

# Role of Body Weight in the Onset and the Progression of Idiopathic Premature Pubarche

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## Keywords

Premature pubarche · Low birth weight · Body mass index · Predictor factors

## Abstract

**Background:** The term premature pubarche (PP) refers to the appearance of pubic hair before age 8 in girls and before age 9 in boys. Although idiopathic PP (often associated with premature adrenarche) is considered an extreme variation from the norm, it may be an initial sign of persistent hyperandrogenism. Factors contributing to PP onset and progression have not been identified to date. **Aims:** The objectives of this study are to describe a group of Italian children with PP, to identify potential factors for its onset, and to define its clinical and biochemical progression. **Methods:** We retrospectively enrolled all infants born between 2001 and 2014 with PP. Children with advanced bone age (BA) underwent functional tests to determine the cause of PP. Hormonal analysis and BA determination were performed annually during a 4-year follow-up period. **Results:** A total of 334 children with PP were identified: idiopathic PP (92.5%, associated with premature adrenarche in some cases); related to

precocious puberty (6.6%); late-onset 21-hydroxylase deficiency (0.9%). Low birth weight was associated with premature adrenal activation. Body mass index (BMI) was the only factor that influenced the progression of BA during follow-up. **Conclusions:** Low birth weight is a predisposing factor for premature adrenal activation. The increase in BMI in patients with idiopathic PP during the 4-years of follow-up was responsible for BA acceleration. We recommend prevention of excessive weight gain in children with PP and strict adherence to follow-up in order to prevent serious metabolic consequences.

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## Introduction

Premature pubarche (PP) refers to the appearance of pubic hair, often accompanied by axillary hair, before the age of 8 in girls and before the age of 9 in boys [1]. It occurs more frequently in females, and the cause of the unequal sex ratio is unknown [2–4]. In the majority of cases, PP is idiopathic (due to premature adrenarche) and can be determined only by diagnosis of exclusion [2, 3, 5].

Other disorders, such as milder and nonclassical congenital adrenal hyperplasia (CAH), Cushing's disease, virilizing tumors originating from the adrenals or the gonads, or more rare conditions, may also manifest with PP [3, 6, 7]. A diagnosis of precocious puberty should be considered in patients with progressive signs of pubertal development (e.g., breast development in girls and penile or testicular growth in boys) besides clinical signs of PP [3].

The pathophysiological basis of idiopathic PP and premature adrenarche is unknown [1]. It is secondary to early and isolated maturation of the zona reticularis of the adrenal cortex and is triggered by the activation of androgen production, resulting in a dramatic increase in serum DHEAS levels [8–10]. DHEAS levels  $\geq 40$ –50  $\mu\text{g/dL}$  are consistent with the onset of adrenarche [3, 11, 12]. DHEAS is converted to active androgens in the gonads and the peripheral target tissues of androgen action, including the skin, leading to the appearance of pubic and axillary hair. Increased androgen production also brings about changes in body odor and oily skin, transient growth spurt, and it contributes to bone maturation [7, 13, 14]. In patients with PP, however, bone age (BA) advancement is linked not only to elevated DHEAS levels but also to obesity, another concurrent factor in these children [12, 13]. Finally, hypersensitivity of hair follicles to steroid hormones has been hypothesized in children with PP and normal androgen levels [5, 15].

Idiopathic PP is considered an extreme variation from the norm; as such, it is a serious source of concern for parents. Furthermore, it may be a risk factor for later dysfunction of the reproductive endocrine system [3, 7, 12]. The main question is whether idiopathic PP (and premature adrenarche) is a form of early adrenal maturation or an initial sign of persistent hyperandrogenism. To answer this question, studies have investigated the potential factors for PP onset; however, none to date has been conclusively identified as a regulator of adrenal androgen secretion. Ethnic origin, elevated body mass index (BMI), and weight gain have all been reported to be factors contributing to adrenarche [3, 8, 12], whereas elevated 17-hydroxyprogesterone (17-OHP) level at newborn screening has been recently excluded as a predictive factor for PP [16]. Furthermore, we do not know which predictor factors are able to identify the clinical and biochemical progression of PP during infancy and puberty. With the abovementioned points in mind, the aims of this study were to describe a group of Italian children with PP, to identify potential factors for its onset, and to define its clinical and biochemical progression.

