

Investigating the role of Lin28/Let7 pathway and RF-amide-related peptides in precocious puberty: therapeutic insights from traditional Chinese medicine

Yuanyuan He^{1*}, Amin Tamadon^{2,3,4}, Nadiar M. Mussin⁵

¹National Children's Medical Center & Children's Hospital of Fudan University, Shanghai 201102, China

²Department of Natural Sciences, West Kazakhstan Marat Ospanov Medical University, Aktobe, Kazakhstan

³Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

⁴PerciaVista R&D Co., Shiraz, Iran

⁵Department of General Surgery, West Kazakhstan Medical University, Aktobe, Kazakhstan

Received: 02/03/2023 Accepted: 21/05/2023 Published: 15/06/2023

Abstract

Precocious puberty, defined as the onset of secondary sexual characteristics before age 8 in girls and 9 in boys, poses significant physical and psychological challenges for affected children. This mini-review investigates the role of the Lin28/Let7 pathway and RF-amide-related peptides (RFRPs) in the regulation of puberty, focusing on their implications for precocious puberty. Recent studies highlight the Lin28/Let7 pathway as a critical regulator of gonadotropin-releasing hormone (GnRH) secretion, with disruptions linked to early pubertal onset. Moreover, RFRPs function as inhibitors of GnRH, acting to delay the activation of the hypothalamic-pituitary-gonadal axis. Importantly, traditional Chinese medicine (TCM) has emerged as a potential therapeutic intervention, demonstrating efficacy in modulating these pathways and delaying precocious puberty in animal models. This review synthesizes current findings to enhance understanding of the biological mechanisms underlying precocious puberty and proposes potential therapeutic strategies informed by TCM and molecular insights.

Keywords: Precocious Puberty; Lin28; Let-7 MicroRNAs; RF-Amide-Related Peptides; Traditional Chinese Medicine

1. Introduction

Precocious puberty is defined as the unusually early onset of puberty, typically occurring before the age of 8 in girls and 9 in boys (1). This condition leads to early physical changes such as breast development, pubic hair growth, and accelerated skeletal maturation, often causing short stature in adulthood due to early closure of the growth plates (2). The early onset of puberty can also have psychological and emotional implications, particularly in young children, who may experience challenges in social interactions, self-esteem, and mental health due to their premature physical development (3).

The increasing incidence of precocious puberty in recent decades has raised concerns about its underlying causes and long-term consequences (4). Several factors, including genetic predisposition, environmental exposures (such as endocrine-disrupting chemicals), and obesity, have been implicated in its onset (5). However, despite the known contributing factors, the precise biological mechanisms that trigger early puberty remain incompletely understood (6). In particular, disruptions in the regulatory pathways controlling the onset of puberty

are thought to play a critical role (6).

The hypothalamic-pituitary-gonadal (HPG) axis is the primary neuroendocrine system governing the initiation of puberty (7). Activation of this axis leads to the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which subsequently stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland, resulting in the production of sex hormones (8). In normal puberty, this process is tightly regulated by several genetic and hormonal factors that delay the activation of the HPG axis until the appropriate time (9). However, in cases of precocious puberty, this regulatory balance is disrupted, leading to early activation of the axis and premature sexual development (10).

Recent advances in molecular and genetic research have shed light on two key regulatory pathways involved in puberty include the Lin28/Let7 pathway (11) and RF-amide-related peptides (RFRPs) (12). These pathways have emerged as critical regulators of GnRH secretion and pubertal timing, offering new insights into the molecular control of puberty and potential therapeutic

*Corresponding author: Yuanyuan He, National Children's Medical Center & Children's Hospital of Fudan University, Shanghai 201102, China. Email: yuanyuan_he@qq.com

targets for managing precocious puberty.

The Lin28/Let7 pathway is known for its role in stem cell biology, metabolism, and the timing of developmental processes (13). In the context of puberty, Lin28 inhibits the Let7 family of microRNAs (miRNAs), which in turn regulates the expression of key genes involved in growth and metabolism (14). Dysregulation of this pathway has been linked to the early onset of puberty in both humans and animal models (15). Overexpression of Lin28a, for example, has been shown to delay puberty onset, while reduced expression accelerates it, suggesting that the Lin28/Let7 axis plays a pivotal role in controlling the timing of sexual maturation (11).

