Journal of the Endocrine Society, 2025, **9**, bvaf065
https://doi.org/10.1210/jendso/bvaf065
Advance access publication 23 May 2025
Clinical Research Article

Serum Phoenixin Levels and Their Diagnostic Significance in Girls With Precocious Puberty

Ye Yang,[1,*] Jun Sun,[1,*] Shuyi Yang,[1] Siqing Li,[1,2] Jingjing Zhang,[1] Fei Zhu,[1,2] Xiaoxiao Pan,[1] Ying Wang,[1] Xiaona Li,[1] Hua Bai,[1] Peiliang Luo,[1,2] and Yingdi Yuan[1,2]

[1]Pediatric Endocrinology Department, The First People's Hospital of Lianyungang, The Affiliated Lianyungang Hospital of Xuzhou Medical University, Lianyungang 222000, China
[2]Pediatric Endocrinology Department, The First People's Hospital of Lianyungang, Lianyungang Clinical College of Nanjing Medical University, Lianyungang 222000, China

Correspondence: Peiliang Luo, Pediatric Endocrinology Department, The First People's Hospital of Lianyungang, The Affiliated Lianyungang Hospital of Xuzhou Medical University, No. 182, N Tongguan Rd, Haizhou District, Lianyungang 222000, China. Email: lpl62502@sina.com; or Yingdi Yuan, PhD, Pediatric Endocrinology Department, The Affiliated Lianyungang Hospital of Xuzhou Medical University, No. 182, N Tongguan Rd, Haizhou District, Lianyungang 222000, China. Email: yuanyingdi@njmu.edu.cn.
*Ye Yang and Jun Sun contributed equally to this work.

Abstract

Context: Phoenixin (PNX), a recently identified neuropeptide, is recognized for its role in reproduction.

Objective: This study aims to explores serum phoenixin expression and its diagnostic value in girls with central precocious puberty (CPP).

Methods: Girls visiting the Pediatric Endocrinology Department of Lianyungang First People's Hospital (February 2023-February 2025) were included and divided into 3 groups: CPP (n = 48), premature thelarche (PT) (n = 58), and healthy controls (NCs, n = 50). Serum phoenixin levels were measured via enzyme-linked immunosorbent assay and compared among groups. Spearman correlation analysis assessed variable relationships, and receiver operating characteristic (ROC) curves evaluated phoenixin's diagnostic value for CPP.

Results: Serum PNX-14 levels of the CPP group (228.42 [193.29-277.22] pg/mL) were considerably higher relative to the PT group (192.58 [151.34-239.48] pg/mL) ($P < .05$), and higher serum PNX-14 levels were found in the PT group compared to the NC group (124.26 [88.78-167.49] pg/mL) ($P < .05$). No statistically significant differences in PNX-20 levels were found among the groups ($P > .05$). PNX-14 levels were positively correlated with height, weight, body mass index, bone age, uterine and ovarian volumes, and pituitary height, and negatively with sex hormone–binding globulin. ROC analysis showed an area under the curve of 0.695 for PNX-14 in identifying CPP, with a cutoff of 195.85 pg/mL (sensitivity: 75.0%; specificity: 69.0%).

Conclusion: Serum PNX-14 is associated with CPP and reflects growth and pubertal development. It may serve as a potential biomarker for adjunct diagnosis of CPP in girls.

Key Words: girls, phoenixin, central precocious puberty

Abbreviations: BMI, body mass index; CNS, central nervous system; CPP, central precocious puberty; CV, coefficient of variation; E2, estradiol; ELISA, enzyme-linked immunosorbent assay; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; MRI, magnetic resonance imaging; NC, normal control; PNX, phoenixin; PP, precocious puberty; PT, premature thelarche; ROC, receiver operating characteristic; SHBG, sex hormone–binding globulin.

In pediatric endocrinology, precocious puberty (PP) is among the most prevalent disorders, typically defined in girls as the onset of breast development (thelarche) before age 7.5 years or menarche prior to age 10 years. This premature activation may lead to accelerated bone age advancement, compromised final adult height, and a spectrum of psychosocial issues. The classification of PP is based on whether the gonadal axis is activated. If the gonadal axis is activated, it is classified as central precocious puberty (CPP); otherwise, it is classified as peripheral PP or incomplete PP. With better living standards, the prevalence of PP has been rising annually. A large-scale study from Denmark reported a significant rise in the annual prevalence of CPP between 1998 and 2017, with incidence rates rising 6-fold in girls and 15-fold in boys [1]. Premature thelarche (PT), the most common subtype of incomplete PP, manifests solely as breast development with a prevalence of 4.8% among Chinese girls. It typically requires no intervention; however, approximately 13% to 18% of PT cases may progress to CPP, necessitating close clinical follow-up [2]. PP often has an insidious onset and a prolonged course, resulting in delayed diagnosis in many affected children, which can severely affect their physical and mental health. In clinical practice, treatment for PP varies based on its classification. Gonadotropin-releasing hormone analogue intervention is considered an effective treatment for CPP, aiming to increase adult height and delay sexual development rate to an age more consistent with peers [3]. Studies have shown that in children diagnosed with CPP, those in the gonadotropin-releasing hormone analogue intervention group