## Patients and Methods

### Patients

For this retrospective study, we reviewed the clinical data of Caucasian patients born between 2001 and 2014 referred to the Pediatric Endocrinology Division of Verona Hospital, Italy, for the appearance of pubic hair before the age of 8 in girls and before the age of 9 in boys. Weight, height, and BMI (weight in kg/height in  $\text{m}^2$ ) were recorded. The height, weight, and BMI standard deviation score (SDS) were derived from Italian cross-sectional growth charts [17]. Pubertal development was evaluated according to Tanner and Whitehouse [18]. For boys, testicular volume was measured using a Prader orchidometer.

Gestational age (GA), birth weight (BW), and birth length (BL) were evaluated, and familiar genetic target was calculated according to the parents' height. Target height was determined by calculating the midparental height as follows:  $([\text{father's height cm} - 13 \text{ cm}] + \text{mother's height cm})/2$  for girls;  $([\text{mother's height cm} + 13 \text{ cm}] + \text{father's height cm})/2$  for boys [19]. Children born small for gestational age (SGA) were defined according to Bertino Neonatal Anthropometric Charts [20]. We defined SGA as a newborn with a birth weight and/or length of  $< -2$  SD according to the Consensus Statement of the International Societies of Pediatric Endocrinology and of the Growth Hormone Research Society [21]. Figure 1 presents the study flowchart showing the number of patients and type of tests and radiological exams performed.

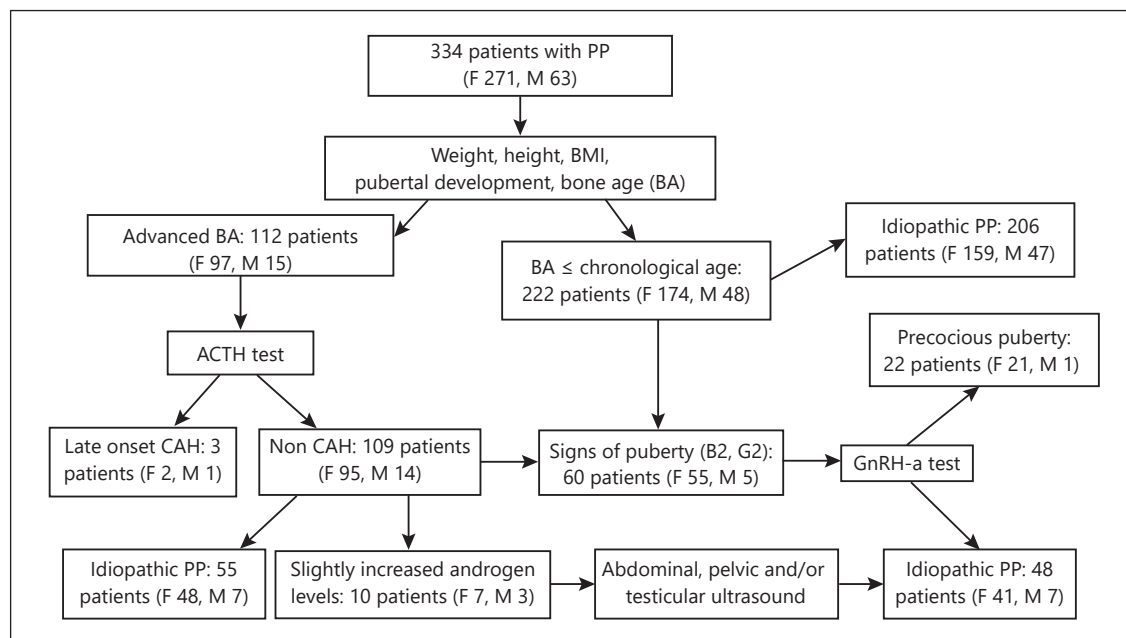
Radiography was performed in all children to determine BA, which was evaluated by the same pediatric endocrinologist using the Greulich and Pyle method [22]. Children with advanced BA (ratio of BA to chronological age  $> 1$ ) underwent the adrenocorticotrophic hormone (ACTH) stimulation test (intravenous administration of soluble Synacthen 250 mg). Serum 17-OHP and cortisol levels were measured at baseline and after 60 min. Data were analyzed using Maria New nomogram [23]. Levels of testosterone, ACTH, DHEAS, and  $\Delta 4$ -androstenedione were also measured.

Children with clinical signs of precocious puberty (Tanner stage  $\geq B2$  in girls and testicular volume  $\geq 4$  mL in boys) were submitted to GnRH-analogue (GnRH-a) stimulation testing (subcutaneous administration of 0.1 mg of GnRH-a (Triptorelin) after overnight fasting). Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol in females, and testosterone in males were measured at baseline and 4 h afterward. A LH peak of  $\geq 15$  U/L was indicative of activation of the hypothalamic-pituitary-gonadal (HPG) axis in both sexes.