Similarly, RFRPs, which belong to the RF-amide peptide family, have been identified as potent inhibitors of GnRH secretion (16). By acting directly on GnRH neurons, RFRPs can delay the activation of the HPG axis and thus postpone puberty (17). Research into the exact role of RFRPs in human puberty is ongoing, but preliminary findings suggest that these peptides may serve as natural regulators of pubertal timing, making them potential targets for therapeutic interventions in precocious puberty (12).

The primary aim of this mini-review is to explore the roles of the Lin28/Let7 pathway and RFRPs in the regulation of puberty, with a specific focus on their implications for precocious puberty. By synthesizing findings from recent studies, this review seeks to provide a deeper understanding of how disruptions in these pathways contribute to the early onset of puberty and to highlight potential therapeutic strategies.

2. Lin28/Let7 pathway and its role in puberty

The Lin28/Let7 pathway is a well-established regulator of development, playing a pivotal role in various biological processes, including stem cell regulation, metabolism, and the timing of puberty (13). It consists of the Lin28 protein family, which includes Lin28a and Lin28b, and the Let7 family of microRNAs (miRNAs), specifically Let7a and Let7b (18). This pathway operates through a tightly controlled feedback loop, where Lin28 proteins inhibit the biogenesis of Let7 miRNAs, preventing them from regulating key developmental genes (19).

2.1. Overexpression of Lin28a and its role in delaying puberty

In the context of puberty, the overexpression of Lin28a has been shown to delay pubertal onset (20). This is supported by findings where experimental models using rats demonstrated that increased expression of Lin28a postponed the occurrence of vaginal opening—a key indicator of puberty in rodents (21). The mechanism behind this delay involves the suppression of Let7a and Let7b miRNAs, which are responsible for promoting developmental processes associated with puberty (22). By inhibiting Let7 miRNAs, Lin28a effectively slows down the activation of the HPG axis, delaying the secretion of GnRH and thus postponing the onset of

puberty (11).

The studies suggest that Lin28a overexpression in the hypothalamus plays a crucial role in maintaining pubertal timing (14). Specifically, Lin28a modulates the transcriptional activity of genes involved in growth and reproduction, preventing premature activation of the hormonal cascades that initiate puberty (23). Furthermore, Lin28a regulates the expression of Lin28b mRNA, which also contributes to hypothalamic control over pubertal onset (24). Lin28b is similarly involved in suppressing Let7 miRNA activity, reinforcing the pathway's overall inhibitory effect on puberty (18).

2.2. Suppression of Let7a and Let7b miRNAs and early puberty

In contrast, the suppression of Lin28a and Lin28b, leading to increased levels of Let7a and Let7b miRNAs, is associated with early puberty (18). In the precocious puberty rat models used in the studies, diminished Lin28 expression allowed for the upregulation of Let7 miRNAs, which in turn accelerated the maturation of the HPG axis (11). This resulted in the early secretion of GnRH, leading to the premature onset of puberty as evidenced by early vaginal opening, increased ovarian weights, and elevated serum levels of sex hormones, such as LH and progesterone (11). These hormonal changes are hallmarks of central precocious puberty and indicate the critical role of the Lin28/Let7 axis in controlling pubertal timing (11).

2.3. Therapeutic intervention using traditional Chinese medicine (TCM)

One of the most compelling aspects of the studies is the investigation of TCM, specifically the “nourishing Yin and purging Fire” herbal mixture, as a therapeutic intervention for precocious puberty (25). This TCM mixture effectively modulates the Lin28/Let7 pathway and delays pubertal onset in rats with induced precocious puberty (11).

The TCM treatment demonstrated several notable effects. In the TCM-treated group, the ovarian weights were significantly lower compared to the precocious puberty model group (26). This suggests that the TCM mixture can reverse some of the physiological changes associated with early puberty (27). One of the primary indicators of pubertal onset in rats, vaginal opening, was delayed in the TCM group, further supporting the hypothesis that the TCM mixture can slow down the pubertal process (11). The TCM-treated rats showed lower serum levels of LH and progesterone compared to the untreated precocious puberty model, indicating that the mixture had a stabilizing effect on the hormonal imbalances that trigger early puberty (27).

