REVIEW

Genetic regulation in pubertal delay

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Abstract

Delayed puberty represents the clinical presentation of a final common pathway for many different pathological mechanisms. In the majority of patients presenting with significantly delayed puberty, there is a clear family history of delayed or disturbed puberty, and pubertal timing is known to be a trait with strong heritability. Thus, genetic factors clearly play a key role in determining the timing of puberty, and mutations in certain genes are recognised as responsible for delayed or absent puberty in a minority of patients. Through the identification of causal genetic defects such as these we have been able to learn a great deal about the pathogenesis of disrupted puberty and its genetic regulation. Firstly, deficiency in key genes that govern the development of the gonadotropin-releasing hormone system during fetal development may result in a spectrum of conditions ranging from isolated delayed puberty to absent puberty with anosmia. Secondly, a balance of inhibitory and excitatory signals, acting upstream of GnRH secretion, are vital for the correct timing of puberty. These act to repress the hypothalamic-pituitary-gonadal axis during mid-childhood and allow it to reactivate at puberty, and alterations in this equilibrium can cause delayed (or precocious) puberty. Thirdly, disturbances of energy metabolism inputs to the kisspeptin-GnRH system may also lead to late onset of puberty associated with changes in body mass.

Introduction

Delayed onset of puberty is one of the commonest conditions presenting to the paediatric endocrinologist, and in both genders is most frequently due to self-limited delayed puberty (Sedlmeyer & Palmert 2002, Abitbol et al. 2016). The term self-limited refers to the fact that these patients will have an onset of puberty before the age of 18 years, either spontaneously or induced with a short course of sex steroids. Moreover, in patients with this phenotype there are no recognised features of hypogonadism or infertility in adulthood. This isolated pubertal delay has historically been termed constitutional delay in growth and puberty, as it is often seen in young people who have had slow growth since early childhood. However, not all those presenting with delayed puberty will display these constitutional features and thus the name may not always be accurately applied to this condition.

Young people who remain prepubertal, at Tanner stage B1 in girls (absence of breast budding) or Tanner stage G1 in boys (with testicular volumes less than 4mL), at an age that is 2–2.5 standard deviations (s.d.) later than the population mean are classified as having delayed puberty (Palmert & Dunkel 2012). Those with delayed or faltering progression through puberty, identification of which can be aided by the use of puberty normograms, need to be carefully reviewed for isolated delayed puberty (Lawaetz et al. 2015). However, it is also important to consider other differential diagnoses including organic causes. Self-limited delayed puberty is currently a diagnosis of
of families with hypogonadotropic hypogonadism or Kallmann syndrome (Table 1). In these pedigrees, family members were seen with isolated delayed puberty, in some cases with the same genetic mutation as the proband with GnRH deficiency or resistance, and in others with less deleterious genetic changes (for example, a heterozygous change whilst the proband was homozygous for the same mutation). Such situations have been seen in families with mutations in the genes FGFR1, GNRHR and HS6ST1 (Lin et al. 2006, Pitteloud et al. 2006, Tornberg et al. 2011). In many of these genes the phenomenon of allelic heterogeneity is displayed, where different mutations in the same gene lead to varying phenotypes along a range of disease severity. For example, complete loss-of-function mutations in the GNRHR gene are associated with severe hypogonadism due to exonic nonsense changes or even imprinting defects such as maternal uniparental isodisomy (Cioppo et al. 2019), whereas mutations leading to partial deficiency show a wide spectrum of clinical manifestations including the ‘fertile eunuch’ phenotype, partial hypogonadotropic hypogonadism and isolated delayed puberty (Pitteloud et al. 2001, Chevrier et al. 2011, Vaaralahti et al. 2011).

Defects in genes further downstream in the hypothalamic–pituitary–gonadal axis may also result in a number of different phenotypes. Mutations in the genes encoding the luteinising hormone and follicle-stimulating hormone beta-subunits and their receptors present with a spectrum of disease severity including delayed puberty, primary amenorrhea, hermaphroditism, infertility and hypogonadism in males and females (Layman et al. 1997, Themmen & Huhtaniemi 2000, Narayan 2015, Potorac et al. 2016, Szymanska et al. 2018).