Abdominal, pelvic, and/or testicular ultrasound studies were performed in patients with elevated androgen levels and advanced BA to exclude organic causes for PP, such as ovarian, testicular, or adrenal tumors. Pelvic ultrasound was performed to measure uterine and ovarian size in females with pubertal signs.

Children with idiopathic PP and advanced BA, born between 2001 and 2012, were followed up for an average period of 4 years. Children born after 2012 were excluded from the present analysis because the follow-up period was not yet complete. Exams at follow-up assessment included repeated BA and hormonal analysis (DHEAS,  $\Delta 4$ -androstenedione, ACTH, and cortisol). Two groups were formed based on DHEAS level at the time of diagnosis.

The present study was conducted in compliance with the terms of the Helsinki II Declaration. The Institutional Ethics Committee of the provinces of Verona and Rovigo, Italy, took note of the retrospective conduct of the study and approved it for publication of



**Fig. 1.** Study flowchart. PP, premature pubarche; BA, bone age; ACTH, adrenocorticotrophic hormone; CAH, congenital adrenal hyperplasia.

the results. Written, informed consent was obtained from the child's parents or legal guardian.

#### Assay

Serum total testosterone, ACTH, cortisol,  $\Delta 4$ -androstenedione, DHEAS, estradiol, FSH, and LH were measured using a solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000; Siemens Healthcare Diagnostic, USA). The analytical sensitivity was 14.4 ng/dL for total testosterone, 5 pg/mL for ACTH, 0.2  $\mu$ g/dL for cortisol, 0.3 ng/mL for  $\Delta 4$ -androstenedione, 0.3  $\mu$ g/dL for DHEAS, 0.1 U/L for FSH, 0.05 U/L for LH, and 15 pg/mL for estradiol.

Intraassay and interassay coefficients of variation were 11.7 and 13.0%, respectively, for testosterone; 9.5 and 10.0%, respectively, for ACTH; 7.4 and 9.4%, respectively, for cortisol; 9.3 and 12%, respectively, for  $\Delta 4$ -androstenedione; 4.1 and 6.3%, respectively, for DHEAS; 2.9 and 4.1%, respectively, for FSH; 3.6 and 6.7%, respectively, for LH; 11.7 and 13%, respectively, for estradiol. Serum 17 $\alpha$ -OHP was assayed using a radioimmunoassay (DSL-5000 Active 17 $\alpha$ -OH Progesterone Coated-Tube Radioimmunoassay, Diasorin, Saluggia, Italy). The assay sensitivity was 0.01 ng/dL, and intra- and interassay coefficients of variation were both 9.0%.

#### Statistical Analysis

Statistical analyses were performed using software R version 3.5.1 (R Core Team [2018] R: A language and environment for statistical computing; R Foundation for Statistical Computing, Vienna, Austria). Since the majority of the variables were not normally distributed, nonparametric statistics were employed. The Mann-Whitney *U* test was used to compare differences between 2 independent groups, and Friedman's nonparametric ANOVA was

employed to compare differences between time points. Multiple linear regression analysis was also performed in order to explore the relationship between DHEAS levels and selected independent variables (BW SDS, GA, BMI SDS, age, and sex) at the time of DHEAS measurement. Furthermore, multiple regression analysis was performed at the last follow-up visit using BA acceleration ( $\Delta$ BA) as the dependent variable and BMI SDS, sex, age, GA, Tanner genital/breast stage, and serum DHEAS as independent variables.

Correlations were evaluated using either the Spearman or the Pearson correlation test, when appropriate. Fisher's exact test was used to compare 2 proportions. Data are expressed as numbers with frequency, median plus range, or mean  $\pm$  SD, as appropriate. Statistical significance was set at  $p \leq 0.05$ .

## Results

Between 2001 and 2014, we identified 334 patients with PP (271 females and 63 males). Table 1 presents the clinical data and the neonatal characteristics of the patients. Forty children (12%) in the cohort were born premature and 8 (2.4%) were SGA; 43.7% of the children were overweight.