The therapeutic mechanism of the TCM mixture appears to be linked to its ability to regulate the hypothalamic expression of Lin28b mRNA and the suppression of Let7a and Let7b miRNAs (11). By enhancing the expression of Lin28b, the TCM treatment inhibits the Let7 miRNAs,

thereby delaying the activation of the HPG axis and postponing puberty (11). This finding highlights the potential for TCM as a non-invasive, natural treatment option for precocious puberty, particularly for patients who may be seeking alternatives to conventional hormone-based therapies (28).

3. RFRP in puberty regulation

3.1. Role of RFRP in puberty

RFRP are a family of neuropeptides that play a crucial role in regulating reproductive function, primarily through their inhibition of GnRH secretion (29). RFRP is the mammalian homolog of gonadotropin-inhibitory hormone (GnIH), first discovered in birds, and is expressed in the hypothalamus (30). These peptides are thought to act as gatekeepers of puberty, inhibiting the premature release of GnRH and thus delaying the onset of puberty (31). GnRH is a key hormone that triggers the release of LH and FSH from the pituitary gland, initiating the cascade of events that lead to sexual maturation and reproductive capability (32). In the HPG axis, RFRP neurons release peptides that bind to specific receptors on GnRH neurons, suppressing their activity (29). This inhibitory action helps prevent the premature activation of the HPG axis, maintaining a delay in pubertal onset until the appropriate developmental stage is reached (10).

RFRP expression decreases as puberty approaches, releasing the inhibition on GnRH neurons and allowing the surge of GnRH that triggers the onset of puberty (33). This indicates that RFRP plays a protective role in guarding against the premature initiation of puberty, particularly in response to environmental, metabolic, or stress-related factors that might otherwise push the body towards early maturation (34).

3.2. Comparison with other neuroendocrine mechanisms

While RFRP acts as a direct inhibitor of GnRH secretion, other neuroendocrine mechanisms also contribute to the regulation of puberty (6). For example, kisspeptin, a potent stimulator of GnRH release, plays an opposing role to RFRP in controlling pubertal timing (31). Kisspeptin signaling increases significantly as puberty approaches, promoting the activation of GnRH neurons and triggering the onset of puberty (31).

In contrast, Leptin, a hormone produced by adipose tissue, indirectly influences puberty by signaling the availability of sufficient energy reserves for reproduction (35). Leptin's effect on GnRH neurons is partly mediated through kisspeptin, indicating a complex interaction of metabolic and neuroendocrine signals in the regulation of puberty (36).

While kisspeptin and leptin are more commonly associated with the promotion of puberty, RFRP serves as a balancing mechanism to delay puberty until environmental and physiological conditions are favorable for reproduction (6). The interplay between these pathways ensures that puberty occurs at the optimal time, with RFRP acting as a critical inhibitor

to prevent early activation of the HPG axis (37).

3.3. Implications for treatment of precocious puberty

The role of RFRP in regulating GnRH secretion offers valuable insights into potential therapeutic strategies for managing precocious puberty (30). Since RFRP acts as an inhibitor of GnRH release, modulating its expression or activity could offer a way to delay the onset of puberty in children experiencing early sexual maturation (6). This is particularly important for preventing the long-term complications associated with precocious puberty, such as short stature, psychological stress, and reproductive issues in adulthood (4).

Although the precise role of RFRP in human puberty is still being studied, animal models provide promising evidence that enhancing RFRP signaling could serve as a therapeutic strategy to delay early puberty (38). Pharmacological agents that mimic the action of RFRP or enhance its expression in the hypothalamus may help suppress the premature release of GnRH and delay pubertal onset (39).

Moreover, combining RFRP modulation with other pathways, such as the Lin28/Let7 axis, may provide a more comprehensive approach to managing precocious puberty (11). While RFRP directly inhibits GnRH secretion, the Lin28/Let7 pathway regulates the overall timing of developmental processes that influence the HPG axis (40). A dual approach targeting both neuroendocrine inhibition (through RFRP) and developmental timing (through Lin28/Let7) could potentially provide more effective treatment options.

