# Behind the scenes: epigenetic mechanisms rule the roost in pubertal timing

In humans, following a mysterious quiescence during childhood, the reactivation of pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus marks the onset of puberty.[1] The precise molecular triggers that govern this dynamic developmental transition remain elusive. Early reactivation of pulsatile GnRH secretion presents clinically as central precocious puberty. Over the past two decades, genetic studies in central precocious puberty have begun to shed light into the crucial molecular determinants that reactivate pulsatile GnRH secretion in humans.[2] However, the full genetic architecture of central precocious puberty and full ensemble of genes contributing to the cause of central precocious puberty remain unclear, especially in sporadic central precocious puberty presentations. Hence, genetic investigation of central precocious puberty provides a unique opportunity to unravel the molecular drivers governing the initiation of puberty.

Altered pubertal timing, both early and late puberty, is often recognised as a constituent phenotype in patients presenting with multisystem syndromic developmental disorders with varied genetic causes.[2] Rett syndrome is a severe progressive neurodevelopmental disorder, characterised by rapid regression in language and motor skills following a period of typical development.[3] Repetitive stereotypical movement (hand-wringing) replaces purposeful hand movements and in addition to these motor symptoms, patients have a complex phenotype with multiple comorbidities. In *The Lancet Diabetes & Endocrinology*, Ana P M Canton and colleagues[4] capitalise on previous phenotypic observations that pubertal timing is accelerated in patients with Rett syndrome.[5] Because 90% of reported cases of Rett syndrome result from mutations in the X-linked *MECP2* gene (encoding methyl-CpG-binding protein 2),[6] Canton and colleagues[4] astutely hypothesised that *MECP2* variants might also be causal for idiopathic central precocious puberty. Through contemporary genetic approaches within a multiethnic central precocious puberty cohort of more than 400 individuals with central precocious puberty, the authors show significant enrichment for rare *MECP2* variants exclusively in girls with central precocious puberty, thus defining a novel X-linked form of central precocious puberty. A total of four heterozygous variants were identified in seven girls including two missense variants in three girls (all occurring de novo), two nine base pair insertion variants (one inherited from unaffected mother, inheritance in other not known) and one non-coding 3′ untranslated region variant in two girls (one de novo and one inherited from her unaffected mother).[4] Although some patients had mild neurodevelopmental features, none of the girls had syndromic features of Rett syndrome, attesting that these alleles impart an attenuated puberty-restricted phenotype, suggesting that central precocious puberty represents a distinct MECP2-related allelic disease. In keeping with this observation, only one of the reported alleles in this current study has been previously reported in the Rett database suggesting that alleles associated with central precocious puberty are mostly distinct to those causing severe Rett syndrome. To establish the relevance of MECP2 in pubertal regulation, the authors then examined hypothalamic tissue sections of pubertal female mice and showed robust staining for Mecp2 in multiple hypothalamic nuclei relevant to GnRH biology (arcuate, suprachiasmatic, and paraventricular nuclei, and in the median eminence) with more than 70% of GnRH neuronal cells shown to be coexpressing *Mecp2*. Thus, identification of multiple de novo variants in unrelated individuals within a well phenotyped central precocious puberty cohort along with relevant tissue expression data firmly establishes a novel role for the *MECP2* gene in hypothalamic GnRH regulation and etiopathogenesis of central precocious puberty.

Although *MECP2* variants only contribute to a small number of central precocious puberty cases, the findings from this study are important. The increasing accessibility and affordability of genetic screening will enable precise genetic diagnoses and appropriate counseling for central precocious puberty families who carry *MECP2* variants. Although initial studies postulated that neuroendocrine circuitry involving hypothalamic kisspeptin signalling might represent primary genetic drivers for central precocious puberty, variants in *KISS1* and *KISS1R* genes (encoding kisspeptin and its cognate receptor) are

Andrii Zastrozhnov/Getty Images

*Lancet Diabetes Endocrinol* 2023
Published **Online**
June 26, 2023
https://doi.org/10.1016/S2213-8587(23)00167-5