Some evidence for mutations in genes known to be associated with congenital hypogonadotropic hypogonadism causing isolated delayed puberty has been found by sequencing these GnRH deficiency genes in delayed puberty cohorts (Fig. 1). Potentially pathogenic variants in GNRHR, TAC3 and its receptor TACR3, IL17RD and SEMA3A have been identified by whole exome sequencing (Zhu et al. 2015). However, without in vitro or in vivo evidence of pathogenicity for these variants, it is difficult to know definitively if they are disease causing.

A study comparing the frequency of mutations in 24 GnRH deficiency genes between a cohort of probands with isolated delayed puberty and a cohort with congenital hypogonadotropic hypogonadism (CHH) found the latter to have a significantly higher proportion of mutations (7% of delayed puberty probands vs 51%
Table 1  Genetic causes of syndromic and non-syndromic delayed puberty.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-syndromic</td>
<td>HS6ST1 (Howard et al. 2018b), TAC3 (Zhu et al. 2015), TACR3 (Zhu et al. 2015), IL17RD (Zhu et al. 2015), GNRHR (Vaaraahti et al. 2011), SEMA3A (Zhu et al. 2015)</td>
</tr>
<tr>
<td>Self-limited delayed puberty or hypogonadotropic hypogonadism</td>
<td>IGSF10 (Howard et al. 2016)</td>
</tr>
<tr>
<td>Self-limited delayed puberty or hypothalamic amenorrhea</td>
<td>EAP1 (Mancini et al. 2019)</td>
</tr>
<tr>
<td>Self-limited delayed puberty with constitutional features</td>
<td>FTO (Howard et al. 2018a)</td>
</tr>
<tr>
<td>Syndromic</td>
<td>Prader–Willi syndrome, deletions within the paternally imprinted 15q 11.2-12 region (Emerick &amp; Vogt 2013)</td>
</tr>
<tr>
<td>Delayed puberty or hypogonadism with learning difficulties and obesity</td>
<td>Bardet-Biedl syndrome, BBS 1-11 (multiple loci) 20p12, 16q21, 15q22.3-23, 14q32.1 (Forsythe &amp; Beales 2013)</td>
</tr>
<tr>
<td></td>
<td>Borjeson–Forssman–Lehmann syndrome, PHF6 (Turner et al. 2004)</td>
</tr>
</tbody>
</table>

of CHH probands, $P=7.6 \times 10^{-11}$). There was also a higher proportion of oligogenicity in the cohort with hypogonadotropic hypogonadism (Cassatella et al. 2018). The low level of mutations in these genes in the delayed puberty group points to a genetic basis of isolated pubertal delay that is in large part different to that of GnRH deficiency disorders.

Over the last few years, two genes involved in the regulation of GnRH neuron development have been implicated in self-limited delayed puberty (Table 1). The first of these is IGSF10, a member of the immunoglobulin superfamily, identified by whole exome and targeted resequencing in a large Finnish delayed puberty familial cohort (Howard et al. 2016). The two pathogenic mutations, found in six unrelated families, affected the extracellular secretion of IGSF10. Whole gene knockdown disturbed the migration of immortalised GnRH neurons in vitro and disrupted in vivo development of the GnRH system in zebrafish embryos. This work proposed the concept that defects of GnRH neuronal migration during embryonic development could present with a phenotype of delayed puberty in adolescence, both without previous constitutional delay in growth, and with subsequent normal reproductive capacity (Fig. 2).

The hypothesis is that an insult to the developing GnRH neurosecretory network would lead to reduced numbers, or mis-timed arrival, of GnRH neurons at the hypothalamus. Thus, there would be a functional impairment of the hypothalamic neuroendocrine system with a higher ‘threshold’ for the onset of puberty, and a subsequent delayed pubertal timing. More recently
**Figure 3**
Overlap between genetic basis of delayed puberty, hypogonadotropic hypogonadism and hypothalamic amenorrhoea.

IGSF10 mutations have been identified in a separate study in a cohort with both premature ovarian insufficiency and neuronal development defects and also in a further proband with a hypogonadotropic hypogonadism-type phenotype (Jolly et al. 2019).

