Genes Underlying Delayed Puberty

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Abstract

The genetic control of pubertal timing has been a field of active investigation for the last decade, but remains a fascinating and mysterious conundrum. Self-limited delayed puberty (DP), also known as constitutional delay of growth and puberty, represents the extreme end of normal pubertal timing, and is the commonest cause of DP in both boys and girls. Familial self-limited DP has a clear genetic basis. It is a highly heritable condition, which often segregates in an autosomal dominant pattern (with or without complete penetrance) in the majority of families. However, the underlying neuroendocrine pathophysiology and genetic regulation has been largely unknown. Very recently novel gene discoveries from next generation sequencing studies have provided insights into the genetic mutations that lead to familial DP. Further understanding has come from sequencing genes known to cause GnRH deficiency, next generation sequencing studies in patients with early puberty, and from large-scale genome wide association studies in the general population. Results of these studies suggest that the genetic basis of DP is likely to be highly heterogeneous. Abnormalities of GnRH neuronal development, function, and its downstream pathways, metabolic and energy homeostatic derangements, and transcriptional regulation of the hypothalamic-pituitary-gonadal axis may all lead to DP. This variety of different pathogenic mechanisms affecting the release of the puberty 'brake' may take place in several age windows between fetal life and puberty.

Highlights:

1. Pubertal timing is strongly determined by genetics, but also by factors such as BMI and psychosocial environment.
2. Self-limited delayed puberty (DP) is the commonest cause of DP in both sexes, but only a small number of genetic causes are known.
3. Other rarer genetic causes of DP include mutations in GnRH deficiency genes and primary hypogonadism.
4. Gene discovery in DP is expanding via next generation sequencing and genome wide association approaches.

Introduction

The development of the hypothalamic-pituitary-gonadal (HPG) axis is remarkable, with GnRH neurons originating in metazoan embryos outside of the central nervous system. These neurons then undergo a coordinated and timely migration alongside olfactory and terminal axons during fetal life. Immature GnRH precursor neurons are first detectable in the olfactory placode in the nose from an early embryological stage, and then begin a complex journey towards the hypothalamus [1, 2]. The HPG axis is measurably active in fetal and again in early infant life, during the so-called 'mini-puberty', but thereafter becomes largely dormant between the age of one and eight-to-nine years [3].

Development of the clinical features of puberty is initiated by the reactivation of the HPG axis after this relative quiescence during childhood. However, the nature of the puberty 'brake' that acts on the axis after the mini-puberty, and how this puberty brake is released – and the how the timing of release is controlled – to allow for puberty onset, is not well understood.

Despite the demonstrated importance of environmental factors such as body mass, psychosocial stressors and endocrine disrupting chemicals (EDCs) [4], genetic influence on the timing of puberty is clearly fundamental. Whilst the timing of pubertal onset varies within and between different populations, it is a highly heritable trait. Twin studies demonstrate that the timing of sexual maturation is highly correlated between highly related individuals, suggesting strong genetic determinants [5]. Previous studies estimate that 60-80% of the variation in pubertal onset is under genetic regulation [6, 7]. However, despite this strong heritability, the key genetic factors that determine human pubertal timing in the normal population and in cases of disturbed pubertal timing remain mostly unknown [8].

Causes of Delayed Puberty

The pathogenesis of delayed puberty (DP) encompasses several conditions, but is most commonly due to self-limited DP. There are two main groups of differential diagnosis of DP (Table 1): hypogonadotropic hypogonadism (HH) due to either functional or primary GnRH deficiency, and disorders causing

primary hypogonadism [8, 9], although up to 30 different aetiologies underlying DP have been identified [10].

Self-limited DP, also known as constitutional delay of growth and puberty (CDGP), represents the commonest cause of DP in both sexes. Up to 83% of boys and 30% of girls with pubertal delay have self-limited DP [9-12]. The underlying reasons for this gender difference are not clear. HH due to GnRH deficiency, such as in Kallmann syndrome (KS) and idiopathic HH, is also seen more commonly in men than in women. In contrast to DP and HH, precocious onset of puberty is approximately five times more common in girls than boys. The prevalence of central precocious puberty (CPP) in girls was found to be 0.2%, but only 0.05% in boys, over a nine year period in one European series [13]. However, an underlying abnormality is found far more commonly in girls with DP, and in boys with CPP, suggesting that many cases of male DP and female CPP may represent the end of the normal spectrum without underlying pathology.

There are fundamental differences between the two sexes in the dynamics of the reactivation of the gonadotropic axis. The biological reactivation of the gonadotropic axis occurs earlier in girls than boys. In boys, the secretion of testosterone increases shortly after the increase in the plasma concentration of luteinising hormone (LH) and follicular stimulating hormone (FSH). In girls, estradiol increases together with increasing LH and FSH. It is unknown how the genetic differences between the sexes contribute to this sexual dimorphism. There is some evidence that the female HPG axis may be more sensitive to environmental factors such as increased fat mass than the male, such as in conditions of functional hypogonadism due to weight loss or excessive exercise, where women tend to be affected more than men.

Individuals with DP have age of pubertal onset outside of the statistical definition of normal pubertal timing, with the absence of testicular enlargement in boys or breast development in girls at an age that is 2 to 2.5 standard deviations (SD) later than the population mean [8]. In addition, DP may also encompass older children with delayed pubertal progression, a diagnosis that is aided by the use of puberty normograms [12]. Self-limited DP may be associated with adverse health outcomes including short stature, reduced bone mineral density and compromised psychosocial health [14].

Differential diagnosis between self-limited DP and HH in adolescents who present with DP is often difficult at the time of referral, as both conditions may present with effectively the same clinical and hormonal features. Whilst a variety of clinical and biochemical investigations are available in such patients, none can reliably distinguish between those patients who will spontaneously enter, and progress in a normal manner, through puberty, and those who will require significant short or long term medical management. As the understanding of the genetic basis of both self-limited and other causes of DP improves, it is likely that genetic testing will be able to help establish a definitive diagnosis in such cases.