Received: 31 December 2024. **Editorial Decision:** 7 April 2025. **Corrected and Typeset:** 26 May 2025

achieved an average increase of 7 cm in final adult height compared to the control group [4].

Currently, refining the clinical diagnosis of PP subtypes is a major research focus. Although pediatric and endocrinology experts worldwide consider sex hormone stimulation testing a key diagnostic tool for CPP, it is complex, requiring multiple blood samples, which often leads to refusal by children and their parents, thus delaying diagnosis and treatment. Therefore, identifying new auxiliary diagnostic markers to streamline the diagnostic process for CPP is an ongoing research priority.

Phoenixin (PNX) is a cleavage product of the Smim20 (C4orf52) protein. Smim20 serves as a component of the mitochondrial translation regulatory assembly intermediate of the cytochrome c oxidase (COX) complex. It participates in the biogenesis of cytochrome c oxidase and stabilizes the COX1 subunit [5]. The predominant cleavage product of C4orf52 is a peptide made up of 14 amino acid residues, referred to as PNX-14. Another isoform of PNX, PNX-20, is a 6-amino acid extension at the N-terminal of PNX-14. Although PNX-14 and PNX-20 differ in sequence structures, they exhibit similar biological activities [6]. The most well-known function of PNX is its role as a reproductive peptide [6]. PNX is considered a gonadotropin-releasing hormone axis stimulator and has the ability to regulate steroid activity [7]. Currently, research on PNX in pediatric PP is limited. As research progresses, PNX is expected to become a potential diagnostic marker for PP and a new therapeutic target for drug development.

Materials and Methods

Inclusion of Study Participants

The study included female children diagnosed with precocious puberty (classified into CPP and PT; the Tanner stages and menarche status of the participants are detailed in Table 1) at Lianyungang First People's Hospital (February 2023-February 2025), and age-matched healthy girls undergoing routine health check-ups during the same period. Currently, there is no clear evidence establishing a relationship between PXN and age. We hypothesize that in girls, as age increases and sexual maturation progresses, plasma PXN levels may increase to a certain extent. Therefore, to eliminate the potential influence of age on plasma PXN levels, we considered age matching when selecting the study participants. Prior to enrollment, all participants and their parents/primary guardians were thoroughly informed about the study objectives and procedures, including repeated blood sampling, pelvic color Doppler ultrasound (uterus and adnexa), and cranial magnetic resonance imaging (MRI), to ensure complete understanding. Written informed consent was obtained in accordance with the Declaration of Helsinki. This study was approved by the local institutional review board (approval No.: KY-20240730001-02). To enhance protocol compliance, fees for the aforementioned examinations (eg, imaging and laboratory tests) were waived both for the PP group and healthy controls (NCs). The NC group declined to undergo the gonadotropin-releasing hormone (GnRH) stimulation test due to its requirement for multiple blood draws, and thus data from this procedure were not obtained.

Ethics Statement

This human studies research received approval from the ethics committee of the First People's Hospital of Lianyungang (approval No.: KY-20240730001-02). All procedures followed local ethical standards and institutional guidelines. These measures ensured the protection of participants' rights and well-being. Before enrollment, participants were informed about the study's purpose, procedures, and risks. Each participant provided written informed consent to confirm their voluntary participation and understanding.

Table 1. Tanner staging and menarche status in girls with precocious puberty

	Tanner staging					Menarche		Total
	1	2	3	4	5	Yes	No	
CPP group	0	23	24	1	0	5	43	48
PT group	0	54	4	0	0	0	58	58

Abbreviations: CPP, central precocious puberty; PT, premature thelarche.

Inclusion criteria

Central precocious puberty group. Participants included girls diagnosed with CPP according to the 2022 consensus guidelines [2]. Diagnosis relied on the early onset of puberty caused by premature hypothalamo-pituitary-gonadal axis activation (GnRH stimulation test: luteinizing hormone [LH] peak/follicle-stimulating hormone [FSH] peak ≥ 0.6, and LH peak $\geq$ 5IU/L), resulting in early secondary sexual characteristic development. Clinical evaluation and hormone testing confirmed the diagnosis. Cranial MRI was performed to rule out central nervous system (CNS) abnormalities.