In the first IGSF10 study, mutations were additionally found in patients with adult-onset functional hypogonadotrophic hypogonadism. In terms of genetic regulation, a potential overlap between hypogonadotropic hypogonadism and other forms of functional hypogonadism such as hypothalamic amenorrhoea has already been identified (Caronia et al. 2011, Cangiano et al. 2019, Dwyer et al. 2019), and this IGSF10 work points also to a potential shared genetic basis of delayed puberty and hypothalamic amenorrhoea (Fig. 3).

The second gene identified, with a mutation in a large family from the same familial delayed puberty cohort, was the GnRH deficiency gene HS6ST1 (Howard et al. 2018b). The pathogenic mutation was found by next-generation sequencing to be carried by six family members from three generations. All had heterozygous carriage of this missense mutation and had typical features of isolated delayed puberty, with no short stature in early childhood or at adult height. Moreover, no family members had phenotypic features of hypogonadotropic hypogonadism. Mouse experiments demonstrated that heterozygous loss of Hs6st1 caused delayed puberty, with female mice having significantly late vaginal opening, a marker of pubertal onset in female rodents. Like the human patients, however, the mice showed no fertility defects, with normal testes size and development in males and normal litter size for both genders.

Interestingly, the mice with heterozygous Hs6st1 deficiency had a similar number of GnRH neurons in the adult hypothalamus as compared to WT mice. As Hs6st1 mRNA is highly expressed in both the arcuate nucleus and paraventricular nucleus, where KNDy (kisspeptin, neurokinin B and dynorphin) neurons and tanycytes are known to regulate GnRH secretion and function (Pielecka-Fortuna et al. 2008, Parkash et al. 2015), HS6ST1 may modulate GnRH neuronal activity or other relevant downstream pathways. Together the findings in these two genetic studies point to a potential fetal origin of isolated delayed puberty. Furthermore, they lend weight to the evidence for allelic heterogeneity of the hypothalamic-pituitary-gonadal axis; where mutation of single allele of a gene can cause self-limited delayed puberty, but more severe defects of the same gene, or in combination with additional gene defects, lead to GnRH deficiency phenotypes (Pitteloud et al. 2007).

**GWAS in pubertal timing**

Very large meta-analyses of GWAS of timing of age of menarche in over 370,000 women using directly sequenced and imputed genotype data have isolated 389 independent signals significant associated with age at puberty ($P < 5 \times 10^{-8}$) (Day et al. 2017). Each of these alleles has an effect size on timing of menarche of between 1 week and 5 months. In total the loci of the largest and most recent study explains ~7.4% of the variation in the timing of age of menarche in the general population. This corresponds to approximately 25% of the estimated heritability of pubertal timing (Day et al. 2017). These results point at a conclusion that the majority of these genetic variants in isolation have a low impact and that there is a large degree of heterogeneity in the genetic determinants of normal pubertal timing. The data are applicable to male puberty as well as to female, as many of the signals show a significant association with Tanner staging in both genders and have consistent effects on age at voice breaking. Of note, the signals identified have stronger effects on early than on late age of female puberty, whilst in male puberty, they have larger effect estimates for relatively late voice breaking (Day et al. 2017).

Interestingly, there is an overlap between those genetic loci identified by GWAS of timing of puberty and the genetic basis of hypogonadotropic hypogonadism and Kallmann syndrome, including the genes TACR3, LEPR and KISS1 (Day et al. 2017). Furthermore, there are also common genes found between these GWAS of age at menarche and voice break, and mutations discovered in pedigrees with precocious puberty, namely MKRN3 and DLK1 (Abreu et al. 2015, Simon et al. 2016, Dauber et al. 2017, Day et al. 2017). It, therefore, would appear plausible that genes, deficiency of which are responsible...
for isolated delayed puberty, may also have been pulled out via genome wide association techniques.

The gene FTO was the first and largest signal to be associated with obesity via GWAS, and has been also identified through GWAS of age at menarche (Yeo 2014). FTO was already known from animal models to regulate food intake and be an important component of the energy homeostasis pathways (McMurray et al. 2013, Merkestein et al. 2015). FTO expression is itself modulated by levels of essential amino acids, and FTO serves to regulate mTORC1 signalling based on these levels (Fischer et al. 2009, Speakman 2015). mTOR in turn acts to couple energy balance and the hypothalamic expression of Kiss1, thus modulating the hypothalamic–pituitary axis on the basis of its metabolic environment (Martinez de Morentin et al. 2014). This is evidenced in rodent studies by delayed vaginal opening, a proxy of the timing of puberty onset, secondary to blockade of mTOR.