Investigating the Inheritance of Self-Limited Delayed Puberty

Self-limited DP segregates within families with complex patterns of inheritance including autosomal dominant, autosomal recessive, bilineal and X-linked [15], although sporadic cases are also observed. The majority of families display an autosomal dominant pattern of inheritance (with or without complete penetrance) (Figure 1) [5, 15]. 50 to 75% of subjects with self-limited DP have a family history of delayed pubertal onset [15].

Some insights into the genetic mutations that lead to familial self-limited DP have come from sequencing genes known to cause GnRH deficiency, most recently via next generation sequencing. Linkage analysis and targeted sequencing strategies that have provided initial insights in this field [16, 17] have been mostly superseded by whole exome and genome sequencing strategies to identify novel candidate genes. Other candidates have been identified from large-scale genome wide association studies in the general population.

Analysis of self-limited DP families is complicated by the fact that this phenotype represents the tail of a normally distributed trait within the population, so it is expected that variants that govern the inheritance of this condition may also be present in the general population at a low level. Thus, the absence of these variants in population databases cannot be used as an exclusion criterion during filtering of sequencing data. Instead, a comparison of prevalence of such variants must be made to identify those that are enriched in patients compared to the

154 general population. To date, in the majority of patients with DP the
155 neuroendocrine pathophysiology and its genetic regulation remain unclear.
156
157 Novel Genetics Discoveries in Self-Limited Delayed Puberty
158 Recently, whole exome and targeted resequencing methods have implicated two
159 pathogenic mutations in *Immunoglobulin superfamily member 10 (IGSF10)* as the
160 causal factor for late puberty in six unrelated families from a large Finnish cohort
161 with familial DP [18]. A further two rare variants of unknown significance were
162 identified in four additional families from the cohort. Mutations in *IGSF10* appear
163 to cause a dysregulation of GnRH neuronal migration during embryonic
164 development (Figure 2), which presents in adolescence as DP without previous
165 constitutional delay in growth. An intact GnRH neurosecretory network is
166 necessary for the correct temporal pacing of puberty. Pathogenic *IGSF10*
167 mutations leading to disrupted IGSF10 signalling potentially result in reduced
168 numbers or mis-timed arrival of GnRH neurons at the hypothalamus; producing
169 a functional defect in the GnRH neuroendocrine network. With this impaired
170 GnRH system there would follow an increased 'threshold' for the onset of
171 puberty, with an ensuing delay in pubertal timing. *IGSF10* loss-of-function
172 mutations were also discovered in patients with a hypothalamic amenorrhoea-
173 like phenotype. Although loss-of-function mutations in *IGSF10* were enriched in
174 patients with HH, these mutations did not alone appear sufficient to cause the
175 phenotype of full GnRH deficiency, in view of lack of complete segregation with
176 trait. These findings represent a new fetal origin of self-limited DP, and reveal a
177 potential shared pathophysiology between DP and other forms of functional
178 hypogonadism.
179 Loss-of-function mutations in a member of the immunoglobulin superfamily,
180 Immuno*globulin superfamily member 1 (IGSF1)*, have been identified in patients
181 with X-linked central hypothyroidism [19]. Notably, male patients with *IGSF1*
182 mutations have a late increase in testosterone levels with a delayed pubertal
183 growth spurt. However, pathogenic mutations in *IGSF1* have not been
184 conclusively found in patients with isolated DP [20].
185
186 Relevance of established GnRH deficiency genes to DP

187 At the extreme end of the spectrum of DP are conditions of GnRH deficiency
188 including congenital hypogonadotropic hypogonadism (CHH), with complete
189 failure to enter puberty. The condition may be due to failure of development of
190 GnRH neurons, lack of activation of GnRH secretion or disrupted GnRH signalling
191 (Figure 3). Because of different causes and incomplete penetrance, there is a
192 wide spectrum of phenotypes, ranging from complete CHH with lack of pubertal
193 development to a partial hypogonadism with an arrest of pubertal development,
194 and reversible CHH in up to 20% of patients post treatment [21-23]. Despite
195 recent advances, with over forty genes linked to this disorder identified, the
196 pathophysiological basis of CHH in approximately 50% of individuals remains
197 unclear (Figure 4) [3].
198 In view of the possible overlap between the pathophysiology of DP and
199 conditions of GnRH deficiency, a few studies have specifically examined the
200 contribution of mutations in CHH genes to the phenotype of self-limited DP.
201 Mutations in *Heparan Sulfate 6-O-Sulfotransferase 1 (HS6ST1)*, *Fibroblast Growth*
202 *Factor Receptor 1 (FGFR1)* and newly in *Klotho Beta (KLB)* have been found in a
203 small number of kindreds of CHH patients and their relatives with DP [24-26].
204 Variants in several HH genes including *Gonadotropin Releasing Hormone*
205 *Receptor (GNRHR)*, *Tachykinin 3 (TAC3)* and its receptor *(TACR3)*, *Interleukin 17*
206 *Receptor D (IL17RD)* and *Semaphorin 3A (SEMA3A)* have been identified by
207 whole exome sequencing in some cases of DP, including self-limited DP [27].
208 However, these variants have not been tested *in vitro* or *in vivo* for pathogenicity,
209 or investigated for segregation with trait within pedigrees, and thus may be an
210 over-estimation. Most recently, a comparative study of the frequency of
211 mutations in 24 GnRH deficiency genes between probands with congenital HH
212 and those with self-limited DP found a significantly higher proportion of
213 mutations in the HH group (51% of HH probands vs 7% of DP probands,
214 p=7.6x10^{-11}), with a higher proportion of oligogenicity in the HH group,
215 suggesting distinct genetic profiles in these two conditions (Cassatella et al, *EJE*
216 *2018, in press*). Mutations in KS genes such as *Anosmin 1 (ANOS1)* and *Nasal*
217 *Embryonic Luteinizing Hormone-Releasing Hormone Factor (NELF)* have not to
218 date been identified in pedigrees with DP. Overall, the current picture indicates