Premature thelarche group. Breast development was noted in the absence of accelerated growth velocity. GnRH stimulation test revealed no evidence of gonadotropin axis activation. Following comprehensive evaluation, patients with other endocrine disorders, CNS pathologies, or chronic exogenous hormone exposure were excluded.

Healthy/Normal control group. Participants included girls undergoing routine health check-ups at Lianyungang First People's Hospital during the same period. These girls were age-matched to the study group and had no history of endocrine or developmental disorders, with normal growth and pubertal development as confirmed by clinical evaluation and lab tests.

Exclusion criteria

Girls who had previously been treated with GnRH analogues were excluded as this could affect hormonal levels and pubertal development. Girls with incomplete data were also excluded to ensure accurate and reliable analysis.

Collection of Clinical Data and Biochemical Indicators

Both in outpatient and inpatient settings, clinical assessments were performed by two experienced pediatric endocrinologists (at or above attending physician level) to evaluate the following parameters in girls: breast development stage (Tanner staging), presence of menarche, and bone age vs chronological age discrepancy. After admission, transabdominal pelvic ultrasound and brain MRI were conducted to assess gonadal

maturation and exclude CNS pathologies. Blood samples were uniformly collected at a standardized time point (3 PM) the day of admission to measure baseline sex hormone levels (LH, FSH, estradiol [E2]). The following morning at 8 AM, a GnRH stimulation test was initiated: intravenous administration of triptorelin (2.5 μg/kg, maximum dose should not exceed 100 μg), followed by serial blood sampling at 0, 30, 60, 90, and 120 minutes post injection to determine peak stimulated gonadotropin levels. Clinical data for patients with PP and children undergoing routine health check-ups, including age, height, and weight, were collected through clinical evaluations. Basal and stimulated levels of FSH and LH were quantified using immunochemiluminescence assays in the hospital's clinical laboratory. Moreover, bone age, uterine volume, ovarian volume, and pituitary height were evaluated based on reports from the radiology department. Following the acquisition of written informed consent from the participants, blood samples were collected. We then separated serum from blood using low-temperature centrifugation, recorded the data, and stored it at −80°C.

The serum levels of PNX-14 and PNX-20 were measured using an enzyme-linked immunosorbent assay (ELISA). The kits, including the Human PNX-14 ELISA Kit (FineTest catalog No. EH4521, RRID:AB_3665841) (intra-assay coefficient of variation: CV = 5.96%, interassay CV = 4.87%) and the Human PNX-20 ELISA Kit (FineTest catalog No. EH4352, RRID:AB_3665827) (intra-assay CV = 4.59%, interassay CV = 5.49%), were purchased from Wuhan Fine Biotech Co, Ltd. First, we determined the optimal serum dilution factor through preliminary experiments to match each kit's optimal detection range. Then, all serum samples were diluted accordingly to obtain new samples. The protein concentration in the samples was quantified using a double-sandwich ELISA, in accordance with the protocol outlined in the manufacturer's guidelines. Finally, the absorbance of the samples at 450 nm was read and recorded using a microplate reader (Tecan), and the concentrations of PNX-14 and PNX-20 in serum samples were calculated based on the absorbance values and predefined dilution factors.

Statistical Analysis

In this study, all data were processed using SPSS 27.0. The first step involved assessing the normality of the data using appropriate tests to determine the distribution type. Based on the results, either parametric or nonparametric tests were selected to compare differences between the variables. Group differences were assessed using one-way analysis of variance for normally distributed data, while the Kruskal-Wallis test was employed for non-normally distributed data. To explore the relationships between variables, Spearman rank correlation was applied. Statistical significance was defined as a P value less than .05 in all analyses conducted in this study. In addition, we constructed receiver operating characteristic (ROC) curves to determine the optimal cutoff value for PNX in diagnosing CPP when the Youden index was maximized. These curves were also used to calculate the sensitivity and specificity of PNX as a potential diagnostic marker for CPP, allowing for an assessment of its diagnostic accuracy.

Results

Comparison of General Data

No statistically significant differences in age were found among the three groups. However, notable differences were observed in height, weight, body mass index (BMI), and predicted adult height [(father's height + mother's height)/2 − 6.5 cm] ($P < .05$). Pairwise comparisons revealed no statistically significant differences in weight and BMI between the CPP and PT groups. The CPP group was taller than the PT ([135.47 ± 6.35] cm vs [131.28 ± 9.64] cm; $P < .05$), but had a lower predicted adult height ([160.29 ± 3.18] cm vs [162.06 ± 3.70] cm; $P < .05$). The height, weight, BMI, and predicted adult height of the CPP group were significantly greater than those of the NC group ($P < .05$). Similarly, the PT group exhibited higher values in height, weight, BMI, and predicted adult height compared to the NC group ($P < .05$). Further details can be found in Table 2.