The first human mutations in FTO associated with pubertal timing were identified recently in patients with self-limited delayed puberty associated with extreme low BMI and maturational delay in growth in early childhood (Howard et al. 2018a). In the same study, a murine model of heterozygous deficiency of FTO displayed significantly delayed puberty timing but without marked reduction in body mass. It is not known, however, if FTO exerts its effect on pubertal onset via its influence on body mass, or by modulation of mTOR signalling, or both.

In contrast, several fascinating candidate genes from the aforementioned GWAS have not, as yet, been found to be causal in human delayed puberty (Tomniska et al. 2010) or early puberty (Silveira-Neto et al. 2012). An example of this is the human gene LIN28B, an orthologue of a controller of developmental timing in the worm Caenorhabditis elegans, which was the first clear signal to be found via age of menarche GWAS (Ong et al. 2009). A SNP rs314276 from intron 2 of the LIN28B gene was associated with an earlier age of both breast bud formation and menarche. This family of proteins regulates, and is regulated by, a key family of miRNAs named let-7 (Liu et al. 2015, Corre et al. 2016). No mutations in LIN28B have as yet been found in patients with pubertal disorders.

**Pubertal timing and metabolic regulation**

There is strong evidence that timing of puberty is linked to energy balance and that higher BMI, particularly in girls, is associated with earlier puberty (He & Karlberg 2001, Wang 2002, Sorensen et al. 2010). This interplay between metabolism and the hypothalamic–pituitary–gonadal axis is thought to go some way to explain the secular trend toward an earlier age of pubertal onset that has been noted for some time in the developed world. One of the important signals of energy sufficiency is the metabolic hormone leptin, which is a permissive signal for puberty. Leptin is required for reproductive health both in humans and mice, where lack of the hormone or its receptor lead to pubertal failure and infertility (Farooqi 2002, Nunziata et al. 2019). Despite the observation that in females serum leptin concentrations rise during puberty (Ahmed et al. 1999), levels are lower in males and decrease during puberty (Garcia-Mayor et al. 1997), and it is not thought to be the key coordinator in the amplification of GnRH signalling pathways at pubertal onset.

Apart from mutations in FTO and in the leptin gene as discussed earlier, rare pathogenic variants in genes which regulate body mass have rarely been shown to lead to delayed puberty in humans. However, a substantial fraction of common variants affecting BMI also have significant associations in genome wide studies of timing of puberty (Day et al. 2015). There is recent evidence that α-MSH signalling may have a key role in the metabolic control of puberty. α-MSH acting on MC3/4 receptors increase Kiss1 expression and mediate the permissive effects of leptin on puberty (Manfredi-Lozano et al. 2016). Ghrelin and other gut-derived peptides may also contribute to the pathways by which energy homeostasis modulates the reproductive axis (Fernandez-Fernandez et al. 2006, Pomerants et al. 2006). A small number of patients with delayed puberty were found to have mutations in the ghrelin receptor or growth hormone secretagogue receptor (GHSR) (Pugliese-Pires et al. 2011).

Mutations in one of the newest GnRH deficiency genes KLB, which encodes beta-klotho and is part of the metabolic FGF21/KLB/FGFR1 pathway, have been demonstrated to cause hypogonadotropic hypogonadism in association with metabolic defects such as obesity and insulin resistance (Xu et al. 2017). In mouse models, both deficiency of Klb and overexpression of Fgf21 lead to delayed puberty and subfertility (Owen et al. 2013, Xu et al. 2017), reinforcing the importance of energy homeostasis for reproductive axis function. Interestingly, a small number of the relatives from these pedigrees carrying KLB mutations had a phenotype of isolated delayed puberty without CHH features.