219 that the genetic background of HH and DP may be largely different, or shared by
220 as yet undiscovered genes [28].
221 Loss-of-function mutations within the GnRH receptor are the most frequent
222 cause of autosomal recessive CHH, accounting for 16% to 40% of patients.
223 Mutations have been found within the extracellular, transmembrane and
224 intracellular domains of the receptor leading to impaired GnRH action [29]. A
225 homozygous partial loss-of-function mutation in *GNRHR* was found in two
226 brothers, one with self-limited DP and one with idiopathic HH [30], and a further
227 heterozygous mutation found in one male with self-limited DP [28].
228
229 Genetic candidates for control of the pubertal 'brake'
230 Our understanding of the reactivation of the gonadotropic axis at the end of the
231 juvenile period, also seen as the release of the inhibitory 'brake' that has been
232 restraining the HPG axis in childhood, remains incomplete. Puberty is marked by
233 the change of the balance of GABA-glutamate signalling in the brain. This is
234 associated with a higher dendritic spine density and a simplification of the
235 dendritic architecture of GnRH neurons. GnRH neuronal activity is under the
236 control of several neurotransmitters and neuropeptides, and the onset of
237 puberty is triggered by a decline in these inhibitory signals and amplification of
238 the excitatory inputs, leading to increased frequency and amplitude of GnRH
239 pulses. However, the neuroendocrine mechanisms that act upstream to control
240 and coordinate this activity remains unknown.
241 Kisspeptin, an excitatory neuropeptide, was identified as a instructive factor in
242 puberty onset by the discovery of patients with GnRH deficiency with loss-of-
243 function mutations in the *Kisspeptin 1 receptor*, *KISS1R* (previously known as *G-*
244 *Protein Coupled Receptor 54, GPR54*) [31, 32]. Mice with knockout of *Kiss1r* were
245 simultaneously discovered to be infertile despite anatomically normal GnRH
246 neurons and normal hypothalamic GnRH levels [32], with a phenotype
247 consistent with normosmic GnRH deficiency. However, despite a large body of
248 evidence for kisspeptin as one of the most important elements of the neural
249 network responsible for GnRH pulse generation, only very rarely have human
250 mutations in *Kisspeptin 1 (KISS1)* been found in patients with delayed or absent
251 puberty [33]. Moreover, kisspeptin neurons in the arcuate nucleus have not been

252 demonstrated as controllers of the release of the puberty brake, but instead are
253 likely to act as a conduit for upstream regulators [34].
254 The excitatory neuropeptide, neurokinin b, also plays a role in the upstream
255 control of GnRH secretion. Identification of this pathway was also via discovery
256 of loss-of-function mutations in *TAC3*, encoding neurokinin b, and its receptor
257 *TACR3*, in patients with normosmic GnRH deficiency and pubertal failure [35].
258 Kisspeptin, neurokinin b and dynorphin are coexpressed in KNDy neurons of the
259 arcuate nucleus of the hypothalamus [36], which project to and directly interact
260 with GnRH neurons. Their expression is downregulated by oestrogen and
261 testosterone as part of the negative feedback regulation of gonadotropin
262 secretion [37, 38]. Despite this, administration of neurokinin b agonists failed to
263 stimulate GnRH release in rodents, and *Tacr3* knockout mice do achieve fertility
264 when mated [39, 40]. However, on closer phenotyping of *Tacr3* mice both males
265 and females demonstrate central reproductive defects with potential for reversal
266 of hypogonadism, highly reminiscent of the human phenotype [41]. With respect
267 to DP, in one study of 50 self-limited DP patients investigated for mutations in
268 *TAC3* and *TAC3R*, only one mutation in a single patient was found in the latter
269 gene [42].
270 The inhibitory role of GABAergic neurotransmission has been clearly shown in
271 primates [43] but is more ambiguous in rodents. Opioid peptides provide
272 additional inhibitory input but this appears to be less critical than the GABAergic
273 signals in restraining the initiation of puberty [44]. Additionally, *RFamide-related*
274 *peptide* (*RFRP*), the mammalian ortholog of the avian peptide *gonadotrophin-*
275 *inhibiting hormone* (*GnIH*), has been identified as a further inhibitory regulator of
276 GnRH neuronal activity in mice [45]. Glial inputs appear to be predominantly
277 facilitatory during puberty and consist of growth factors and small diffusible
278 molecules, including TGFβ1, IGF-1 and neuregulins, that directly or indirectly
279 stimulate GnRH secretion [46].
280 Upstream regulation of GnRH transcription is less well established. Candidate
281 transcriptional regulators identified from a systems biology approach and
282 animal models include *OCT-2*, *TTF*-1 and *EAP1* [47] (Figure 5). *Oct-2* mRNA is
283 upregulated in the hypothalamus in juvenile rodents, blockage of Oct-2 synthesis
284 delays age at first ovulation whilst activation of Oct-2 expression (e.g.