Comparison of Laboratory and Imaging Data

Notable differences were found between the three groups regarding bone age, uterine and ovarian volumes, pituitary height, E2 levels, sex hormone–binding globulin (SHBG), and the basal levels of LH and FSH. Pairwise comparisons revealed no statistically significant differences in pituitary height, SHBG, and peak FSH levels between the CPP and PT groups. However, the CPP group showed significantly higher bone age, uterine volume, ovarian volume, E2 levels, basal LH, basal FSH, peak LH (pLH), and pLH/peak FSH (pFSH) ratio compared to the PT group ($P < .05$). Moreover, the CPP group exhibited significantly higher values in bone age, uterine and ovarian volumes, pituitary height, E2 levels, as well as basal LH and FSH, in comparison to the NC group, while SHBG levels in the CPP group were significantly lower than those in the NC group ($P < .05$). There were no statistically significant differences in E2, basal LH, and basal FSH levels between the PT and NC groups. However, the PT group exhibited significantly higher bone age, uterine volume, ovarian volume, and pituitary height compared to the NC group ($P < .05$), and SHBG levels were lower in the PT group than in the NC group ($P < .05$). Further details can be found in Table 3.

Comparison of Serum Phoenixin Levels

Serum PNX-14 levels differed significantly among the three groups ($P < .05$). Subgroup analyses revealed that serum PNX-14 levels were significantly higher in the CPP group compared to the PT group (228.42 [193.29-277.22] pg/mL vs 172.93 [129.82-229.44] pg/mL; $P < .05$). Additionally, PNX-14 levels of the PT group were higher than in the NC group (172.93 [129.82-229.44] pg/mL vs 124.26 [88.78-167.49] pg/mL; $P < .05$). In contrast, no notable differences in serum PNX-20 levels were found among the three groups ($P > .05$). Further details can be found in Table 4.

Correlation Analysis of Phoenixin-14 and Clinical Data

In all study participants, the following observations were made: Serum PNX-14 levels exhibited positive correlations with height, weight, BMI, bone age, uterine volume, ovarian volume, and pituitary height. Conversely, PNX-14 levels exhibited a negative correlation with SHBG levels. Further details can be found in Table 5.

Prediction Model Construction and Evaluation

ROC curve analysis revealed that the area under the curve for PNX-14 in diagnosing CPP was 0.695, with a cutoff value of

Table 2. Comparison of general data among the central precocious puberty, premature thelarche, and normal control groups

Project	CPP, 48	PT, 58	NC, 50	*P*1	*P*2	*P*3	*P*4
Age, y	8.00 ± 0.75	7.64 ± 0.84	7.87 ± 1.47	.062	.927	.695	.206
Height, cm	135.47 ± 6.35	131.28 ± 9.64	116.95 ± 8.54	.011[a]	.000[a]	.000[a]	.000[a]
Weight, kg	31.75 (28.00-36.38)	29.00 (25.00-34.25)	21.25 (17.38-24.33)	.220	.000[a]	.000[a]	.000[a]
BMI	16.88 (15.76-19.25)	17.62 (15.60-19.24)	14.82 (13.80-16.58)	≥.999	.000[a]	.000[a]	.000[a]
Predicted adult height, cm	160.29 ± 3.18	162.06 ± 3.70	158.33 ± 3.13	.008[a]	.005[a]	.000[a]	.000[a]

For normally distributed variables, values are expressed as mean ± SD (X ± SD). For nonnormally distributed variables, values are presented as median and interquartile range (median [P25-P75]).
*P*1 = CPP vs PT; *P*2 = PT vs NC; *P*3 = PT vs NC; *P*4 = comparison among the CPP, PT, and NC groups.
Abbreviations: BMI, body mass index; CPP, central precocious puberty; NC, normal control; PT, premature thelarche.
Statistical significance is indicated as [a]*P* less than .05.

