Pubertal Development and Menarche

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Puberty is the developmental process that culminates in reproductive capability and is the result of a complex series of molecular and physiological events. The release of gonadotropin-releasing hormone from specialized neurons of the hypothalamus begins the hormonal cascade that causes gonadal activation and the physical changes of puberty. Several factors have been proposed to influence the activation of the hypothalamus to trigger puberty, but the involved pathways have not been fully elucidated. The recent observations that the age of pubertal onset may be lowering in American girls calls attention to the lack of knowledge of modulating factors that affect the pubertal process. Genes necessary for puberty have been found by studying persons who do not achieve puberty; such studies have provided insights into the pathways necessary for pubertal development. A multidisciplinary focus is required to elucidate the complex mechanisms involved in the initiation and progression of puberty.

Key words: **gonadotropin-releasing hormone; isolated hypogonadotropic hypogonadism; menarche; puberty**

Menarche is a memorable event for women, a symbol of the end of childhood and the beginning of adulthood. It is a defining event in puberty, the developmental process that transforms a sexually immature child to a reproductively capable adult. Menarche is a late event in puberty and can only occur once the complex hormonal cascade of the reproductive system is fully operational. Puberty begins when the gonadotropin-releasing hormone (GnRH) neurons of the hypothalamus secrete GnRH in a pulsatile manner. Pulsatile GnRH causes pituitary gonadotrophs to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH in turn induce steroidogenesis and gametogenesis in the gonads. Steroid production subsequently causes the dramatic physical changes associated with puberty. Research during the last 30 years has firmly established that puberty is initiated by the pulsatile release of GnRH. A single factor or pathway responsible for inducing GnRH neuronal activity at puberty has not been firmly established, although several pathways have been identified that affect GnRH neuron function during puberty. This chapter will first discuss the physiology of the reproductive axis in childhood and then discuss the signals and pathways that affect puberty. The recent trends in the timing of female puberty and the proposed factors involved will also be discussed. Finally, the chapter will discuss the genes that are necessary for puberty and reproduction.

Physiological Processes

The reproductive axis undergoes dramatic changes during fetal life, infancy, and childhood (FIG. 1). Because the hypothalamic-pituitary-gonadal (HPG) axis is active in the fetus and newborn and then enters a quiescent state during childhood, a discussion of the factors affecting puberty is best started with a review of the development of the neuroendocrine axis and the activity of the axis in early infancy. GnRH neurons are found in the olfactory pit at week 6 post conception. They then migrate via the forebrain, arriving at the hypothalamus by week 9.[1] Migration is supported by the protein anosmin, which is encoded by the Kal1 gene. The pituitary begins to secrete LH and FSH into the fetal circulation by week 12.[2] The activity of the axis continues to increase, and LH and FSH reach peak levels at midgestation, at about 20–24 weeks. Why the axis demonstrates this robust activity at midgestation is unclear; possibly the adult LH and FSH levels achieved contribute to normal ovarian development.[3] By late gestation, placental estrogen production provides negative feedback to the axis to cause LH and FSH levels to decrease.[2,4] At birth, LH and FSH levels are low, but begin to increase again upon withdrawal of

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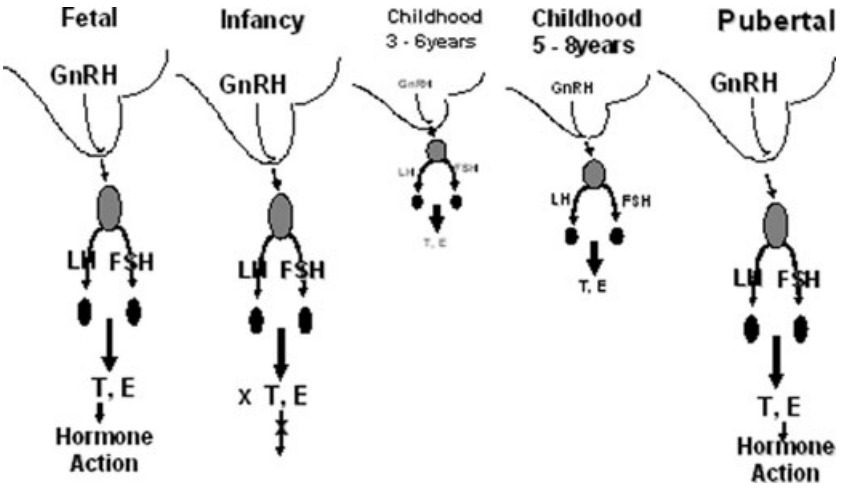