4. Conclusion

In conclusion, the modulation of the Lin28/Let7 pathway and the regulation of RFRP present promising avenues for controlling precocious puberty. The studies discussed in this review provide important insights into how these pathways regulate the onset of puberty and demonstrate the potential of TCM as a therapeutic strategy. The TCM mixture used in the rat model effectively delayed puberty by influencing key hormonal regulators, suggesting that similar treatments could be developed for clinical use in humans. However, the findings from these studies lay the groundwork for developing novel therapeutic approaches that could improve the management of precocious puberty and contribute to the broader field of reproductive biology.

References

1. Stephen MD, Zage PE, Waguespack SG. Gonadotropin-dependent precocious puberty: neoplastic causes and endocrine considerations. *Int J Pediatr Endocrinol*. 2011;2011(1):184502. <https://doi.org/10.1155/2011/184502>
2. Soliman A, De Sanctis V, Elalaily R, Bedair S. Advances in pubertal growth and factors influencing it: Can we increase pubertal growth? *Indian J Endocrinol Metab*. 2014;18(Suppl 1):S53-62. <https://doi.org/10.4103/2230-8210.145075>
3. Mendle J, Turkheimer E, Emery RE. Detrimental

- Psychological Outcomes Associated with Early Pubertal Timing in Adolescent Girls. *Dev Rev.* 2007;27(2):151-71. <https://doi.org/10.1016/j.dr.2006.11.001>
4. Street ME, Ponzi D, Renati R, Petraroli M, D'Alvano T, Lattanzi C, et al. Precocious puberty under stressful conditions: new understanding and insights from the lessons learnt from international adoptions and the COVID-19 pandemic. *Front Endocrinol (Lausanne).* 2023;14:1149417. <https://doi.org/10.3389/fendo.2023.1149417>
5. Gupta R, Kumar P, Fahmi N, Garg B, Dutta S, Sachar S, et al. Endocrine disruption and obesity: A current review on environmental obesogens. *Current Research in Green and Sustainable Chemistry.* 2020;3:100009.
6. Livadas S, Chrousos GP. Molecular and Environmental Mechanisms Regulating Puberty Initiation: An Integrated Approach. *Front Endocrinol (Lausanne).* 2019;10:828. <https://doi.org/10.3389/fendo.2019.00828>
7. Acevedo-Rodriguez A, Kauffman AS, Cherrington BD, Borges CS, Roepke TA, Laconi M. Emerging insights into hypothalamic-pituitary-gonadal axis regulation and interaction with stress signalling. *J Neuroendocrinol.* 2018;30(10):e12590. <https://doi.org/10.1111/jne.12590>
8. Howard SR. Interpretation of reproductive hormones before, during and after the pubertal transition—identifying health and disordered puberty. *Clinical Endocrinology.* 2021;95(5):702-15.
9. Abreu AP, Kaiser UB. Pubertal development and regulation. *Lancet Diabetes Endocrinol.* 2016;4(3):254-64. [https://doi.org/10.1016/S2213-8587\(15\)00418-0](https://doi.org/10.1016/S2213-8587(15)00418-0)
10. Han XX, Zhao FY, Gu KR, Wang GP, Zhang J, Tao R, et al. Development of precocious puberty in children: Surmised medicinal plant treatment. *Biomed Pharmacother.* 2022;156:113907. <https://doi.org/10.1016/j.biopha.2022.113907>
11. He Y, Han X, Sun W, Yu J, Tamadon A. Precocious Puberty and the Lin28/Let7 Pathway: The Therapeutic Effect of the Nourishing “Yin” and Purging “Fire” Traditional Chinese Medicine Mixture in a Rat Model. *Evid Based Complement Alternat Med.* 2018;2018:4868045. <https://doi.org/10.1155/2018/4868045>
12. He Y, Sun W, Yu J. Is precocious puberty linked to hypothalamic expression of arginine-phenylalanine-amide-related peptide? *Iran J Basic Med Sci.* 2017;20(10):1074-8. <https://doi.org/10.22038/IJBMS.2017.9397>
13. Thornton JE, Gregory RI. How does Lin28 let-7 control development and disease? *Trends Cell Biol.* 2012;22(9):474-82. <https://doi.org/10.1016/j.tcb.2012.06.001>