285 hamartomas) induces precocious puberty [48]. *Ttf-1* (thyroid transcription
286 factor-1) enhances GnRH expression, with increased expression in pubertal
287 rhesus monkeys [49]. *Eap1* mRNA levels also increase in the primate and rodent
288 hypothalamus during puberty. Eap1 transactivates the GnRH promoter, and
289 Eap1 knockdown with siRNA caused DP and disrupted estrous cyclicity in a
290 rodent model [50]. Recent data suggests Eap1 regulates GnRH expression
291 independent of Kiss1 signalling [51]. No published mutations in these upstream
292 or regulatory factors have been reported in patients with DP. However, our
293 group is completing functional annotation of two potentially pathogenic variants
294 in *EAP1* found in our cohort of Finnish patients with self-limited DP (manuscript
295 *in submission*).
296 Epigenetic regulators are potential modulators of pubertal timing. Recent
297 evidence highlights the importance in mice of microRNAs (particularly the miR-
298 200/429 family and miR-155) in the epigenetic up-regulation of GnRH
299 transcription during the critical period (murine comparator of the mini-puberty)
300 [52]. Moreover, miR-7a2, has been demonstrated to be essential for normal
301 pituitary development and HPG function, with deletion in mice leading to
302 hypogonadotropic infertility [53]. The effects of environmental changes on the
303 hypothalamic regulation of puberty may be mediated in part via epigenetic
304 mechanisms, and several studies have shown that the pubertal brain epigenome
305 is affected by environmental perturbations. Moreover, maternal exposure to
306 EDCs in rodents have been shown to cause epigenetic modifications in testis and
307 other systemic effects, and thus epigenetic changes during foetal life are also a
308 potential mechanism for the hypothalamic effects of EDCs in utero [54].
309
310 <u>Pituitary genes controlling puberty</u>
311 Downstream mutations in the GnRH signalling pathway can also present with
312 DP. LH and FSH are glycoprotein hormones encoded by a common α-subunit
313 gene and a specific β-subunit gene. Mutations of the β-subunits genes of LH or
314 FSH are extremely rare causes of pubertal abnormalities [55]. Males with
315 inactivating mutations of the *LHB* have absent pubertal development with Leydig
316 cell hypoplasia leading to T deficiency and azoospermia. Females with
317 inactivating mutations of *LHB* present with onset of normal puberty, but with

318 normal or late menarche followed by infertility due to lack of ovulation [55].
319 Individuals with inactivating *FSHB* mutations present with incomplete pubertal
320 development and azoospermia in males and primary amenorrhea in females
321 [56].
322 Genetic defects affecting the development of the anterior pituitary may cause
323 pubertal delay or failure. The pituitary transcription factors *LIM Homeobox 3*
324 *(LHX3)*, *SRY-Box 2 (SOX2)* and *HESX homeobox 1 (HESX1)* are vital for early
325 patterning of the forebrain and pituitary, and mutations in these developmental
326 genes result in syndromic hypopituitarism with gonadotropin deficiency in
327 humans [57]. *Paired like homeodomain factor 1 (PROP1)* is important for the
328 development of gonadotropin-secreting cells [58], and patients with *PROP1*
329 mutations have variable GnRH deficiency ranging from DP to CHH [57].
330 Mutations in *Orphan nuclear receptor Dax-1 (DAX1)* cause X-linked adrenal
331 hypoplasia congenita with associated HH, but have not been found in isolated DP
332 [59].
333 Gonadotropin deficiency may also be associated with other conditions,
334 particularly with neurological phenotypes. Mutations in *RNA polymerase III*
335 *subunit A and B (POLR3A/B)* result in the 4H syndrome (Hypomyelination,
336 Hypodontia and Hypogonadotropic Hypogonadism) [60] whilst those in *Ring*
337 *finger protein 216 (RNF216), OTU deubiquitinase 4 (OTUD4)* and *Patatin like*
338 *phospholipase domain containing 6 (PNPLA6)* produce the phenotypic
339 combination of HH and ataxia (also known as Gordon-Holmes syndrome) [61,
340 62]. *DMX like 2 (DMXL2)* mutations are associated with congenital HH, other
341 endocrine deficiencies and polyneuropathies [63]. Dysregulation of the RAB3
342 cycle, such as with mutations in *RAB3 GTPase activating protein catalytic subunit*
343 *1 (RAB3GAP1)*, lead to Warburg Micro syndrome with ocular,
344 neurodevelopmental and central reproductive defects [64, 65].
345
346 <u>Delayed puberty due to primary gonadal failure</u>
347 In gonadal dysgenesis in both males and females, delayed or absent pubertal
348 development may be the presenting complaint, although associated features
349 usually predominate. In Turner syndrome, the most common form of
350 hypergonadotropic hypogonadism in females, puberty is delayed and usually

351 followed by progressive ovarian failure [66]. Importantly, however, up to 30% of
352 girls will undergo spontaneous pubertal development and 2 to 5% will have
353 spontaneous menses [67]. About half of girls with Turner syndrome have the
354 45,X karyotype. Other causes of ovarian dysgenesis include X isochromosome,
355 where abnormal chromosome division results in duplication of identical
356 chromosome arms, most commonly of the long (q) arm. Various deletions and
357 duplications of the short and long arm of the X chromosome are also found in
358 women with primary ovarian insufficiency, with several genes implicated
359 including *Fragile X mental retardation 1 (FMR1), Premature ovarian failure 1B*
360 *(POF1B)*, *Diaphanous related formin 2 (DIAPH2)*, *Forkhead box L2 (FOXL2)* and
361 *Bone morphogenetic protein 15 (BMP15)* [68]. Point mutations in the extra-
362 cellular domain of the FSH receptor are mostly restricted to the Finnish
363 population and result in inactivation of the receptor function with primary or
364 secondary amenorrhea [69].
365 In males, testicular abnormalities are characterized by elevated gonadotropin
366 and low inhibin-B concentrations, and may present as pubertal delay. The
367 commonest condition underlying hypergonadotropic hypogonadism in males is
368 Klinefelter syndrome (47,XXY), with a prevalence of 1 in 667 live births. The
369 majority of those affected will enter puberty spontaneously at a normal age [70],
370 but testosterone levels become increasingly deficient by Tanner stages 4-5,
371 possibly as a result of secondary regression. DP may be seen in those with a
372 more complex karyotype (48,XXYY, 48,XXXY, 49,XXXXY).
373
374 <u>Genetics of pubertal timing in the general population</u>
375 Over the last decade there have been several large genome wide association
376 studies (GWAS) of age-at-menarche, examining pubertal timing in healthy
377 females, and more latterly also in males [71-73]. These studies have sought to
378 identify key genetic regulators of the timing of puberty in humans and have
379 demonstrated that there is significant genetic heterogeneity in pubertal timing in
380 the general population. These data suggest that the genetic architecture of the
381 timing of puberty in healthy subjects is likely to involve at least hundreds of
382 common variants. The first of many loci associated with age of menarche was the
383 gene *Lin-28 homolog B (LIN28B)* [74]. *LIN28B* is a human ortholog of the gene