FIGURE 1. Schematic of the hypothalamic-pituitary-gonadal axis during developmental stages of childhood. The axis is active in fetal life and infancy, becoming quiescent at 1 year of age. In early childhood, the axis is minimally active, but becomes increasingly active in later childhood. Finally, at the time of puberty, the axis fully "reawakens" to mature to the activity present in adults.

placental estrogens. LH and FSH levels rise in the first few months of life, with FSH levels higher in female infants than in male infants. Negative steroid regulation of the axis is intact during this time, because agonadal infants exhibit LH and FSH levels similar to that of agonadal adults.[5,6] In girls, LH and FSH levels fall to nearly undetectable levels by the age of 2 years. This effect is independent of steroid feedback because agonadal toddlers also experience the decrease in gonadotropin levels.[5] This fall in gonadotropin levels is thought to be due to suppressive influences of the central nervous system that develop during the first years of life.

Although significantly suppressed in early and mid-childhood, the HPG axis has detectable activity. Multiple groups have shown that prepubertal children as young as 5 years of age exhibit infrequent, low-level LH and FSH peaks during sleep.[7–9] Using ultrasensitive immunofluorometric assays to measure LH and FSH, peaks have been detected in girls three years prior to the first physical sign of puberty.[9] FSH peaks are higher than LH peaks, and the gonadotropin peaks do not result in detectable steroid hormone levels. About one year prior to breast budding, prepubertal girls begin to experience higher peaks of LH exclusively during sleep. Brief nocturnal increases in estradiol, barely above the 2 pg/mL limits of the assay, are detectable. At the time that breast budding becomes detectable, LH peak amplitude increases about 10-fold, while FSH pulse amplitude doubles. These changes produce a smaller FSH/LH ratio.[7,9] The augmentation of LH pulse amplitude occurs because of increased pituitary responsiveness to GnRH. There is a priming effect of GnRH on LH release by the gonadotroph and an increase in the number of GnRH receptors on the gonadotroph. Pulse frequency increases to a lesser extent, and pulses become diurnal. Increases in estradiol also become more prolonged.[9]

Subsequent changes in hormonal patterns are most readily discussed with reference to standards developed by Tanner for staging female puberty on the basis of physical changes of the breast and the appearance of pubic hair.[10] Multiple groups have characterized the hormonal patterns present at each stage.[11,12] The pubertal progression from Tanner stage 2 to 3 is marked by further increases in LH pulse amplitude, 20–40-fold increases from levels detected prepubertally. Although the greatest pulse amplitudes are during sleep, significant detectable LH peaks are present during the day, and basal LH levels are detectable.[7] Estradiol levels increase, becoming detectable at all hours. Upon advancement to Tanner stage 4, the pattern of gonadotropin release is not appreciably different, but estadiol levels continue to increase.[11] This phenomenon suggests that ovarian responsiveness to gonadotropins has increased, possibly via gonadotropin-induced increases in enzymes necessary for estradiol production. Inhibin levels also rise during this time, possibly mediating some degree of negative feedback on the axis.[13] Menarche occurs near the end of stage 4 after a year-long rise in daily estradiol output.[14] High estradiol levels at this time can exert negative feedback on the

axis to suppress it, leading to cyclic estrogen levels and uterine bleeding.[12] At the time of menarche, positive feedback of estradiol on the axis is not established, and therefore, ovulation rarely occurs. Uterine bleeding occurs with varying cyclicity, until the mechanism of estrogen-induced LH surge is mature and ovulatory cycles occur. This process takes a variable amount of time in girls, often taking a year or more after menarche to become fully established.