Table 3. Comparison of laboratory and imaging data among the central precocious puberty, premature thelarche, and normal control groups

Project	CPP, 48	PT, 58	NC, 50	*P*1	*P*2	*P*3	*P*4
Bone age, y	10.2 (9.13-11.00)	9.00 (8.95-10.00)	6.50 (5.00-8.00)	.016[a]	.000[a]	.000[a]	.000[a]
Uterine volume, mm^3	7746.00 (4235.00-10466.00)	2830.5 (1895.00-3870.00)	1434.00 (1035.00-2604.00)	.000[a]	.000[a]	.000[a]	.000[a]
Ovarian volume, mm^3	7550.00 (5038.50-11843.50)	5697.00 (3875.75-7392.00)	2857.00 (1394.50-4608.00)	.011[a]	.000[a]	.000[a]	.000[a]
Pituitary height, mm	5.25 (4.93-6.00)	5.00 (4.60-5.93)	4.45 (3.90-4.90)	≥.999	.000[a]	.000[a]	.000[a]
E2, pg/mL	19.48 (15.00-40.53)	15.00 (15.00-15.00)	15.00 (15.00-15.00)	.000[a]	.000[a]	.937	.000[a]
Sex hormone-binding globulin, nmol/L	50.05 (35.70-75.93)	58.00 (44.05-86.53)	101.80 (80.13-121.55)	.365	.000[a]	.000[a]	.000*
Baseline LH, MIU/mL	1.30 (0.70-3.25)	0.43 (0.20-0.94)	0.29 (0.2-0.64)	.000[a]	.000[a]	.665	.000[a]
Baseline FSH, MIU/mL	3.78 (2.49-6.82)	2.92 (1.92-4.80)	3.20 (2.02-4.14)	.047[a]	.025[a]	1.000	.015[a]
pLH, MIU/mL	18.42 (12.27-29.10)	4.69 (2.84-6.12)		.000[a]			
pFSH, MIU/mL	17.08 (13.78-20.18)	17.75 (14.83-21.76)		.393			
pLH/pFSH	1.09 (0.79-1.54)	0.24 (0.17-0.35)		.000[a]			

For normally distributed variables, values are expressed as mean ± (X ± SD). For nonnormally distributed variables, values are presented as median and interquartile range (median [P25-P75]).
*P*1 = CPP vs PT; *P*2 = CPP vs NC; *P*3 = PT vs NC; *P*4 = comparison among the CPP, PT, and NC groups.
Abbreviations: CPP, central precocious puberty; E2, estradiol; NC, normal control; pFSH, peak follicle-stimulating hormone during stimulation test; pLH, peak luteinizing hormone during stimulation test; PT, premature thelarche.
Statistical significance is indicated as [a]*P* less than .05.

Table 4. Comparison of serum phoenixin levels among central precocious puberty, premature thelarche, and normal control groups

Project	CPP, 48	PT, 58	NC, 50	*P*1	*P*2	*P*3	*P*4
PNX-14, pg/mL	228.42 (193.29-277.22)	172.93 (129.82-229.44)	124.26 (88.78-167.49)	.006[a]	.000[a]	.004[a]	.000[a]
PNX-20, pg/mL	299.07 (124.60-1277.22)	215.53 (118.51-1291.01)	176.16 (112.38-720.54)				.403

For normally distributed variables, values are expressed as mean ± (X ± SD). For nonnormally distributed variables, values are presented as median and interquartile range (median [P25-P75]).
*P*1 = CPP vs PT; *P*2 = CPP vs NC; *P*3 = PT vs NC; *P*4 = comparison among the CPP, PT, and NC groups.
Abbreviations: CPP, central precocious puberty; NC, normal control; PT, premature thelarche.
Statistical significance is indicated as [a]*P* less than .05.

195.85 pg/mL, sensitivity of 75.0%, and specificity of 69.0%, as illustrated in Fig. 1.

Discussion

Children with CPP are characterized by the early development of secondary sexual characteristics compared to healthy children. This includes the early onset of menarche and taller stature during early development, but a shorter final adult height [8]. Such early maturation can lead to psychological health issues and hinder social integration. The etiology of CPP is complex and multifactorial, involving both congenital and acquired factors related to structural or functional brain changes. CPP onset results from early pulsatile GnRH secretion from the hypothalamus, triggering premature activation of the hypothalamo-pituitary-gonadal axis [9].This study aimed to identify such children at an early stage for timely intervention.

This study introduces PNX, a newly discovered neuropeptide initially isolated from the rat hypothalamus and cattle heart [10]. It has since been found in various mammals and

Table 5. Correlation analysis of phoenixin-14 with clinical data

		Ht	Wt	BMI	Predicted adult height	Bone age	Uterine volume	Ovarian volume	Pituitary height	E2	SHBG	Basal LH	Basal FSH
PNX-14	*r*	0.365	0.380	0.263	0.155	0.325	0.324	0.324	0.233	0.157	−0.233	0.235	0.051
	P	.000[a]	.000[a]	.001[a]	.054	.000[a]	.000[a]	.000[a]	.003[a]	.051	.003[a]	.003[a]	.524

Abbreviations: BMI, body mass index; E2, estradiol; FSH, follicle-stimulating hormone; Ht, height; LH, luteinizing hormone; PNX-14, phoenixin-14; SHBG, sex hormone–binding globulin; Wt, weight.
Statistical significance is indicated as [a]*P* less than .05.