384 that controls, through microRNAs, developmental timing in the Caenorhabditis
385 elegans. However, mutations in *LIN28B* have not to date been identified in
386 human patients with DP [75] or precocious puberty [76].
387 The largest study of this type comprises 1000 Genomes Project-imputed
388 genotype data in up to ~370,000 women, and identifies 389 independent signals
389 ($P < 5 \times 10^{-8}$) for age at menarche [77]. Per-allele effect sizes ranged from 1 week
390 to five months. These signals explain ~7.4% of the population variance in age at
391 menarche, corresponding to ~25% of the estimated heritability. Many of these
392 signals have concordant effects on the age at voice breaking, a corresponding
393 milestone in males. However, in women the signals identified had stronger
394 effects on early than on late age of menarche, but in contrast had larger effect
395 estimates for relatively late than relatively early voice breaking in males [77].
396 Around 250 genes were identified via coding variation or associated expression,
397 particularly those expressed in neural tissues. Importantly, genes already
398 implicated in rare disorders of puberty were identified, including *Leptin receptor*
399 *(LEPR)*, *Gonadotropin releasing hormone 1 (GNRH1)*, *KISS1* and *TACR3*, and
400 signals in or near several further genes with relevance to pituitary development
401 and function including *POU Class 1 Homeobox 1(POU1F1)*, *Teneurin*
402 *Transmembrane Protein 2 (TENM2)* and *Leucine Rich Repeat Containing G*
403 *Protein-Coupled Receptor 4 (LGR4).* Two imprinted genes were also reported:
404 *Makorin ring finger protein 3 (MKRN3)*, paternally-inherited mutations in which
405 have been identified as causal in pedigrees of central precocious puberty (CPP)
406 [78]; and *Delta Like Non-Canonical Notch Ligand 1 (DLK1)* [79]. *MKRN3* is
407 thought to contribute to the puberty 'brake' restraining the HPG axis via
408 inhibition of GnRH release. However, neither *MKRN3* nor *DLK1* mutations have
409 been described in the pathogenesis of DP (Figure 4).
410
411 <u>Metabolism and timing of puberty</u>
412 Nutritional changes play an important role in the observed secular trend
413 towards an earlier age of pubertal onset in the developed world [80], as shown
414 by the positive correlation between age at puberty onset and childhood body
415 size, particularly in girls [81]. In contrast, under-nutrition in females, for

416 example in chronic disease or anorexia nervosa, can result in delay in both the
417 onset and tempo of puberty [82].
418 This relationship between fat mass and pubertal timing is partly mediated
419 through the permissive actions of the metabolic hormone leptin, a key regulator
420 of body mass, produced from white adipose tissue [83]. Humans and mice
421 lacking leptin (Lep ob/ob) or the leptin receptor (LepR db/db) fail to complete
422 puberty and are infertile [84]. However, whilst self-limited DP in boys is
423 associated with hypoleptinaemia [85], there have been no identified association
424 of specific leptin or leptin receptor polymorphisms with DP [86]. GWAS studies
425 of pubertal timing found, in addition to leptin signalling, overlap with several
426 genes implicated in body mass index including *Fat mass and obesity-associated*
427 *protein (FTO)*, *SEC16 homolog B (SEC16B)*, *Transmembrane protein 18 (TMEM18)*,
428 and *Neuronal growth regulator 1 (NEGR1)* [77].
429 Very recently, rare heterozygous variants in *FTO* have been identified in
430 pedigrees with self-limited DP associated with extreme low BMI and
431 maturational delay in growth in early childhood [87]. Notably, mice that are
432 heterozygous for *FTO* gene knockout displayed significantly delayed timing of
433 puberty, without significant reduction in body mass. FTO is known to function as
434 a RNA demethylase linking amino acid availability, via mTOR, to appropriate
435 levels of growth and translation [88], although may also act in concert with other
436 genes in the nearby region to exert effects on body weight. There is evidence that
437 mTOR plays a central role in the coupling of energy balance and HPG axis
438 activation, via modulation of hypothalamic expression of Kiss1 [89, 90]. Blockade
439 of mTOR caused delayed vaginal opening in rodents with blunting of the positive
440 effects of leptin on puberty onset in food-restricted females. It remains to be
441 determined if the effect of *FTO* on pubertal timing in self-limited DP is mediated
442 via effects on body mass, via mTOR signaling, or both.
443 α-MSH signalling via MC3/4 receptors, acting to increase Kiss1 expression and
444 mediate the permissive effects of leptin on puberty, has also been implicated
445 recently as an important element in the metabolic control of puberty [91].
446 Ghrelin and other gut-derived peptides may also form part of the mechanism by
447 which energy homeostasis regulates reproductive development [92, 93]. A small
448 cohort of 31 patients was analysed for mutations in the ghrelin receptor *Growth*

449 *Hormone Secretagogue Receptor (GHSR)* and 5 patients were found to have point
450 mutations in this gene [94].
451 Children with CDGP have a dual phenotype of slow growth in childhood with DP.
452 In contrast, both low birth weight and prematurity are associated with earlier
453 onset of puberty [95], particularly in those children with rapid increase in length
454 or weight in the first two years of life [96]. It is not clear, however, if childhood
455 obesity, insulin resistance, excess androgens or underlying genetic or epigenetic
456 factors may explain this association [97].
457
458 Conclusions
459 The mystery of which are the key controllers of the duration of dormancy of the
460 HPG axis after the mini-puberty, and what triggers the release of this puberty
461 'brake', has yet to be answered. A wide variety of genetic and epigenetic defects
462 affecting different aspects of the HPG axis at different time periods in fetal and
463 postnatal life may result in delayed and disordered puberty. Whilst familial self-
464 limited in DP is a highly heritable trait with evidence for a genetic basis, the
465 majority of these genes remain unknown. Although our understanding of the
466 highly complex underlying biological network remains imperfect, results to date
467 demonstrate the importance of defects in GnRH neuronal development and
468 function, GnRH receptor and LH/FSH abnormalities, transcriptional regulation of
469 the HPG axis and metabolic and energy homeostasis derangements in the
470 pathogenesis of self-limited DP. This review serves to highlight the high degree
471 of heterogeneity in the genetic basis of self-limited DP.
472 Clinically it is important to distinguish between the conditions of DP and
473 idiopathic CHH in adolescents presenting with DP. However, this diagnosis is
474 often a difficult one as both disorders can present with a picture of functional
475 hypogonadism. There is still no definitive test to accurately discriminate
476 between the two diagnoses. More complex and involved management is required
477 in patients with CHH to achieve both development of secondary sexual
478 characteristics and to maximize the potential for fertility [98]. Genetic testing
479 may inform diagnosis of associated syndromic features, likelihood of reversal
480 and inheritance in family members. Rapid and efficient diagnosis of patients in
481 clinic would represent a huge leap forward in patient care and a likely significant