Coincident with estradiol production by the gonad during puberty, the adrenal glands begin to secrete androgens that cause pubic and axillary hair development in girls. Pubic hair generally develops at breast Tanner stage 3 in females. This androgen output by the adrenal gland, so called adrenarche, is associated with the development of the zona reticularis of the adrenal gland. DHEA-S is the predominant ketosteroid produced by the mature adrenal gland. The factors causing development of the zona reticularis and subsequent adrenal gland steroid production are unknown. Although androgen production by the adrenal gland and estradiol production by the ovary coincide, they appear to be separate processes and are regulated independently. If timing of adrenarche varies from the norm, it can be indicative of pathologic adrenal androgen secretion.

Factors That Modulate the Onset of Puberty

The necessity of pulsatile GnRH in regulating the reproductive axis was established in the late 1970s. A study by Wildt and Knobil established the essential role of pulsatile GnRH in initiating puberty.[15] They induced puberty in a prepubertal monkey by administering GnRH in pulses. Puberty progressed and menarche occurred in these monkeys with continued administration of pulse GnRH. Research during the last 25 years has centered on what factors induce the "reawakening" of the GnRH neuron at puberty. TABLE 1 is a listing of the factors believed to be involved in the pubertal reawakening of the GnRH neuron. Inhibitory, stimulatory, and nutrition-dependent signals have been identified and the evidence behind the role of each signal in the onset of puberty is discussed below.

TABLE 1. Factors involved in induction of the GnRH pulse generator at puberty

Inhibitory Factors
Gamma-aminobutyric acid (GABA)
Stimulatory Factors
Kisspeptin
Glutamate
Norepinephrine
Growth factors

Inhibitory Signals

Gamma-Aminobutyric Acid (GABA)

GABA is made by specialized neurons of the hypothalamus and is a major inhibitory molecule of the hypothalamus. Studies have demonstrated that GABA release into the median eminence decreases at the onset of puberty in primates.[16] In the same study, release of GABA inhibition with $GABA_A$ receptor antagonists in prepubertal primates allowed for pulsatile release of GnRH into the pituitary portal system. Pulsatile infusion of the same GABA receptor antagonists to prepubertal monkeys causes precocious menarche.[17] Infusion of GABA after puberty has started is also effective in inhibiting GnRH release.[16] Evidence suggests that $GABA_A$ receptors undergo changes in subunit composition at the time of puberty, contributing to the release of inhibition of GnRH secretion by GABA at the time of puberty.[18] Whether GABA accounts for the inhibition of GnRH release in the neonatal period has not been studied in primates.

Neuropeptide Y

Neuropeptide Y (NPY) is an abundant hypothalamic peptide that controls food intake behavior and reproductive function in adults. Studies investigating the role of NPY in puberty have been conflicting; both inhibitory and stimulatory effects have been observed. Ventricular administration of NPY in agonadal male and female monkeys inhibits GnRH release postpubertally.[19] Ventricular infusion of NPY antagonist stimulated LH release and resulted in precocious puberty in juvenile monkeys.[20] NPY mRNA levels were found to be inversely related to GnRH release in infancy, prepubertally, and pubertally.[20] These studies suggest an inhibitory effect of NPY on the neuroendocrine reproductive function. Other laboratories found that NPY levels in the median eminence increase at the onset of puberty in monkeys,[21] and infusion of NPY into the median eminence increases GnRH release.[22] In addition, the infusion of NPY antiserum into the median eminence did not stimulate GnRH release in prepubertal monkeys,[21] suggesting that NPY does not exert an inhibitory effect on GnRH release. Differences in the infusion site of NPY and therefore site of action of NPY—ventricular versus median eminence—could account for the differences between the studies. More studies are needed to clarify the role of NPY in puberty.

Stimulatory Signals

Kisspeptin

In 2003, two groups independently reported that the neuropeptide kisspeptin and its receptor, GPR54, were essential for GnRH neuronal function.[23,24] Humans with mutations in the GPR54 gene that lead to loss of receptor function have hypothalamic hypogonadism, as do mice lacking the GPR54 gene. Thus GPR54 and its ligand, kisspeptin, moved to the forefront in neuroendocrine reproductive research as possible regulators of GnRH neuron function and a stimulatory pathway for puberty.