**Regulatory control of the GnRH network**

After the fetal and postnatal (known as the mini-puberty) activity of the hypothalamic–pituitary–gonadal axis, there
is a long period of mid-childhood quiescence where the GnRH network is minimally active (Lanciotti et al. 2018). Around the time of puberty onset, central inhibition of this neuroendocrine system decreases, and there is a marked upregulation in the GnRH pulse generator activity. This change is brought about by a number of factors. Firstly, there are alterations in the balance of GABA-glutamate signalling in the brain (Bourguignon et al. 1997). Secondly, GnRH neurons undergo morphological changes with increased dendritic spine density and a simplification of their dendritic architecture. Thirdly, and perhaps most importantly, kisspeptin signalling, one of the key stimulatory input to GnRH activity, is intensified at this time. This is due (at least in mouse and primate models) to increased kisspeptin synthesis in KNDy neurons in the arcuate nucleus and to an increase in the GnRH neuronal responsiveness to kisspeptin stimulation (Plant & Barker-Gibb 2004).

However, mutations in Kiss1 and its receptor have not been commonly found to be responsible for disorders of pubertal timing (Chan et al. 2009, Silveira et al. 2010, Topaloglu et al. 2012). This would suggest that kisspeptin is not the ultimate controller of pubertal timing and that we instead need to search for what triggers this upregulation of kisspeptin biosynthesis in the hypothalamus at this critical time. Kisspeptin is influenced by and acts to coordinate a further cascade of upstream stimulators and repressors, the balance of which also contribute to the puberty brake and its release (Fig. 4) (Plant 2015). This includes a complex system of transcription factors which act as repressors and activators of Kiss1 and GnRH1 transcription, and which is in turn regulated by a number of different epigenetic mechanisms including DNA methylation, histone modification and non-coding RNAs (Kurian et al. 2010, Lomniczi et al. 2013, Messina et al. 2016, Toro et al. 2018).

Both systems biology approaches (Ojeda et al. 2010) and animal models (Plant 2015) have proposed several candidates for these upstream controllers, but there have been little data from human subjects. Potential key regulators include transcriptional repressors, particularly those containing zinc finger motifs, which act to regulate gene expression in mammals (Lomniczi et al. 2015, Gabriele et al. 2017). Two homeobox genes, Oct-2 and Ttf-1, have been shown to have increased expression in the early juvenile period in animal models (Lee et al. 2001). Oct-2 deficiency leads to delayed age at first ovulation in mice whilst overexpression induces precocious puberty (Ojeda et al. 1999). Ttf-1 acts to increase GnRH expression (Mastronardi et al. 2006).

Ttf-1 activates a further nuclear transcription factor, Eap1, and levels of Eap1 mRNA also increase in the hypothalamus of primates and rodents during puberty. EAP1 binding to the GnRH1 promoter increases in monkey hypothalamus at the onset of puberty (Mancini et al. 2019). Furthermore, knockdown of Eap1 with siRNAs causes delayed puberty and disrupted estrous cyclicity in rodents (Heger et al. 2007, Dissen et al. 2012, Lomniczi et al. 2012, Xu & Li 2016, Li & Li 2017). Eap1 has dual transcriptional activity: it trans-activates the...
GnRH promoter, leading directly to increased GnRH secretion, and inhibits the preproenkephalin promoter, thus preventing repression of GnRH secretion. This points to a multifaceted system where activators can act directly on GnRH transcriptional activity or via hypothalamic Kiss1 expression to promote the initiation of puberty (Li & Li 2017). Eap1 gene expression is itself regulated by repression by two further transcriptional regulators, Yy1 and Cux1 (Mueller et al. 2012).

EAPI is the first of these proposed upstream regulators in which mutations have been found in human subjects with pubertal disorders (Mancini et al. 2019). Deleterious mutations in EAPI carried by members of two families with classical clinical and biochemical features of self-limited delayed puberty were identified by whole exome sequencing. Both probands had late onset of puberty after 15.5 years with delayed peak height velocity, without syndromic features. Hypogonadotropic hypogonadism was excluded on the basis that the subjects had spontaneous pubertal development by the age of 18 years. The two mutations were both found at highly conserved residues and were translated into mutant proteins that had a reduced ability to trans-activate the GnRH promoter as compared to WT.