14. Sangiao-Alvarellos S, Manfredi-Lozano M, Ruiz-Pino F, Navarro VM, Sanchez-Garrido MA, Leon S, et al. Changes in hypothalamic expression of the Lin28/let-7 system and related microRNAs during postnatal maturation and after experimental manipulations of puberty. *Endocrinology.* 2013;154(2):942-55. <https://doi.org/10.1210/en.2012-2006>
15. Holder MK, Blaustein JD. Puberty and adolescence as a time of vulnerability to stressors that alter neurobehavioral processes. *Front Neuroendocrinol.* 2014;35(1):89-110. <https://doi.org/10.1016/j.yfrne.2013.10.004>
16. Salehi MS, Tamadon A, Jafarzadeh Shirazi MR, Namavar MR, Zamiri MJ. The Role of Arginine-Phenylalanine-Amide-Related Peptides in Mammalian Reproduction. *Int J Fertil Steril.* 2015;9(3):268-76. <https://doi.org/10.22074/ijfs.2015.4540>
17. Odetayo AF, Akhigbe RE, Bassey GE, Hamed MA, Olayaki LA. Impact of stress on male fertility: role of gonadotropin inhibitory hormone. *Front Endocrinol (Lausanne).* 2023;14:1329564. <https://doi.org/10.3389/fendo.2023.1329564>
18. Piskounova E, Polyarchou C, Thornton JE, LaPierre RJ, Pothoulakis C, Hagan JP, et al. Lin28A and Lin28B inhibit let-7 microRNA biogenesis by distinct mechanisms. *Cell.* 2011;147(5):1066-79. <https://doi.org/10.1016/j.cell.2011.10.039>
19. Stefani G, Chen X, Zhao H, Slack FJ. A novel mechanism of LIN-28 regulation of let-7 microRNA expression revealed by in vivo HITS-CLIP in *C. elegans*. *RNA.* 2015;21(5):985-96. <https://doi.org/10.1261/rna.045542.114>
20. Corre C, Shinoda G, Zhu H, Cousminer DL, Crossman C, Bellissimo C, et al. Sex-specific regulation of weight and puberty by the Lin28/let-7 axis. *J Endocrinol.* 2016;228(3):179-91. <https://doi.org/10.1530/JOE-15-0360>
21. Zhu H, Shah S, Shyh-Chang N, Shinoda G, Einhorn WS, Viswanathan SR, et al. Lin28a transgenic mice manifest size and puberty phenotypes identified in human genetic association studies. *Nat Genet.* 2010;42(7):626-30. <https://doi.org/10.1038/ng.593>
22. Ma Y, Shen N, Wicha MS, Luo M. The Roles of the Let-7 Family of MicroRNAs in the Regulation of Cancer Stemness. *Cells.* 2021;10(9). <https://doi.org/10.3390/cells10092415>
23. Shim YS, Lee HS, Hwang JS. Genetic factors in precocious puberty. *Clin Exp Pediatr.* 2022;65(4):172-81. <https://doi.org/10.3345/cep.2021.00521>
24. Leinonen JT, Chen YC, Penonen J, Lehtonen L, Junna N, Tukiainen T, et al. LIN28B affects gene expression at the hypothalamic-pituitary axis and serum testosterone levels. *Sci Rep.* 2019;9(1):18060. <https://doi.org/10.1038/s41598-019-54475-6>
25. Park SC, Trinh TA, Lee WY, Baek JY, Lee S, Choi K, et al. Effects of estrogen inhibition formula herbal mixture for danazol-induced precocious puberty in female rats: an experimental study with network pharmacology. *Integr Med Res.* 2021;10(3):100708. <https://doi.org/10.1016/j.imr.2020.100708>
26. He Y, Han X, Sun W, Yu J, Tamadon A. Precocious puberty and the Lin28/Let7 pathway: the therapeutic effect of the nourishing “Yin” and purging “Fire” traditional chinese medicine mixture in a rat model. *Evidence-Based Complementary and Alternative Medicine.* 2018;2018(1):4868045.
27. Bai GL, Hu KL, Huan Y, Wang X, Lei L, Zhang M, et al. The Traditional Chinese Medicine Fuyou Formula Alleviates Precocious Puberty by Inhibiting GPR54/GnRH in the Hypothalamus. *Front Pharmacol.* 2020;11:596525. <https://doi.org/10.3389/fphar.2020.596525>
28. Yu CH, Liu PH, Van YH, Lien AS, Huang TP, Yen HR. Traditional Chinese medicine for idiopathic precocious puberty: A hospital-based retrospective observational study. *Complement Ther Med.* 2014;22(2):258-65. <https://doi.org/10.1016/j.ctim.2014.01.002>
29. Mohapatra SS, Mukherjee J, Banerjee D, Das PK, Ghosh PR, Das K. RFamide peptides, the novel regulators of mammalian HPG axis: A review. *Vet World.* 2021;14(7):1867-73. <https://doi.org/10.14202/vetworld.2021.1867-1873>
30. Tsutsui K, Ubuka T. Discovery of gonadotropin-inhibitory hormone (GnIH), progress in GnIH research on reproductive physiology and behavior and perspective