GPR54 receptor protein was found to co-localize specifically with GnRH neurons in rodents[25,26] and nonhuman primates,[27] and kisspeptin was found to be expressed in a subpopulation of neurons in the hypothalamus, with highest levels of expression at puberty. This supports the notion that kisspeptin acts on GnRH neurons via its receptor, GPR54 to affect their function. Subsequent studies found that infusion of kisspeptin intraventricularly resulted in a dramatic increase in GnRH neuron activation[25] and induced gonadotropin secretion in rodents.[26] Intravenous administration induced gonadotropin secretion in humans.[28] Administration of kisspeptin to prepubertal rats[29] and prepubertal monkeys[27] induced LH release into the serum, suggesting that kisspeptin is a stimulatory signal for puberty. Male and female GPR54 knockout mice do not enter puberty and are infertile, but a subset of female kisspeptin knockout mice enter puberty and experience estrous cycling.[30] This suggests that other unknown factors modulate puberty in these mice, possibly via the GPR54 receptor. Cumulatively, these studies indicate that the kisspeptin-GPR54 pathway is an important stimulator of GnRH neuron activity and puberty. Factors that affect the kisspeptin–GPR54 pathway have yet to be determined.

Glutamate

Glutamate is a major excitatory neurotransmitter in the hypothalamus. Glutamate stimulates GnRH release via *N*-methyl-D-aspartic acid (NMDA) receptors *in vivo* and *in vitro*.[31] Glutamate stimulates GnRH release, as inferred by LH release, in prepubertal monkeys[32] and rodents.[31] Stimulation of NMDA receptors leads to precocious puberty in rats[33] and monkeys.[34] In contrast, NMDA receptor blockers delay, but do not prevent the onset of puberty in rodents.[35] There is evidence that sensitivity to glutamate stimulation increases at puberty. Studies found that low and 10-fold higher doses of NMDA stimulate GnRH release in postpubertal monkeys, whereas only the 10-fold higher dose of NMDA stimulates GnRH release in prepubertal monkeys.[36] Thus, these results suggest that glutamate plays a role in the onset of puberty, but whether it is required for puberty induction is unknown.

Norepinephrine

Although norepinephrine is a strong inducer of GnRH release postpubertally,[37] it seems to have a facilitative, rather than primary role in puberty. When adrenal chromaffin cells releasing norephinephrine are transplanted into the third ventricle of macaques, puberty is not induced. However, monkeys with transplantation of chromaffin cells experienced menarche significantly earlier,[38] suggesting a role for norephinephrine in pubertal maturation, but not in pubertal onset.

Growth Factors

Astroglia adjacent to GnRH neurons can synthesize and release growth factors such as transforming growth factor-alpha and -beta (TGF-α and -β), fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1).[37] The hypothalamic expression of TGF and its receptor, erbB1, increases at the time of puberty.[39] Transgenic mice overexpressing TGF-α in the hypothalamus experience precocious puberty. Additionally, defective erbB1 signaling has been implicated in the pathogenesis of hypothalamic hamartomas.[40]

IGF-1 has also been found to play a role in puberty in rodents. Intraventricular infusion of IGF-1 induces puberty in rats,[41] while intraventricular infusion of IGF-1 antibody delays puberty.[42] IGF-1 has been shown by our laboratory to increase GnRH expression *in vitro*.[43] These data suggest that integration of growth, nutrition, and reproduction may occur at the level of the GnRH neuron. More studies are needed to elucidate the level of IGF-1 signaling in puberty.

Nutritional Factors

In 1970, Frisch hypothesized that a threshold weight and fat mass must be achieved before menarche can occur,[44] providing an explanation for the decrease in age of menarche over the previous century. Mechanistic pathways underlying this hypothesis have been debated. Many candidate metabolic signals have been studied for their role in the function of the reproductive axis and puberty. The physiological effect of nutritional extremes will be discussed as well as a few of the candidate signals.