Regulation of the Kiss1 gene by the polycomb complex repressors EED and Cbx7 and the trithorax group of activators have been implicated as a further component of this system of transcriptional control (Fig. 4) (Lomniczi & Ojeda 2016). Overexpression of EED represses GnRH expression and compromises fertility in rodents, an effect that is mediated via kisspeptin. These are termed bivalent genes, as their expression is repressed at some points during development and upregulated at others. Prior to puberty onset there is increased methylation of the promoters of these polycomb genes, resulting in a reduction in expression, as well as a decrease in the binding of EED on the Kiss1 promoter. In contrast, binding of the trithorax group protein MLL3 to the Kiss1 enhancer upstream of the promoter increases at puberty and leads to the recruitment of other components of the trithorax group, allowing these enhancer sites to be modified (Toro et al. 2018).

Moreover, there is also reorganisation of the chromatin status and changes in histone methylation to accompany the loss of these polycomb complex proteins from the Kiss1 promoter (Lomniczi et al. 2013), mediated in part via SIRT1. Deacetylation of histones (via SIRT1) leads to decreased Kiss1 mRNA expression, and overexpression of SIRT1 leads to delayed puberty in a rodent model (Vazquez et al. 2018). Interestingly, nutritional status in the rodent model influences SIRT1 levels, and this may be a further mechanism by which metabolic inputs regulate pubertal timing. Studies on both rats and goats also provide data on changes in histone acetylation and gene methylation resulting in alterations in gene expression during puberty (Morrison et al. 2014, Yang et al. 2016).

Imprinting, non-coding RNAs and chromosomal anomalies

Imprinting is an important mechanism for the regulation of several key human developmental stages including weaning and adrenarche. In general, genes which are expressed from the paternal allele encourage later childhood maturation, whilst maternally expressed genes support earlier development (Peters 2014). The two principal parent-of-origin-specific signals that have been identified to date from GWAS of pubertal timing are both paternally inherited; however, maternally inherited polymorphisms may also exert effects on the timing of puberty (Day et al. 2017). The two paternally inherited genes identified via GWAS of timing of puberty, MKRN3 and DLK1, are both associated with central precocious puberty (Abreu et al. 2013, Dauber et al. 2017, Day et al. 2017). Notably, MKRN3 encodes a further zinc finger protein with E3 ubiquitin ligase activity (Jong et al. 1999, Simon et al. 2016). MKRN3 expression in the arcuate nucleus declines in mice at weaning and in humans serum concentrations fall at puberty onset, suggesting an inhibitory role of MKRN3 on the GnRH network during mid-childhood which is relaxed at puberty (Hagen et al. 2015, Busch et al. 2016). However, although clearly implicated in human precocious puberty, neither MKRN3 or DLK1 mutations have been found in patients with delayed pubertal timing.

Prader–Willi syndrome (PWS) is classically caused by a disorder of imprinting, and patients with this condition often have either absent or delayed puberty (Hirsch et al. 2015). PWS is most commonly due to deletion of a cluster of imprinted genes (including MKRN3) on the paternally inherited copy of chromosome 15 (paternal deletion) or maternal uniparental disomy where both copies of this cluster are inherited from the mother (Butler 2009). Precocious puberty is unusual in PWS (Lee & Hwang 2013), whereas most individuals show some pubertal disturbance ranging from poor growth in puberty, hypogonadotropic hypogonadism, descended testes, underdeveloped genitalia or primary amenorrhea (Crino et al. 2003). The lack of MKRN3 expression is superseded by the inactivation of other genes in this complex,
in particular MAGEL2, which act to delay pubertal timing (de Smith et al. 2009, Kanber et al. 2009). The clinical phenotype of pubertal abnormalities in PWS syndrome appears to be dependent on tissue-specific expression and timing of expression during development of these imprinted genes (Butler 2009, Peters 2014).

Non-coding RNAs also act as important epigenetic modulators of pubertal timing. Again, the evidence for this at present comes mostly from animal models, where particular miRNAs are important for upregulation of GnRH transcription during the murine equivalent of the human mini-puberty (Messina et al. 2016). The miRNAs (miR-200 and miR-155) appear to regulate Gnrh1 synthesis in GnRH neurons, through regulation of two transcriptional repressors, Zeb-1 and Cebpb. miR-200 and miR-155 activity lead to a reduction in Zeb-1 and Cebpb with a resultant increase in the transcriptional activation of GnRH1. There is also, as with the transcriptional regulators Eap1 and Ttf1, in-built regulation of Zeb1 by Cebpb (Messina et al. 2016). Another miRNA, miR-7a2, is vital for normal pituitary development in mice, with deficiency resulting in a phenotype of hypogonadotropic infertility (Ahmed et al. 2017). Evidence for the role of these non-coding RNAs in human puberty is still to be discovered.