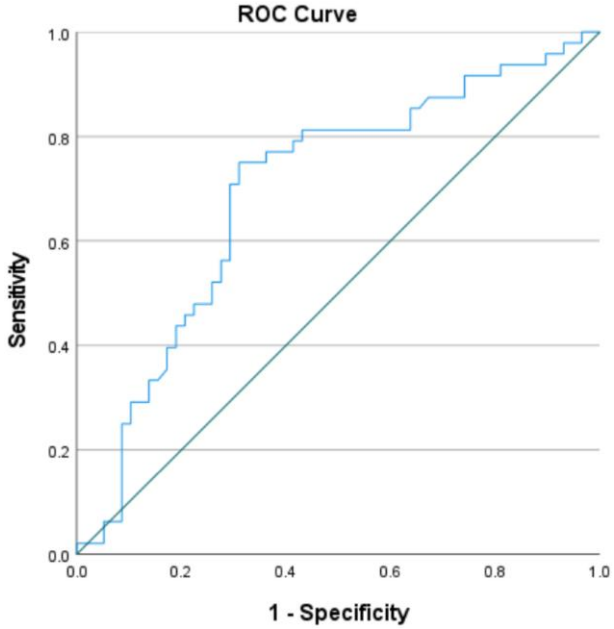

Figure 1. Reference receiver operating characteristic curve for serum PNX-14 in diagnosing central precocious puberty.

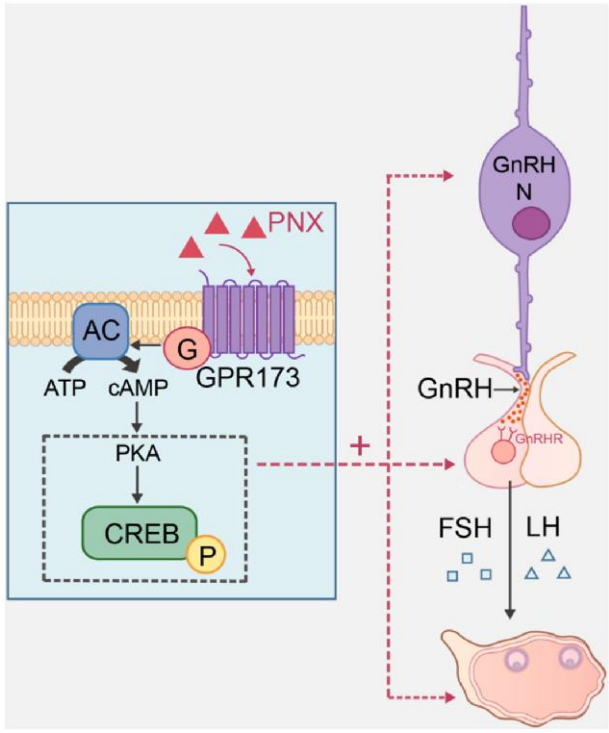

Figure 2. The effects of phoenixin (PNX) at various levels of the gonadal axis. PNX binds to GPR173, activating the cyclic adenosine monophosphate/protein kinase A pathway and increasing CREB phosphorylation, thereby mediating biological functions. At the hypothalamic, PNX enhances the synthesis and secretion of gonadotropin-releasing hormone. At the pituitary, PNX increases the synthesis of gonadotropin-releasing hormone receptor and upregulates the expression of gonadotropins in pituitary gonadotrope cells. At the ovary, PNX promotes the production of estradiol and follicular maturation.

nonmammals, such as humans, mice, dogs, pigs, fish, and shrimp [11]. The PNX amino acid sequence is highly conserved across different species, with only one amino acid difference between humans and rodents in PNX-20 [10]. Animal studies have demonstrated that PNX is highly expressed in the hypothalamus [12], and is also present in peripheral tissues, including the heart [13], pancreas [14], adipose tissue, and ovaries [15], where it is secreted. Research on PNX and its association with the human disease spectrum has demonstrated that PNX plays multiple roles in the human body, affecting various organ systems and being closely linked to the development and progression of several diseases, including polycystic ovary syndrome [16], hypertension [17], cerebral infarction [18], and stress-related disorders [19].