482 economic advantage. While presently next generation sequencing in individuals
483 presenting with delayed or incomplete pubertal development is only a
484 reasonable option in a research setting, future progress in gene discovery and
485 technical developments may facilitate the availability of genetic diagnosis as part
486 of clinical care for patients with both GnRH deficiency and self-limiting DP.
487

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991 Table 1: Differential Diagnoses of Self-Limited Delayed Puberty

	Hypergonadotropic Hypogonadism	Primary Hypogonadotropic Hypogonadism	Functional Hypogonadotropic Hypogonadism
Common Causes:	Klinefelter Syndrome Gonadal dysgenesis including Turner's syndrome Chemotherapy/ Radiation Therapy	Isolated Hypogonadotropic Hypogonadism Kallmann syndrome Combined Pituitary Hormone Deficiency Chemotherapy/ Radiation Therapy CNS Tumours/ Infiltrative Diseases	Inflammatory Bowel Disease Coeliac Disease Anorexia Nervosa Hypothyroidism Excessive Exercise

992 Table modified and reprinted with permission from Palmert MR, Dunkel L. Clinical
993 practice. Delayed puberty. N Engl J Med 2012;366:443-53. [99]

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997 Figures

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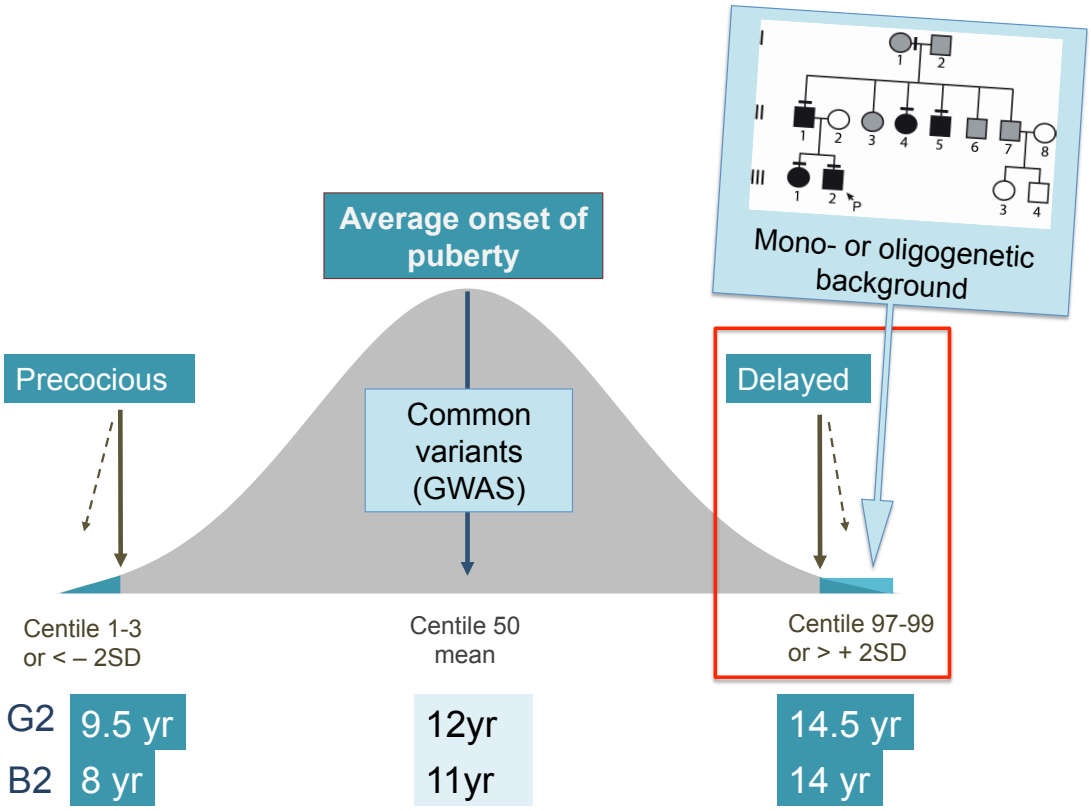

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1000 **Figure 1 – The Genetics of Pubertal Timing.** In the general population there is
1001 a near-normal distribution of the timing of pubertal onset, with the definitions of
1002 precocious and delayed being statistically determined (+/- 2 standard
1003 deviations, SD). Cut-off ages for Tanner genital stage G2 (boys) and B2 (girls)
1004 defining precocious and delayed puberty are given (thick black lines represent
1005 3rd and 97th centiles and dotted lines represent 1st and 99th centiles). Strategies
1006 to determine key genetic determinants in the timing of puberty include large
1007 genome wide association studies (GWAS) of age-at-menarche and voice breaking
1008 in the general population (common variants box), and identification of rare high-
1009 impact variants causing early, late or absent puberty in patients and their
1010 families. Patients with familial self-limited DP often display an autosomal
1011 dominant mode of inheritance, likely with a mono- or oligogenetic basis.