The chromosomal anomalies associated with pubertal disorders are commonly recognised in Turner and Klinefelter syndrome, and a full discussion of the causes of primary hypogonadism is beyond the scope of this text. However, clinicians also need to be aware of more subtle chromosomal anomalies such as isochromosome Yp, i(Yp), detectable via array comparative genomic hybridisation, which has a clinical presentation very similar to self-limited delayed puberty with pubertal delay and slow growth (Gaudino et al. 2019).

Endocrine-disrupting chemicals and their influence on pubertal timing

There is increasing concern about the role of endocrine-disrupting chemicals (EDCs) in human pubertal timing (Mouritsen et al. 2010). These EDCs are found in a wide variety of commonly used products, including polybrominated biphenyls, glyphosate and bisphenol A, as well as in common medicines including paracetamol and betamethasone (Parent et al. 2015, Pinson et al. 2017, Drobeta et al. 2018, Milesi et al. 2018). There is evidence that EDC exposure in early or late childhood, or in utero, may influence the hypogonadal–pituitary–gonadal axis. Exposure to the oestrogenic insecticide DTT leads to premature onset of puberty in internationally adopted children (Krstevska-Konstantinova et al. 2001). Male infants exposed to phthalates during fetal development have increased rates of underdeveloped genitalia (Swan et al. 2005). In mouse models, there are data to support that EDCs given to pregnant mothers produce epigenetic changes in fetal testis as well as other systemic effects (Parent et al. 2015).

Evidence for EDCs affecting the central control of GnRH secretion is more limited, but female mice given arsenic in utero had altered hypothalamic expression not only of GnRH and LH but also of the upstream transcriptional regulators discussed earlier, such as Oct-2 and Ttf-1, with subsequent precocious puberty (Li et al. 2018). Whilst there is evidence that the human brain epigenome at puberty is affected by environmental disturbances (Morrison et al. 2014), in many cases, subjects are exposed to varying doses and combination of EDCs affecting oestrogenic, androgenic or other pathways, at different time points, so complicating the analysis of the effects of these chemicals (van den Driesche et al. 2015).

Importantly, most recently there is evidence from rodent models that the effects of EDCs may be heritable, as they are also present in the next two or more generations (Rissman & Adli 2014). In particular, studies with vinclozolin, a commonly used fungicide, in mice show that it produces transgenerational changes to the epigenome (Nilsson et al. 2018). Second-generation female mice had significantly delayed puberty, and third-generation males had increased rates of testis, prostate and kidney disease, as well as changes in the age of puberty onset.

Conclusions

The genetic control of human puberty comprises an intricate and multifaceted system with many interacting elements. A clear understanding of the genetic defects that result in abnormalities of pubertal timing, including delayed puberty, is hugely important to allow the optimal management of patients with these conditions, but also informs the understanding of the biology of normal pubertal timing. As we have seen from this overview, there is no one single gene that is capable of the hypothalamic control of puberty onset. Rather, a complex and multi-tiered balance of inhibitory and activating signals act together to apply the repressive control of the HPG axis seen in mid-childhood and to allow the brake to be released at puberty onset. Implicitly, the integrity of the GnRH neuronal network upon which this brake is
applied is also key for the correct timing of puberty, and gene defects resulting in erroneous fetal development of this neuroendocrine system can produce phenotypes from isolated delayed puberty to GnRH deficiency. Additionally, energy homeostasis has a vital influence on pubertal timing and deficiency of genes related to metabolic balance can lead to aberrations including delayed puberty. Moreover, gene–environment interactions mediated via epigenetic mechanisms also exert influence on the hypothalamic regulation of puberty, which may be heritable. The potential for translation of this knowledge has yet to be realised, but the hope is that it may aid rapid genetic diagnosis of different types of pubertal disorders, improve treatment options and inform the natural history of these conditions.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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