Of the total of 334 patients, 112 (97 females and 15 males) had a BA to chronological age ratio of  $>1$  (Fig. 1). The difference between BA and chronological age was  $1.3 \pm 1.0$  years for the females and  $1.4 \pm 1.0$  years for the

**Table 1.** Clinical characteristics of all patients at diagnosis and at birth

	Females ( <i>n</i> = 271)	Males ( <i>n</i> = 63)
Age, years	7.2±1.7 (0.5 to 10.3)	7.8±2.1 (0.4 to 9.9)
Weight SDS	3.1±2.1 (-1.3 to 11.4)	3.0±2.4 (-1.6 to 7.9)
Height SDS	2.0±1.2 (-1.7 to 5.4)	1.8±1.2 (-1.3 to 5.3)
BMI SDS	2.0±1.2 (-2.2 to 9.9)	2.5±2.7 (-2.2 to 8.6)
Target height SDS	0.2±0.9 (-2.4 to 2.9)	0.4±0.9 (-1.5 to 2.4)
Pubic hair, <i>n</i> (%)		
Tanner Ph 2	247 (91.1)	57 (90.5)
Tanner Ph 3	24 (8.9)	6 (9.5)
Tanner Ph 4	0	0
Tanner Ph 5	0	0
Axillary hair, <i>n</i> (%)		
Tanner A1	249 (91.9)	60 (95.2)
Tanner A2	20 (7.4)	2 (3.2)
Tanner A3	2 (0.7)	1 (1.6)
Breast, <i>n</i> (%)		
Tanner B1	216 (79.7)	-
Tanner B2	43 (15.9)	
Tanner B3	12 (4.4)	
Tanner B4	0	
Tanner B5	0	
Genitalia, <i>n</i> (%)		
Tanner G1	-	58 (92)
Tanner G2		5 (8)
Tanner G3		0
Tanner G4		0
Tanner G5		0
GA, weeks	38.6±2.5 (25.0 to 42.1)	38.1±2.7 (29.0 to 41.0)
BW, g	3,045.9±638.0 (787.0 to 5,000.0)	3,081.0±746.9 (950.0 to 4,260.0)
BL, cm	49.3±2.8 (25.0 to 54.5)	49.9±3.5 (35.0 to 55.0)

Data are expressed as mean ± SD and range in the brackets. SDS, standard deviation score; GA, gestational age; BW, birth weight; BL, birth length.

males. The mean age was 7.3 ± 1.8 years for the females and 8.6 ± 1.3 years for the males. Table 2 presents the results of blood analysis to identify the etiology of PP and the results of the GnRH-a test to determine activation of the HPG axis in the 60 children presenting clinical signs of puberty.

PP was defined as idiopathic in 92.5% (248 females and 61 males), related to precocious puberty in 6.6% (21 females and 1 male) and a manifestation of late-onset CAH due to 21-hydroxylase deficiency (21-OHD) in 0.9% (2 females and 1 male). In 25 children (24 females and 1 male), the 17-OHP level measured at the ACTH test was compatible with being heterozygous for 21-OHD based on Maria New nomogram [22].

Comparison between the females with precocious puberty and those with idiopathic PP and advanced BA showed that those with precocious puberty were older

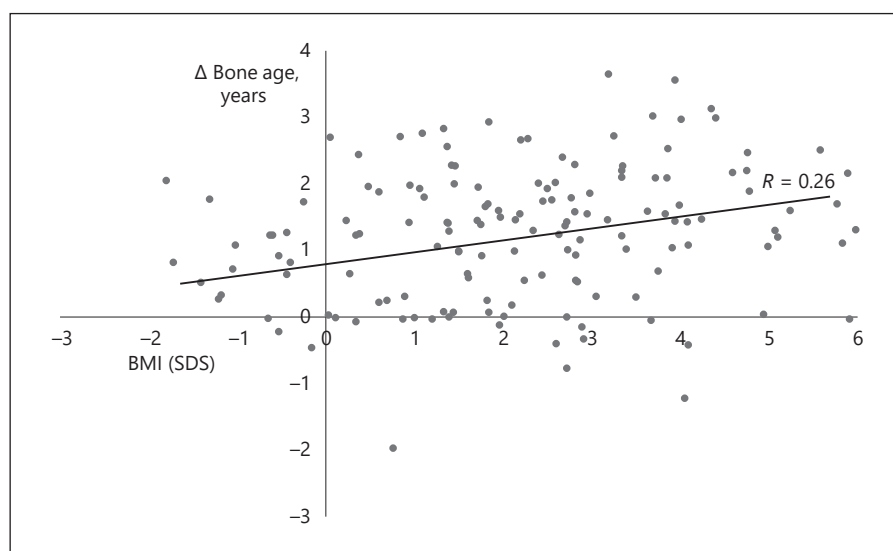
(8.0 ± 0.9 vs. 7.2 ± 1.2 years, *p* = 0.043) and had higher FSH and LH basal levels (FSH 4.2 ± 2.5 vs. 1.8 ± 1.6 U/L; LH 1.3 ± 1.5 vs. 0.2 ± 0.9 U/L; *p* < 0.001 for all comparisons). Basal LH >2.2 U/L and basal FSH >8.7 U/L levels were predictive of precocious puberty (sensitivity 99.3%). Owing to the small number of males, these data were analyzed only for the females.