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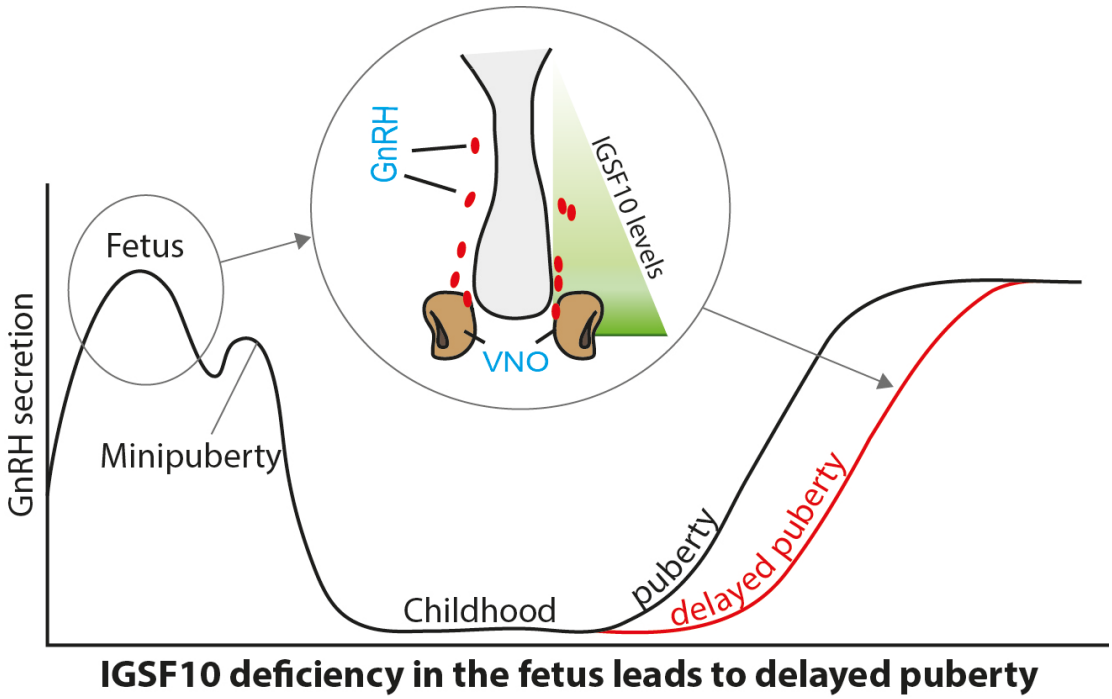

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1020 **Figure 2 – Schematic of the mechanism by which *IGSF10* mutations lead to**
1021 **DP.** Reduced levels of *IGSF10* expression during embryogenesis (represented by
1022 green triangle) in the corridor of nasal mesenchyme from the vomeronasal organ
1023 (VNO) to the olfactory bulbs (in a murine model) result in delayed migration of
1024 GnRH neurons (represented by red ovals) to the hypothalamus. This presents for
1025 the first time in adolescence as a phenotype of DP due to abnormalities of the
1026 GnRH neuronal network (grey arrows linking fetal pathogenesis to adolescent
1027 phenotype).
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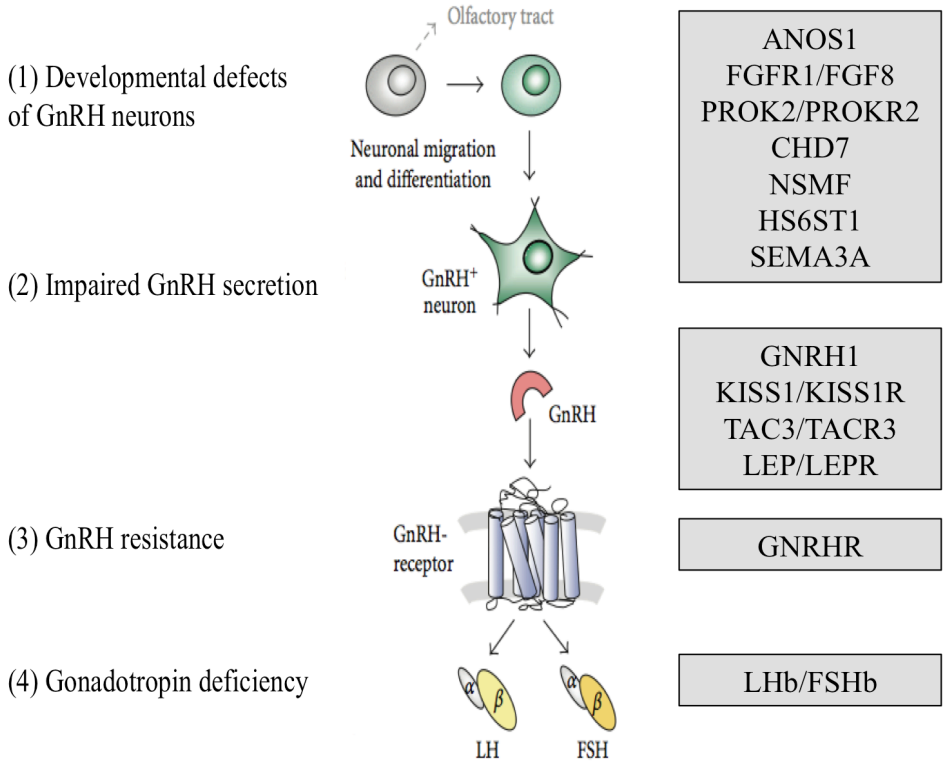
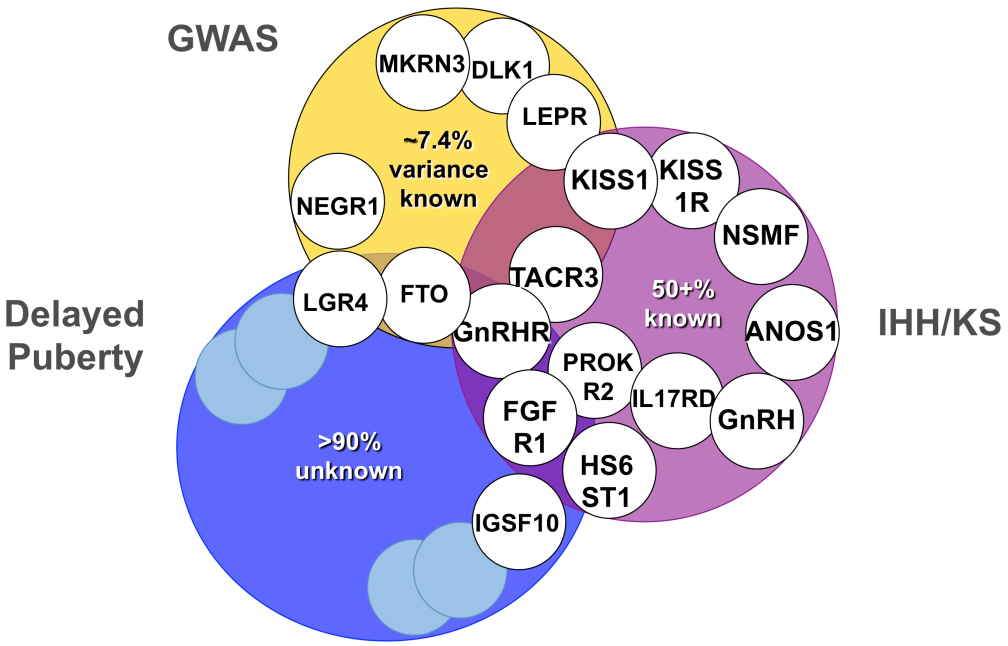