The effect of low caloric intake on the neuroendocrine control of reproduction has been formally studied in human and nonhuman primates. Prolonged fasting with weight loss can lead to hypogonadotropic

hypogonadism in women.[45] Even a short-term, 72-hour fast leads to a 20% decrease in LH pulse frequency in women, with maintenance of LH pulse amplitude.[46] Of interest, men who undergo a shorter, 48-hour fast experience a 50% decrease in LH pulse frequency,[47] suggesting that women are protected from the neuroendocrine consequences of short-term caloric deprivation. The effect of fasting also occurs in the absence of sex steroids; in agonadal adult male monkeys, missing one meal results in loss of LH secretion.[48] In humans, children with chronic malnutrition and disease states associated with malabsorption experience delayed puberty.[49] The site of integration of metabolic signals to the reproductive axis is thought to be at the hypothalamus, as studies in nonprimates indicate that GnRH pulse frequency is severely dampened with food deprivation.[50]

Many different metabolic signals have been proposed to play a role in the nutritional regulation of reproduction and puberty. The discovery of leptin and its role as a signal of nutritional status to the hypothalamus sparked a flurry of studies investigating the effect of leptin on GnRH neuronal activity and puberty. Studies have yielded divergent results. Some studies found that leptin induced puberty in normal prepubertal mice,[51,52] whereas an equal number of studies did not find an effect of leptin on puberty in normal mice.[53,54] The differences in the studies may be due to different methodologies. However, leptin prevents a delay in puberty in malnourished rats.[54] The role of leptin in primate puberty is not as clear. Leptin concentrations do not change with puberty in monkeys.[55] In humans, leptin concentrations increase gradually during childhood, and continue to gradually rise during puberty,[56,57] and boys with constitutional delay can enter puberty without an increase in leptin.[58] Patients with lipoatrophic diabetes with resultant leptin deficiency experience normal timing of puberty.[59] Leptin has not been found to advance puberty in primates. These observations suggest that leptin plays a permissive, rather than primary role in onset of primate puberty.

Numerous other metabolic factors have been proposed to play a role in the nutritional regulation of reproduction in rodents and primates, but their role in puberty onset has not been specifically studied. These factors include insulin,[60,] ghrelin,[61] galanin-like peptide (GALP),[62] glucose, and free fatty acids.[37] Whether these factors interact with the stimulatory or inhibitory signals identified above has not been established. However, there is some evidence that nutritional status regulates kisspeptin because kisspeptin administration can induce an LH surge in both food-deprived mice[63] and streptozotocin-treated (insulin-deficient diabetic) mice.[64] Clearly, more studies are needed to determine the role of these hormones on the neuroendocrine regulation of puberty.

Puberty can only begin when signals from the central nervous system and signals from the periphery indicating systemic health and adequate nutritional status converge in the hypothalamus to stimulate GnRH pulsatile secretion. Although research of the last 25 years has yielded significant knowledge about the pathways involved, the interactions between the pathways have only just begun to be elucidated. The critical role of kisspeptin in puberty was just recently discovered, suggesting that more novel pathways are likely involved, thus increasing the complexity of the task at hand.

Timing of Pubertal Onset

Even though the tempo and series of events in puberty are remarkably conserved in different populations, the onset of puberty occurs across a wide range of ages in normal girls. Longitudinal studies performed by Tanner in the 1980s established the average age of puberty onset in North American girls, as indicated by breast budding, at 10.7 years with a standard deviation (SD) of 1 year.[65] The average age of menarche is 12.7 years with an SD of 1.3 years. Precocious puberty is then defined as breast budding earlier than 2.5 standard deviations from the mean, or in a child younger than 8 years of age, whereas delayed puberty is defined as no breast budding 2.5 SD later than the mean, or by 13 years of age.

The timing of puberty in the developing world was thought to be stable over the last 50 years, but a controversial study published in 1997 put that belief into question. Herman-Giddens *et al*. published a cross-sectional study of 17,000 girls in the Pediatric Research in Office Settings (PROS) network.[66] Upon visual inspection of girls seen for regular office visits, 6.5% of white girls and 27.2% of black girls had breast or pubic hair development before the age of 8 years. Therefore, application of the traditional definitions of precocious puberty would result in a high proportion of potentially normal girls undergoing extensive and expensive testing for precocious puberty. Although some in the field have supported this study resulting in revised guidelines,[67] others have cited the uncertainty of the conclusions because of concerns of ascertainment bias and the lack of palpation to verify the visual inspection.[68,69] Subsequent studies have shown ethnic and racial differences in pubertal development and the trend toward earlier pubertal development, though not as pronounced as in the Herman-Giddens study.[70,71] Concerns about the lack of palpation also exist with