Clinical and biochemical evaluation was performed annually for 4 years in all patients with idiopathic PP and advanced BA (87 females and 13 males). Ten children in the cohort (10%) were born preterm, and one (1%) was SGA. Three children (2 females and 1 male) were excluded from this analysis because they were born after 2012. At each assessment, hormonal levels, auxological data, and BA were recorded with a particular focus on 2 parameters: growth rate and ΔBA (difference between bone and chronological age). A growth rate of >5.5 cm/year and an

**Table 2.** Blood exams (ACTH test and GnRH-analogue test) at the time of diagnosis

	Females ( <i>n</i> = 112)	Males ( <i>n</i> = 16)
Δ4-androstenedione, ng/mL	0.6±0.5 (0.3–3.0)	0.7±0.4 (0.3–1.9)
DHEAS, μg/dL	66.6±42.5 (0.4–248.0)	117.8±58.3 (15.0–291.0)
ACTH, pg/mL	29.1±44.8 (6.2–373.0)	27.2±26.3 (8.7–135.0)
ACTH test		
Cortisol basal, μg/dL	11.8±8.6 (1.6–6.0)	9.5±4.5 (6.1–29.6)
Cortisol peak, μg/dL	26.8±5.4 (1.0–42.9)	24.4±5.8 (4.7–33.3)
17 OHP basal, ng/dL	1.2±2 (0.1–15.7)	1.7±3.7 (0.3–18.0)
17 OHP peak, ng/dL	4.7±8.9 (1.1–76.0)	7.5±20.7 (1.5–110.0)
GnRH-analogue test		
FSH basal, U/L	2.2±1.9 (0.1–9.7)	1.2±1.1 (0.3–4.3)
FSH peak, U/L	22.9±13.1 (1.7–83.6)	9.5±4.6 (3.7–19.4)
LH basal, U/L	0.4±1.1 (0.1–10.0)	0.3±0.4 (0.1–1.8)
LH peak, U/L	8.1±16.3 (0.1–111.0)	6.5±6.8 (0.9–27.3)
Estradiol basal, pg/mL	38.2±28.1 (80.2–195.0)	–
Estradiol peak, pg/mL	40.1±35.2 (19.8–264.0)	–
Testosterone basal, ng/dL	–	20.3±6.8 (0.3–39.6)
Testosterone peak, ng/dL	–	22.3±14.7 (2.1–72.8)

Data are expressed as mean ± SD and range in the brackets. ACTH, adrenocorticotrophic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.



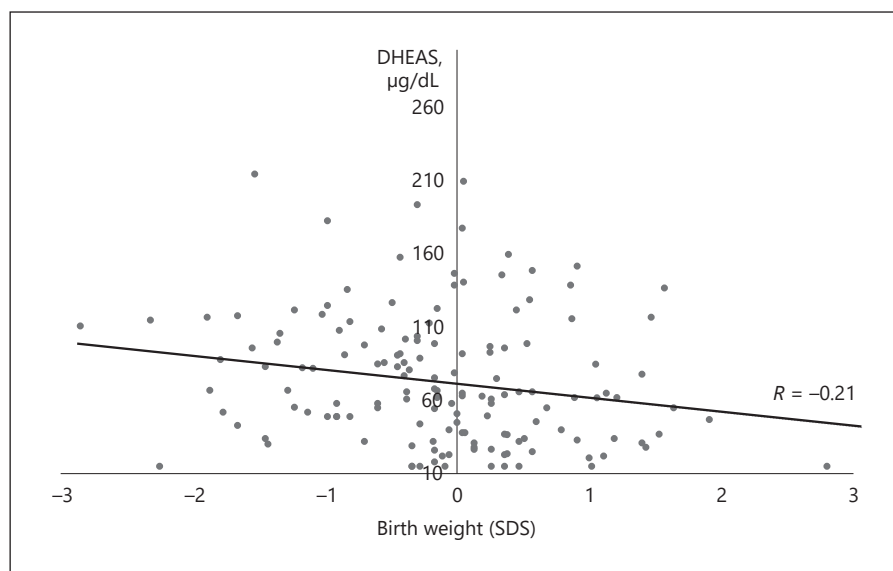
**Fig. 2.** Relationship between BMI SDS and the difference between bone age and chronological age ( $\Delta$ BA) ( $R = 0.26$ ;  $p = 0.011$ ). The bone age used for the  $\Delta$ BA was determined during the last follow-up visit, and the BMI shown in the figure was determined at the same visit. BMI, body mass index; SDS, standard deviation score.

advanced BA of >1.5 years were considered a marker of progression of the condition. No predictive factor for growth rate was identified; the only factor that was positively and significantly ( $p = 0.011$ ) associated with  $\Delta$  BA was BMI (Fig. 2). This association remained significant ( $p = 0.007$ ) even after having adjusted for the effects of sex, age, GA, Tanner genital/breast stage, and serum DHEAS.