The most important and earliest studied role of PNX is its involvement in reproduction, where it acts at various levels of the gonadal axis (Fig. 2). A study by Treen et al [20] highlighted that in the hypothalamus, PNX activates the cyclic adenosine monophosphate/protein kinase A pathway through GPR173, and may upregulate the expression of GnRH and the GnRH receptor, ultimately stimulating reproductive function. In vitro studies have shown that PNX may directly affect the pituitary [21], enhancing gonadotropin expression in pituitary cells [22]. Additionally, PNX is expressed in the ovaries and follicles [21], where it promotes the production of E2 [23]. PNX can also stimulate the expression of genes involved in follicular development, affecting follicular maturation and increasing the number of oocytes [6]. Given the widespread role of PNX in sexual development, we anticipate that it may serve as a potential biomarker for adjunct diagnosis of CPP in girls. This study evaluated their expression levels and differences of PNX in girls with CPP, PT, and NCs. It also analyzed the correlation between PNX and clinical data, further exploring the diagnostic value of PNX for CPP. Our findings indicated that serum PNX-14 levels in the CPP group were significantly elevated relative to the PT group, with the PT group also showing higher levels than the NC group. However, serum PNX-20 levels did not show statistically significant differences across the three groups. We also found positive associations between serum PNX-14 levels with height, weight, BMI, bone age, uterine volume, ovarian volume, and pituitary height. These findings

suggest that serum PNX-14 reflects the physical and sexual development of girls. To further identify children with CPP among those with PP, we constructed a ROC curve to determine the optimal cutoff value. The results suggest that serum PNX-14 could be a potential biomarker for the adjunct diagnosis of CPP in girls. Sexual development in children begins with the activation of the gonadal axis, which triggers the onset of puberty and the subsequent maturation of reproductive organs. However, the mechanisms through which PNX contributes to PP are still being explored. PNX is expressed in various tissue systems, and for this analysis, we used serum PNX levels as an indicator of the overall PNX expression in the body. Future studies measuring tissue-specific PNX concentrations could offer a more thorough understanding of its role in various pathways. Our data indicate a progressive increase in serum PNX-20 levels from NC through PT to CPP groups. However, unlike PNX-14, this trend did not reach statistical significance. Potential explanations include differential bioactivity between PNX isoforms (analogous to 3,5,3′-triiodothyronine/thyroxine dynamics in hyperthyroidism) or tissue-specific distribution (eg, higher hypothalamic vs circulating concentrations). This hypothesis requires validation through larger-scale studies. Our findings demonstrate a statistically significant association between PNX-14 and pubertal progression in girls. To further investigate this relationship, we are conducting longitudinal follow-up with regular monitoring of developmental milestones during clinical visits and collecting serial blood samples at predetermined intervals (particularly around menarche) for future PNX-14 analyses. Additionally, we observed that ovarian volume, uterine volume, and pituitary height have good potential in differentiating between CPP and PT. Since these parameters are closely related to age, future studies could explore their relationships further through age stratification.

Acknowledgments

We sincerely thank all the parents and children who selflessly contributed to this study on behalf of all members of our research team.

Funding

This work was supported by the National Natural Science Foundation of China (grant No. 81900772) and the China Postdoctoral Science Foundation (grant No. 2023M741501). The financial support provided by these organizations was instrumental in facilitating the study and enabling the completion of the research. The authors express their sincere gratitude for the funding, which played a critical role in advancing the scientific investigation presented in this paper.

Author Contributions

Ye Yang and Shuyi Yang handled data curation and drafted the original version of the manuscript. Siqing Li, Jingjing Zhang, and Fei Zhu contributed to data curation and performed the investigation, gathering the necessary data for the study. Xiaoxiao Pan, Xiaona Li, and Ying Wang handled the data analysis, ensuring accurate interpretation of the results. Hua Bai and Peiliang Luo took charge of the conceptualization of the study and reviewed the manuscript, providing critical feedback and guidance. Jun Sun and Yingdi Yuan initiated the research, conceived the overall study design, and supervised all aspects of the project, overseeing its execution and ensuring the scientific rigor of the work.

Disclosures

The authors confirm that this research was conducted independently, with no commercial or financial ties posing a conflict of interest.

Data Availability

Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding authors on reasonable request.