these studies. In a European multicenter retrospective study, the revised guidelines were applied to 443 girls previously diagnosed with central precocious puberty (CPP). The authors determined that the application of the revised guidelines would miss 4 of the 35 girls who had cranial pathology.[72] These results have been interpreted in two ways. Some experts, citing the statistic that 12% of girls with a CNS abnormality associated with precocious puberty are over age 7, are cautious of the revised guidelines.[73] Other experts state that since that fewer than 2% (6/329) of girls over age 6 had an abnormal brain imaging study and thus only 1 in 50 brain imaging studies would be abnormal in these girls, the monetary and emotional cost of performing cranial imaging in all girls over age 6 with CPP is quite high; thus these investagators support the revised guidelines.[74] The clinician, then, must continue to weigh the risks and benefits of cranial imaging of 6–7-year-old girls with precocious puberty on a case-by-case basis. More, well-designed, large prospective studies are needed to confirm the observed trends and assess the rate of pathology among American girls aged 6–8 years to further guide the physician in clinical decision making.

If North American girls are entering puberty earlier, what would be the causal factors? Obesity is an obvious target, as the rate of obesity in children has dramatically increased since the late 1980s. In the Herman-Giddens study, girls with earlier onset of breast budding had higher BMI scores than age-matched girls without budding.[66] In another study, white girls with earlier onset of puberty had higher BMI scores than girls with normal onset of menarche.[75] The association was not significant for black girls, and thus other factors likely account for the higher prevalence of early puberty in African American girls. The molecular mechanism explaining these clinical observations has not been elucidated.

Genetics of Puberty and Pubertal Timing

Genes necessary for puberty and reproduction have been found by analysis of patients with isolated hypogonadotropic hypogonadism (IHH) and are summarized in TABLE 2. These mutations are rare, and are found in only about 30% of IHH patients, implying that many unidentified genes are necessary for proper hypothalamic-pituitary-gonadal function.[76] Mutations in KAL1, FGFR1, or GNRHR account for most of the known causes of IHH. There may be variable penetrance and marked phenotypic variance among affected family members; some individuals with these mutations have complete IHH, whereas while others have partial IHH or delayed puberty. Mutations in KAL1 and FGFR1 are associated with anosmia. These genes encode for proteins important for GnRH neuronal migration from the olfactory placode to the hypothalamus. KAL1 encodes anosmin-1, a neural cell adhesion molecule that has been proposed to provide a scaffold for GnRH and olfactory neuron migration. FGFR1 (KAL2) encodes the transmembrane fibroblast growth factor receptor-1 (FGFR1) that binds FGF1 and FGF2 with high affinity. FGFR1 is thought to interact with anosmin-1, as they are co-expressed at the same embryonic developmental stages and tissues.[77] The presence of a dominant negative mutant that blocks FGF receptor action has been shown to attenuate GnRH neurite outgrowth and cell proliferation *in vitro*.[78] Mutations in the gene GNRHR, which encodes for the GnRH receptor, are not associated with anosmia. Similar to observed differences in phenotype among affected family members, response to exogenous GnRH may also be variable.[79]

TABLE 2. Genes necessary for puberty and reproduction

Gene	Gene Product	Phenotype with Gene Disruption
Kal1	Anosmin-1	Anosmia, HH[a]
FGFR1	FGF Receptor	HH
LEP	Leptin	Obesity, delayed puberty
LEPR	Leptin receptor	Obesity, delayed puberty
GPR54	GPR54 receptor	HH
PCSK1	Prohormone convertase	HH, obesity, including proinsulin and corticotropin
GNRHR1	GnRH receptor	HH
NROB1	DAX-1	HH, AHC[b]
LHβ	LHβ subunit	HH
FSHβ	FSHβ subunit	HH

[a]Hypothalamic hypogonadism.
[b]Adrenal hypoplasia congenital.