The cohort was divided into 2 groups according to serum DHEAS level: one group with PP and elevated DHEAS ( $\geq 40$  μg/dL) and the other with PP and normal DHEAS ( $< 40$  μg/dL). Table 3 presents the characteristics of the 2 groups. The group with elevated DHEAS was noted to have had a lower weight at birth ( $p < 0.05$ ), suggesting an association between lower BW and higher adrenal androgen secretion (Fig. 3). Multiple linear regression analysis confirmed this observation; serum levels of



**Fig. 3.** Inverse relationship between DHEAS level at the time of diagnosis and birth weight (BW SDS): patients with lower BW had higher serum DHEAS levels ( $R = -0.21$ ;  $p < 0.01$ ). SDS, standard deviation score.



**Table 3.** Main characteristics of the 2 groups based on DHEAS levels at the time of diagnosis

	DHEAS <40 µg/dL (n = 38)	DHEAS ≥40 µg/dL (n = 62)	p value
GA, weeks	38.8±2.6 (26.0–41.0)	38.3±2.6 (25.0–41.0)	0.261
BW, g	3,248.6±564.2 (1,000.0–5,000.0)	2,956.5±666.4 (787.0–4,660.0)	<b>0.003</b>
BL, cm	49.9±2.9 (51.0–54.0)	49.4±2.4 (41.0–55.0)	0.368
BMI, kg/m <sup>2</sup>	18.8±2.5 (14.8–26.2)	16.9±6.6 (13.3–28.3)	0.810
Δ4-androstenedione, ng/mL	0.4±0.2 (0.3–1.1)	0.6±0.4 (0.3–3.0)	<b>0.007</b>
ACTH, pg/mL	32.7±53.7 (9.0–373.0)	27.6±38.3 (6.2–344.0)	0.494
Testosterone, ng/dL	6.5±9.4 (0.2–20.0)	7.1±10.9 (0.2–20.0)	0.748
Cortisol, µg/dL	11.3±5.5 (3.6–26.3)	10.5±6.0 (1.6–36.4)	0.420

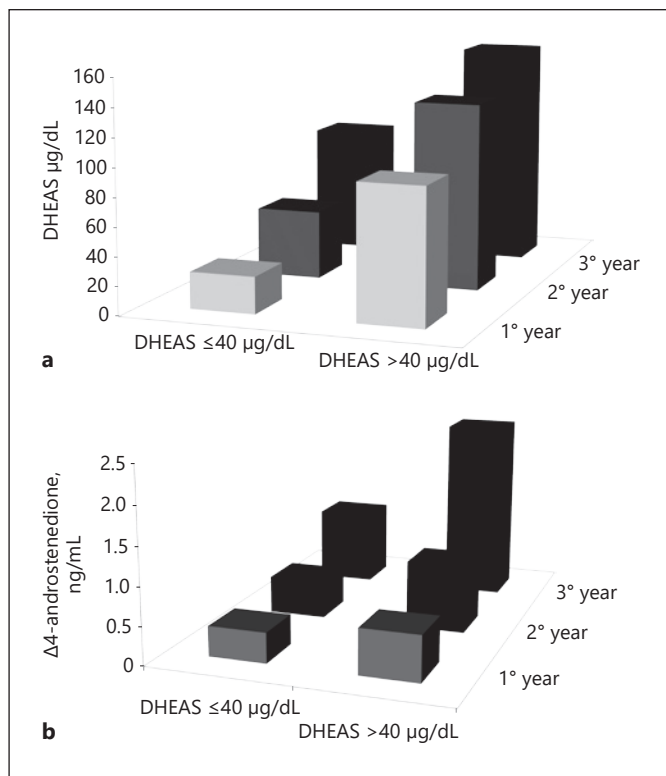
Data are expressed as mean ± SD and range in the brackets. Bold values represent statistical significance. GA, gestational age; BW, birth weight; BL, birth length; ACTH, adrenocorticotrophic hormone.