Figure 3 – Mutations in single genes at many levels of the HPG axis can cause hypogonadotropic hypogonadism (adapted from [3])



1037 **Figure 4 – Overlap between genetic regulation in the general population**
1038 **and extreme phenotypes.**
1039 Examples of genes implicated in timing of puberty from genome wide association
1040 studies in the general population (GWAS), conditions of GnRH deficiency such as
1041 idiopathic hypogonadotropic hypogonadism (IHH) and Kallmann Syndrome
1042 (KS), and self-limited delayed puberty. Pale blue unfilled circles represent as yet
1043 undiscovered genes.
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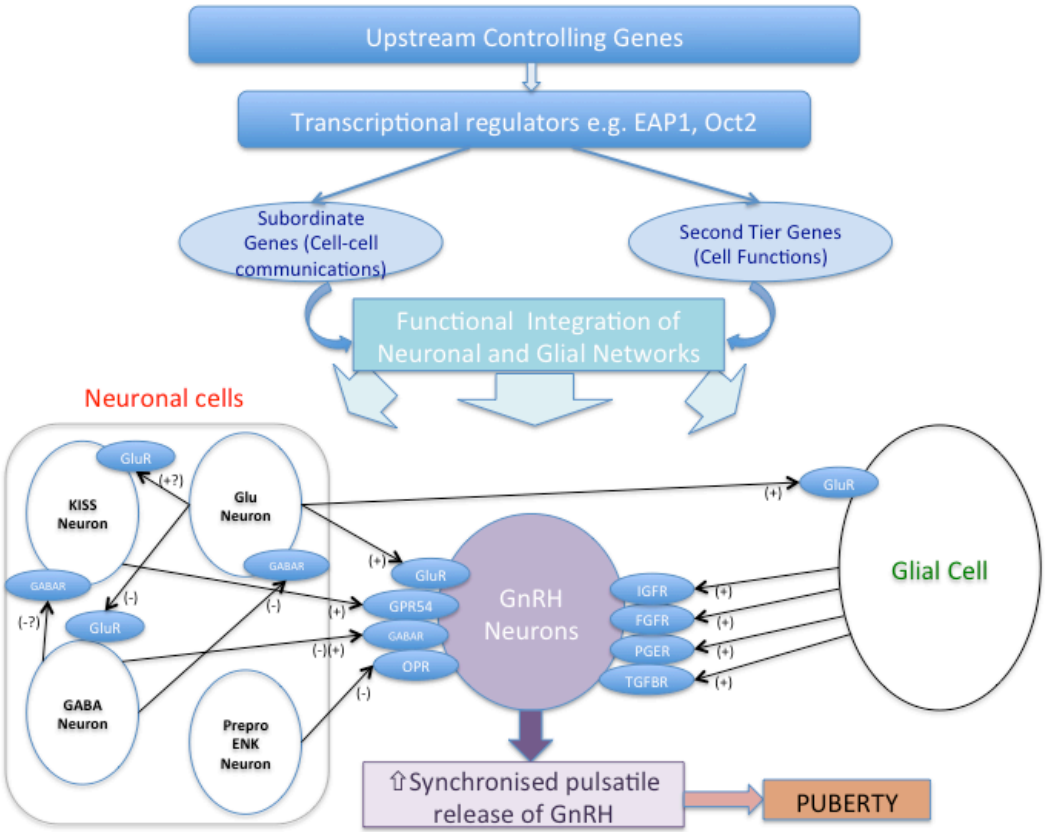

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1049 **Figure 5 - Genetic regulators in the trans-synaptic and glial control of GnRH**
1050 **neurons during puberty, adapted from [44]**
1051 This schematic represents a model whereby key transcriptional regulators
1052 govern a plethora of other genes (termed "subordinate genes" and "second tier
1053 genes", controlling cell-cell communications and cell functions respectively). This
1054 hierarchy, itself controlled by as yet unknown upstream controlling genes,
1055 integrates the neuronal and glial networks influencing GnRH neuronal function.

1056 Inhibitory inputs are primarily from GABAergic (GABA Neuron) and opiatergic
1057 neurons (preproenkephalinergic neurons, Prepro ENK), whilst glutamate (Glu
1058 neurons) and kisspeptin (KISS Neuron) are the central excitatory neuronal
1059 signals. Glial cell inputs are primarily facilitatory.
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