See **Online/Articles**
https://doi.org/10.1016/S2213-8587(23)00131-6

For the **Rett database** see
http://mecp2.chw.edu.au/

now shown to be extremely rare in central precocious puberty.[5] By contrast, loss-of-function mutations in *MKRN3* (encoding Makorin Ring Finger Protein 3, a ubiquitin ligase), a maternally imprinted gene located in the Prader-Willis syndrome locus (chromosome 15q11.2), accounts for a prevalence of about 9% in central precocious puberty and up to 19% in familial central precocious puberty.[3] Studies have revealed that MKRN3 ubiquitination regulates the transcription of *GNRH1* gene by disrupting another epigenetic reader protein: methyl-CpG binding domain family protein 3 (MBD3).[7] Additionally, loss-of-function mutation of *DLK1*, another maternally imprinted gene, encoding delta-like homologue 1, a non-canonical ligand of the Delta–Notch signalling pathway, has also been implicated in central precocious puberty.[8] The global methylation profile of girls with central precocious puberty has been shown to display a pattern of hypermethylation during the pubertal period compared with the prepubertal period.[9] Finally, two crucial microRNAs (miR-200 and miR-155) have been shown to act as key switch that regulates the increase in hypothalamic GnRH production before puberty.[10] Stitching these observations together, the aggregation of causal central precocious puberty genes within imprinted regions, importance of DNA methylation and microRNAs during pubertal transition, and Canton and colleagues finding regarding *MECP2*, a key epigenetic machinery gene in central precocious puberty, strongly places epigenetic regulation at the helm of affairs as being the primary switch that orchestrates the reactivation of GnRH secretion at puberty.

The findings also raise additional questions worthy of deeper study. What are the key MECP2 target genes and how do they intersect with the excitatory or inhibitory signals that regulate hypothalamic neuroendocrine activity? Cellular consequences of Rett-related mutations versus those detected in central precocious puberty must be studied to discern the differential biologic effects that could shed light on potential genotype–phenotype correlations. The authors in this study expanded their search beyond the central precocious puberty cohort and examined the prevalence and pubertal phenotypes of central precocious puberty-related MECP2 variants within the UK Biobank but did not find any significant association. There are no signals relating to MECP2 gene locus in published genome-wide association studies relating to human pubertal timing. Thus, it remains to be seen if MECP2 or its targets are also a determinant of pubertal timing within the general population.

I declare no competing interests.

*Ravikumar Balasubramanian*
**rbalasubramanian@mgh.harvard.edu**

The Harvard Massachusetts General Hospital Center for Reproductive Medicine and Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114, USA

1 Balasubramanian R, Crowley WF Jr. Isolated GnRH deficiency: a disease model serving as a unique prism into the systems biology of the GnRH neuronal network. *Mol Cell Endocrinol* 2011; **346:** 4–12.
2 Argente J, Dunkel L, Kaiser UB, et al. Molecular basis of normal and pathological puberty: from basic mechanisms to clinical implications. *Lancet Diabetes Endocrinol* 2023; **11:** 203–16.
3 Leonard H, Cobb S, Downs J. Clinical and biological progress over 50 years in Rett syndrome. *Nat Rev Neurol* 2017; **13:** 37–51.
4 Canton APM, Tinano FR, Guasti L, et al. Rare variants in the *MECP2* gene in girls with central precocious puberty: a translational cohort study. *Lancet Diabetes Endocrinol* 2023; published online June 26. https://doi.org/10.1016/S2213-8587(23)00131-6.
5 Killian JT, Lane JB, Cutter GR, et al. Pubertal development in Rett syndrome deviates from typical females. *Pediatr Neurol* 2014; **51:** 769–75.
6 Good KV, Vincent JB, Ausió J. MeCP2: The genetic driver of Rett syndrome epigenetics. *Front Genet* 2021; **12:** 620859.
7 Li C, Lu W, Yang L, et al. MKRN3 regulates the epigenetic switch of mammalian puberty via ubiquitination of MBD3. *Natl Sci Rev* 2020; **7:** 671–85.
8 Macedo DB, Kaiser UB. DLK1, notch signaling and the timing of puberty. *Semin Reprod Med* 2019; **37:** 174–81.
9 Bessa DS, Maschietto M, Aylwin CF, et al. Methylome profiling of healthy and central precocious puberty girls. *Clin Epigenetics* 2018; **10:** 146.
10 Messina A, Langlet F, Chachlaki K, et al. A microRNA switch regulates the rise in hypothalamic GnRH production before puberty. *Nat Neurosci* 2016; **19:** 835–44.