DHEAS (dependent variable) were significantly ( $p < 0.001$ ) associated with BW SDS even after having adjusted for the effects of sex, age, GA, and BMI SDS. In group with DHEAS ≥40 µg/dL, 16% had a BW of <2,500 g, whereas in the other group (DHEAS <40 µg/dL), only 2.6% had a BW of <2500 g ( $p < 0.05$ ). The serum Δ4-androstenedione levels were higher in the group with elevated DHEAS, with a positive correlation between the serum DHEAS and Δ4-androstenedione levels measured during the follow-up ( $p < 0.05$ ). The serum ACTH and cortisol levels remained stable during the follow-up period. We found a significant difference between DHEAS levels in the 2 groups at each assessment during the 4 years of follow-up ( $p < 0.05$ ) (Fig. 4). The growth rate in both groups was regular without a significance difference during the follow-up; there was a slight increase in the percentage of

overweight children (from 50.5% at year 1 to 51.8% at year 4), without significance differences between the 2 groups. Pubertal development began at age  $8.9 \pm 0.7$  years in the females and at age  $10.8 \pm 1.2$  years in the males. These data did not differ between the children born pre-term and those born at term.

## Discussion

For this retrospective study, we analyzed a cohort of patients of Caucasian origin with PP followed up for a minimum of 4 years at a single center in northeast Italy. Idiopathic PP was diagnosed in the majority of patients, and body weight played a role in the onset and the clinical progression of PP: low BW was associated with prema-



**Fig. 4.** Mean serum DHEAS (panel **a**) and 4-androstenedione (panel **b**) levels during 3 years of follow-up in the 2 groups (DHEAS >40 or <40 µg/dL at the time of diagnosis). We found a significant difference between DHEAS levels during the follow-up in the 2 groups at each evaluation ( $p < 0.05$ ). There was a significant difference in  $\Delta 4$ -androstenedione levels between the 2 groups only at the third year of follow-up.

ture adrenal activation and increased BMI was the only factor that influenced BA acceleration during the follow-up period.

Only 3 patients in the cohort presented late-onset CAH, consistent with a prevalence estimated between 0 and 40% [7, 24–27]. Furthermore, 7.5% presented 17-OHP levels at the ACTH test indicative of heterozygosity for 21-OHD. Although genetic analysis was not performed, we hypothesized an association between heterozygosity for 21-OHD and the presence of PP. This association has been variously studied [16, 28–30] with some studies reporting a similar risk for mutation carriers and normal subjects [31, 32] and others underlining the association, especially in subjects carrying the V281L mutation [33, 34]. The mechanism behind this association remains unknown.

Precocious puberty was noted in 6.6% of the patients with PP. PP may be secondary to or concomitant with

precocious puberty. The incidence of precocious puberty was higher in the females, as reported elsewhere [35]. There was a significant difference between basal LH and FSH levels in the girls with precocious puberty compared to those with idiopathic PP. LH >2.2 U/L was associated with activation of the HPG axis (sensitivity 99%). The role of basal LH in the diagnosis of precocious puberty has been discussed in the literature; however, the consensus statement on precocious puberty recommends luteinizing hormone-releasing hormone or GnRH-a testing to identify central precocious puberty [36]. While we doubt that baseline LH is sufficient for a diagnosis of precocious puberty, LH levels >2.2 U/L in girls with PP and thelarche should arouse suspicion. Previous studies showed in fact that elevated basal LH levels were significant predictors of central precocious puberty and suggested a threshold of LH >1.6 U/L [37–39].

Although PP is a relatively common, usually nonprogressive, clinical sign, its natural history is not well known [8]; it may be correlated with metabolic disorders including obesity, hyperinsulinism, hyperandrogenism, and polycystic ovary syndrome in females [1, 3, 7]. No factors contributing to PP onset and progression have been identified to date. Numerous studies found a relationship between low BW and androgen excess [3, 8, 12, 40]. Weight gain during infancy seems to trigger adrenarche [1]. Some studies have hypothesized that malnutrition in prenatal life could lead to metabolic programming of fetal organs and result in metabolic alterations, such as hyperinsulinemia and premature androgen excess [8, 41]. While French, Portuguese, Finnish, and Scottish studies reported no association with low BW in children with premature adrenarche [42–45], other studies from Spain, Italy, France, and Australia found a correlation between low BW and adrenarche [46–49], which our data confirm. A role for low BW in the onset of adrenarche in relation to country of origin has not been established, but genetic diversity might be responsible for the difference [9]. The relationship that we found between premature adrenarche and BW was adjusted for sex, age, GA, and BMI. Consequently, it is possible to exclude a role of preterm delivery in the onset of premature adrenarche. Although SGA patients may also experience premature adrenarche [12, 49], only 1 patient in the cohort was born SGA, precluding definitive conclusions about its role.

