de Mendonca BB, de Castro M, Lofrano A, Hall JE, Bolu E, Ozata M, Quinton R, Amory JK, Stewart SE, Arlt W, Cole TR, Crowley WF, Kaiser UB, Latronico AC, Seminara SB: TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab* 2010;95:2857–2867.
- 41 Dode C, Hardelin JP: Kallmann syndrome. *Eur J Hum Genet* 2009;17:139–146.
- 42 Legouis R, Hardelin JP, Levilliers J, Claverie JM, Compain S, Wunderle V, Millasseau P, Le Paslier D, Cohen D, Caterina D, et al: The candidate gene for the X-linked kallmann syndrome encodes a protein related to adhesion molecules. *Cell* 1991;67:423–435.

- 43 Dode C, Levilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pecheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler ML, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C, Hardelin JP: Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet* 2003;33:463–465.
- 44 Miura K, Acierno JS Jr, Seminara SB: Characterization of the human nasal embryonic LHRH factor gene, NELF, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism. *J Hum Genet* 2004;49:265–268.
- 45 Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toubanc JE, Wolczynski S, Delpech M, Petit C, Young J, Hardelin JP: Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* 2006;2:e175.
- 46 Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, Jacobson-Dickman EE, Eliseenkova AV, Ma J, Dwyer A, Quinton R, Na S, Hall JE, Huot C, Alois N, Pearce SH, Cole LW, Hughes V, Mohammadi M, Tsai P, Pitteloud N: Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. *J Clin Invest* 2008;118:2822–2831.
- 47 Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, Ha KS, Itokawa Y, Meliciani I, Wenzel W, Lee D, Rosenberger G, Ozata M, Bick DP, Sherins RJ, Nagase T, Tekin M, Kim SH, Kim CH, Ropers HH, Gusella JF, Kalscheuer V, Choi CY, Layman LC: WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet* 2010;87:465–479.
- 48 Hanchate NK, Giacobini P, Lhuillier P, Parkash J, Espy C, Fouveaut C, Leroy C, Baron S, Campagne C, Vanacker C, Collier F, Cruaud C, Meyer V, Garcia-Pinero A, Dewailly D, Cortet-Rudelli C, Gersak K, Metz C, Chabrier G, Pugeat M, Young J, Hardelin JP, Prevot V, Dode C: SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with kallmann syndrome. *PLoS Genet* 2012;8:e1002896.
- 49 Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, Beenken A, Clarke J, Pers TH, Dworzynski P, Keefe K, Niedziela M, Raivio T, Crowley WF Jr, Seminara SB, Quinton R, Hughes VA, Kumanov P, Young J, Yialamas MA, Hall JE, Van Vliet G, Chanoine JP, Rubenstein J, Mohammadi M, Tsai PS, Sidis Y, Lage K, Pitteloud N: Mutations in FGFR1, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet* 2013;92:725–743.
- 50 Villanueva C, de Roux N: FGFR1 mutations in Kallmann syndrome. *Front Horm Res* 2010;39:51–61.
- 51 Pitteloud N, Acierno JS Jr, Meysing AU, Dwyer AA, Hayes FJ, Crowley WF Jr: Reversible Kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene. *J Clin Endocrinol Metab* 2005;90:1317–1322.
- 52 Raivio T, Avbelj M, McCabe MJ, Romero CJ, Dwyer AA, Tommiska J, Sykiotis GP, Gregory LC, Diaczok D, Tziaferi V, Elting MW, Padidela R, Plummer L, Martin C, Feng B, Zhang C, Zhou QY, Chen H, Mohammadi M, Quinton R, Sidis Y, Radovick S, Dattani MT, Pitteloud N: Genetic overlap in Kallmann syndrome, combined pituitary hormone deficiency, and septo-optic dysplasia. *J Clin Endocrinol Metab* 2012;97:E694–E699.
- 53 Sedlmeyer IL, Hirschhorn JN, Palmert MR: Pedigree analysis of constitutional delay of growth and maturation: determination of familial aggregation and inheritance patterns. *J Clin Endocrinol Metab* 2002;87:5581–5586.
- 54 Wehkalampi K, Widen E, Laine T, Palotie A, Dunkel L: Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. *J Clin Endocrinol Metab* 2008;93:723–728.
- 55 Grumbach MM, Styne DM: Williams Textbook of Endocrinology, ed 9. Philadelphia, Saunders, 1998, pp 1550–1551.
- 56 Traggiai C, Stanhope R: Disorders of pubertal development. *Best Pract Res Clin Obstet Gynaecol* 2003;17:41–56.
- 57 Hochberg Z, Belsky J: Evo-devo of human adolescence: beyond disease models of early puberty. *BMC Med* 2013;11:113.
- 58 Fraietta R, Zylberstein DS, Esteves SC: Hypogonadotropic hypogonadism revisited. *Clinics* 2013;68(suppl 1):81–88.
- 59 Raivio T, Wikstrom AM, Dunkel L: Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. *Eur J Endocrinol* 2007;156:105–111.
- 60 Bouvattier C, Maione L, Bouligand J, Dode C, Guiochon-Mantel A, Young J: Neonatal gonadotropin therapy in male congenital hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 2012;8:172–182.
- 61 Zitzmann M, Nieschlag E: Hormone substitution in male hypogonadism. *Mol Cell Endocrinol* 2000;161:73–88.
- 62 Han TS, Bouloux PM: What is the optimal therapy for young males with hypogonadotropic hypogonadism? *Clin Endocrinol (Oxf)* 2010;72:731–737.
- 63 Fukami M, Maruyama T, Dateki S, Sato N, Yoshimura Y, Ogata T: Hypothalamic dysfunction in a female with isolated hypogonadotropic hypogonadism and compound heterozygous TACR3 mutations and clinical manifestation in her heterozygous mother. *Horm Res Paediatr* 2010;73:477–481.