Less common causes of IHH include mutations in the gene GPR54, which encodes for the transmembrane receptor GPR54. Mutations in the ligand for GPR54, kisspeptin, have not been found. Mutations in NROB1, which encodes for the transcription factor DAX1, cause adrenal hypoplasia congenita and HH. DAX1 is required for normal adrenal and gonadotrope development. Mutation in the gene PCSK1 causes childhood obesity, IHH, elevated pro-opiomelanocortin but hypocortisolism, and elevated proinsulin, but low insulin levels. PCSK1 encodes for prohormone convertase, and dysfunction of this

protein prevents normal production of selected hormones. It is unclear whether prohormone convertase is necessary for processing of a factor important in reproduction or whether the associated metabolic derangements contribute to the reproductive phenotype. Mutations in leptin or leptin receptor cause disruption in the hypothalamic regulation of satiety, leading to severe obesity and associated hyperinsulinemia. These patients also exhibit HH, but it is unclear whether the HH is primarily from disruption in leptin signaling or secondarily from the metabolic derangements.

Mutations in genes encoding transcription factors necessary for pituitary development are also important causes of HH. These genes include HESX1, PROP1, LHX3, and LHX4. Individuals with these mutations, however, also exhibit other features of hypopituitarism, including growth hormone deficiency, cortisol deficiency, secondary hypothyroidism, and prolactin deficiency.

Finally, individuals have been identified with mutations in the LHβ or FSHβ genes.[76] The patients identified with mutations in LHβ have been males with hypogonadism and undetectable LH but elevated FSH. One case of an LHβ mutation has been found in a woman with infertility and anovulation.[80] Mutations in FSHβ have been found in females with hypogonadism and low estrogen, low FSH, and elevated LH. Interestingly, males with this mutation do not have as dramatic a phenotype, but exhibit infertility.

Evidence suggests that IHH and constitutional delay of puberty lie on a spectrum of disorders characterized by delayed activation of the GnRH pulse generator. The phenotypic variability of persons with mutations in KAL1, FGFR1, and GNRHR1, varying from complete IHH to delayed puberty, suggests that environmental factors, gene-modulating factors, or gene dosage play a role in the manifestation of the disorder. In a recent study, males with complete IHH, including some with mutations in FGFR1 and GNRHR1, experienced sustained reversal of IHH after variable periods of androgen replacement.[81] This suggests that either sex steroids modulate the HPG axis to promote activation of the GnRH pulse generator, or that some patients diagnosed with IHH at the age of 18 actually have extreme constitutional delay of puberty. These individuals then may have disorders in as yet unidentified genes necessary for normal pubertal timing.

In efforts to identify the genes that play a role in the variation of pubertal timing and menarche across the population, genome-wide linkage scans and association analysis are beginning to be applied. Constitutional delay of puberty is generally inherited in an autosomal dominant manner, suggesting that single genes may be defective in these families.[82] In one study using sequence analysis and haplotype-based association of individuals with delayed puberty, genetic variation in GNRHR or GNRH1 was not associated with delayed puberty.[83] Another study using sequence analysis and disequilibrium analysis to investigate for genetic association, determined that leptin and leptin receptor gene polymorphisms do not account for delayed puberty.[84] Genome-wide linkage scans have been performed in large female populations to identify regions that may play a role in timing of menarche. One study of Caucasian women found loci on 22q13, 22q11, and 11q23 that were linked to age at menarche.[85] These same authors found an association between age at menarche and a single nucleotide polymorphism (SNP) in the IGF1 gene.[86] A study of European sisters used linkage analysis and found trait loci on chromosomes 1, 7, 8, 16, 19, and 20 associated with age at menarche.[87] Although in its early stages, genome-wide linkage analysis may be a powerful tool to identify genes important for puberty and normal pubertal timing.

The molecular mechanisms regulating initiation and timing of puberty are still largely unknown. In a recent anniversary issue of *Science* magazine, the editors labeled "What triggers puberty?" as one of the 100 most compelling questions facing science in the next century.[88] The answer will be ascertained only with step-wise advances in knowledge by scientists in the fields of physiology, biochemistry, molecular biology, population genetics, and clinical investigation. Clinical observation and characterization of patients with disorders of pubertal development has yielded information that has provided a new direction in the field of puberty research for basic scientists to explore. This underscores the importance of the bedside-to-bench approach of translational investigation in advancing our knowledge of this very critical process—a process that is necessary for the very propagation of our species.

Conflicts of Interest

The authors declare no conflicts of interest.

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