PP has been associated with obesity, hyperinsulinemia, and hyperandrogenism; however, it is not clear whether the primum movens is overweight or hyperandrogenism. A higher prevalence of overweight among children with PP has been recently documented [47, 50–52], and body

weight is thought to be involved in the onset of pubarche [1, 3]. Some studies have shown that overweight girls tend to experience an earlier onset and a more rapid evolution of puberty and pubarche [53–55]. Other studies have described PP as an early clinical feature of metabolic syndrome [50, 56]. Our data suggest that hyperandrogenism is a primary factor and overweight a secondary factor. Although overweight was predominant in this cohort, both at diagnosis and during follow-up, we found no association between PP and overweight. On the contrary, during the follow-up, when adrenal androgen secretion was already active, the rise in BMI played an important role in the advancement of BA. Obese children are noted to present advanced BA secondary to increased adipose tissue aromatization of androgens into estrogens, which are the sex hormones critically involved in skeletal maturation [12, 50]. In addition, hyperinsulinemia and high leptin levels, both consequences of obesity, can contribute to bone maturation. Hyperinsulinemia can act on the IGF-1 receptor and stimulate growth, while leptin appears to act as a skeletal growth factor [57, 58]. Furthermore, androgen action, which potentiates the effect of obesity on advancement of BA, has been described in children with obesity and/or pubarche [13, 59, 60] but not confirmed by the data for our cohort.

The role of androgens in PP is debated. DHEA and DHEAS are the best serum markers of adrenal androgen secretion [3]. The variable peripheral conversion of these circulating adrenal androgen precursors to the biologically more active androgens, testosterone and dihydrotestosterone, is essential for androgen action. Normal ACTH secretion and action are needed for adrenarche [3, 8, 12]. The data for our cohort show normal testosterone, ACTH, and cortisol concentrations. Moreover, we observed a linear rise in DHEAS and  $\Delta 4$ -androstenedione levels, which gradually increased over the 4-year follow-up period. We believe that in addition to DHEAS, the elevated  $\Delta 4$ -androstenedione levels were also a marker of adrenarche in our cohort; however, this hypothesis needs to be confirmed in larger patient populations in prospective clinical trials. Furthermore, since  $\Delta 4$ -androstenedione can be formed peripherally from DHEAS, as well as directly from the gonads, circulating  $\Delta 4$ -androstenedione levels do not necessarily reflect the adrenal production rate [3]. Finally, we found no direct relationship between androgen concentrations and obesity, advancement of BA, and increased growth rate.

During the 4-year follow-up, the children in the cohort presented a regular and linear growth rate, confirming previous data which described only a transient accelera-

tion in growth in children with PP. Moreover, pubertal development began at normal age in both sexes, in line with previous studies [3, 12, 14, 61, 62].

The limitations of this study are its retrospective design, the lack of a control group, the absence of metabolic data, the absence of final height and weight, and a population sample consisting only of patients of Caucasian origin. Nevertheless, the objective was to describe a cohort of Italian children with PP and to identify potential predictive factors for its onset and progression. Evaluation of metabolic disorders or final height and weight was beyond the scope of the present study but will be an area of focus in a future study. Ethnicity seems to play a role in the onset of PP, with a higher prevalence reported among Hispanic and African-American girls [1, 8]. Data for other ethnic groups would have been interesting to evaluate, but given the lack of ethnic diversity in our area, we could not have drawn any meaningful conclusions.

In conclusion, the mechanism underlying PP remains unclear and is probably of multifactorial etiology. Low BW is a predisposing factor for premature adrenarche among children born in northeast Italy; the clinical and biochemical progression of PP depends on diverse factors, including increased BMI, which is responsible for BA acceleration. We recommend prevention of excessive weight gain in children with PP and strict adherence to follow-up in order to prevent serious metabolic consequences.

### Statement of Ethics

The study was conducted in compliance with the terms of the Helsinki II Declaration. The Institutional Ethics Committee of the provinces of Verona and Rovigo, Italy, took note of the retrospective conduct of the study and approved it in order to publish the results. Written informed consent was obtained from the parents of each patient.

### Conflict of Interest Statement

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the affirmation reported.

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## Author Contributions

All the authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Moreover, all authors read and approved the final

manuscript. P.C. and M.M.: study conception and acquisition, analysis, and interpretation of data; P.C., M.M., and R.G.: drafting the manuscript; R.M.: statistical analysis; P.C., F.A., R.G., R.M., and G.P.: critical revision of the manuscript; P.C. and F.A.: coordination of study.

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