References

1. Bräuner EV, Busch AS, Eckert-Lind C, Koch T, Hickey M, Juul A. Trends in the incidence of central precocious puberty and normal variant puberty among children in Denmark, 1998 to 2017. *JAMA Netw Open*. 2020;3(10):e2015665.
2. Subspecialty Group of Endocrinologic, Hereditary and Metabolic Diseases, the Society of Pediatrics, Chinese Medical Association. [Expert consensus on the diagnosis and treatment of central precocious puberty(2022)]. *Zhonghua Er Ke Za Zhi*. 2023;61(1):16-22.
3. Zevin EL, Eugster EA. Central precocious puberty: a review of diagnosis, treatment, and outcomes. *Lancet Child Adolesc Health*. 2023;7(12):886-896.
4. Knific T, Lazarevič M, Žibert J, *et al*. Final adult height in children with central precocious puberty—a retrospective study. *Front Endocrinol (Lausanne)*. 2022;13:1008474.
5. Dennerlein S, Oeljeklaus S, Jans D, *et al*. MITRAC7 acts as a COX1-specific chaperone and reveals a checkpoint during cytochrome c oxidase assembly. *Cell Rep*. 2015;12(10):1644-1655.
6. Billert M, Rak A, Nowak KW, Skrzypski M. Phoenixin: more than reproductive peptide. *Int J Mol Sci*. 2020;21(21):8378.
7. Pałasz A, Janas-Kozik M, Borrow A, Arias-Carrión O, Worthington JJ. The potential role of the novel hypothalamic neuropeptides nesfatin-1, phoenixin, spexin and kisspeptin in the pathogenesis of anxiety and anorexia nervosa. *Neurochem Int*. 2018;113:120-136.
8. Berberoğlu M. Precocious puberty and normal variant puberty: definition, etiology, diagnosis and current management. *J Clin Res Pediatr Endocrinol*. 2009;1(4):164-174.
9. Brito VN, Canton APM, Seraphim CE, *et al*. The congenital and acquired mechanisms implicated in the etiology of central precocious puberty. *Endocr Rev*. 2023;44(2):193-221.
10. Yosten GL, Lyu RM, Hsueh AJ, *et al*. A novel reproductive peptide, phoenixin. *J Neuroendocrinol*. 2013;25(2):206-215.
11. Clarke SA, Dhillo WS. Phoenixin and its role in reproductive hormone release. *Semin Reprod Med*. 2019;37(4):191-196.
12. Lyu RM, Cowan A, Zhang Y, *et al*. Phoenixin: a novel brain-gut-skin peptide with multiple bioactivity. *Acta Pharmacol Sin*. 2018;39(5):770-773.
13. Rocca C, Scavello F, Granieri MC, *et al*. Phoenixin-14: detection and novel physiological implications in cardiac modulation and cardioprotection. *Cell Mol Life Sci*. 2018;75(4):743-756.
14. Billert M, Kołodziejski PA, Strowski MZ, Nowak KW, Skrzypski M. Phoenixin-14 stimulates proliferation and insulin secretion in insulin producing INS-1E cells. *Biochim Biophys Acta Mol Cell Res*. 2019;1866(12):118533.
15. Kalamon N, Błaszczyk K, Szlaga A, *et al*. Levels of the neuropeptide phoenixin-14 and its receptor GRP173 in the hypothalamus, ovary

and periovarian adipose tissue in rat model of polycystic ovary syndrome. *Biochem Biophys Res Commun*. 2020;528(4):628-635.
16. Cundubey CR, Cam SD. Serum phoenixin-14 levels of women with polycystic ovary syndrome increase proportionally with BMI. *Eur Rev Med Pharmacol Sci*. 2023;27(8):3519-3525.
17. Akdu S, Can U, Polat E. Investigation of serum phoenixin levels in patients with hypertension. *Rev Assoc Med Bras (1992)*. 2022;68(6):814-819.
18. Wang S, Liang R, Liu H. Phoenixin-20 ameliorates brain infarction by promoting microglia M2 polarization in an ischemic stroke model. *Metab Brain Dis*. 2022;37(5):1517-1526.
19. Friedrich T, Stengel A. Current state of phoenixin-the implications of the pleiotropic peptide in stress and its potential as a therapeutic target. *Front Pharmacol*. 2023;14:1076800.
20. Treen AK, Luo V, Belsham DD. Phoenixin activates immortalized GnRH and kisspeptin neurons through the novel receptor GPR173. *Mol Endocrinol*. 2016;30(8):872-888.
21. Parlak Ak T, Yaman M, Bayrakdar A, Bulmus O. Expression of phoenixin-14 and nesfatin-1 in the hypothalamo-pituitary-gonadal axis in the phases of the estrous cycle. *Neuropeptides*. 2023;97:102299.
22. Ullah K, Ur Rahman T, Wu DD, *et al*. Phoenixin-14 concentrations are increased in association with luteinizing hormone and nesfatin-1 concentrations in women with polycystic ovary syndrome. *Clin Chim Acta*. 2017;471:243-247.
23. Prinz P, Scharner S, Friedrich T, *et al*. Central and peripheral expression sites of phoenixin-14 immunoreactivity in rats. *Biochem Biophys Res Commun*. 2017;493(1):195-201.