- of GnIH research on neuroendocrine regulation of reproduction. *Mol Cell Endocrinol.* 2020;514:110914. <https://doi.org/10.1016/j.mce.2020.110914>
31. Xie Q, Kang Y, Zhang C, Xie Y, Wang C, Liu J, et al. The Role of Kisspeptin in the Control of the Hypothalamic-Pituitary-Gonadal Axis and Reproduction. *Front Endocrinol (Lausanne).* 2022;13:925206. <https://doi.org/10.3389/fendo.2022.925206>
32. Zakharova L, Sharova V, Izvolskaia M. Mechanisms of reciprocal regulation of gonadotropin-releasing hormone (GnRH)-producing and immune systems: the role of GnRH, cytokines and their receptors in early ontogenesis in normal and pathological conditions. *International Journal of Molecular Sciences.* 2020;22(1):114.
33. Semaan SJ, Kauffman AS. Daily successive changes in reproductive gene expression and neuronal activation in the brains of pubertal female mice. *Mol Cell Endocrinol.* 2015;401:84-97. <https://doi.org/10.1016/j.mce.2014.11.025>
34. McCartney CR, Marshall JC. Neuroendocrinology of reproduction. *Yen and Jaffe's Reproductive Endocrinology: Elsevier;* 2019. p. 1-24. e8.
35. Childs GV, Odle AK, MacNicol MC, MacNicol AM. The Importance of Leptin to Reproduction. *Endocrinology.* 2021;162(2). <https://doi.org/10.1210/endo/bqaa204>
36. Qiu X, Dao H, Wang M, Heston A, Garcia KM, Sangal A, et al. Insulin and Leptin Signaling Interact in the Mouse Kiss1 Neuron during the Peripubertal Period. *PLoS One.* 2015;10(5):e0121974. <https://doi.org/10.1371/journal.pone.0121974>
37. Naule L, Maione L, Kaiser UB. Puberty, A Sensitive Window of Hypothalamic Development and Plasticity. *Endocrinology.* 2021;162(1). <https://doi.org/10.1210/endo/bqaa209>
38. Evans MC, Anderson GM. The Role of RFRP Neurons in the Allostatic Control of Reproductive Function. *Int J Mol Sci.* 2023;24(21). <https://doi.org/10.3390/ijms242115851>
39. Chen Z, Si L, Shu W, Zhang X, Wei C, Wei M, et al. Exogenous Melatonin Regulates Puberty and the Hypothalamic GnRH-GnIH System in Female Mice. *Brain Sci.* 2022;12(11). <https://doi.org/10.3390/brainsci12111550>
40. Navarro VM. Metabolic regulation of kisspeptin - the link between energy balance and reproduction. *Nat Rev Endocrinol.* 2020;16(8):407-20. <https://doi.org/10.1038/s41574-020-0363-7>