Adrenarche and Puberty in Children with Classic Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency

Thomas M.K. Völkl^a Lisa Öhl^a Manfred Rauh^a Christof Schöfl^b
Helmuth G. Dörr^a

^aDivision of Pediatric Endocrinology and Diabetology, Department of Pediatrics and Adolescent Medicine, and

^bDivision of Endocrinology and Diabetology, First Department of Internal Medicine, Friedrich Alexander University of Erlangen-Nuremberg, Erlangen-Nuremberg, Germany

Key Words

Congenital adrenal hyperplasia · 21-Hydroxylase · Adrenarche, puberty

Abstract

There have been only a few studies on adrenarche in girls with classic congenital adrenal hyperplasia (CAH) showing that dehydroepiandrosterone sulfate (DHEAS) levels did not rise at the physiological age of adrenarche. **Objective:** Longitudinal analysis of serum DHEAS levels and Tanner stages in CAH children. **Design:** We studied 98 CAH patients (52 females), aged between 1 month and 18.0 years. All patients had genetically proven classic CAH and received steroid substitution therapy. **Results:** Serum DHEAS levels did not differ between CAH children and healthy children from the age of 1 year until 5–6 years. Beginning at the age of 7–8 years, there was a continuous but blunted increase in DHEAS levels in CAH boys and girls compared to healthy children. There was no correlation of DHEAS levels with the genotype, glucocorticoid dosage, auxological data, or quality of metabolic control. Pubarche (PH2) as well as gonadarche (G2) and thelarche (B2) occurred significantly earlier in CAH boys and girls than in the reference group, but timing of menarche

was normal. **Conclusions:** Pubarche and adrenarche are dissociated in classic CAH: earlier pubarche, gonadarche and thelarche, respectively, in both sexes contrast with the absence of typical adrenarche.

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Introduction

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is associated with abnormally low cortisol and low (salt wasting, SW-CAH) or normal aldosterone (simple virilizing, SV-CAH) production [1, 2]. Due to an intact feedback system with activation of the CRH-ACTH axis, the production of androgens and steroid precursors prior to the enzyme defect is increased [1, 2]. Adequate substitution therapy with glucocorticoids and mineralocorticoids is a prerequisite in order to avoid short-term and/or long-term complications such as elec-

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trolyte imbalances, addisonian crisis, accelerated bone maturation, short stature, hirsutism and virilization, decreased fertility, obesity, and hypertension [3–5].

There are contradictory reports on the onset and course of puberty in CAH. Earlier data show that the onset of puberty in treated patients is more or less normal [6–8]. However, these studies only report the onset of puberty, i.e. Tanner stages B2 or G2, and not their follow-up or pubic hair stages [6–8]. According to two recent papers, there is an earlier onset of pubarche and thelarche in treated children with classic CAH [9, 10], whereas timing of menarche is normal [10].

The increase in the secretion of dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) typically at the age of 5–6 years reflects the development of the zona reticularis of the adrenal cortex and is called adrenarche [11]. The mechanisms that initiate adrenarche are not known in detail to date. However, the increase in 17,20-lyase activity, IGF-1, and insulin concentrations, and the nutritional status play a role besides CRH and ACTH [11, 12]. Pubarche, the development of pubic hair, is the clinical sign of adrenarche. Both terms, pubarche and adrenarche, are usually used as synonyms, but a dissociation between pubarche and biochemical markers of adrenarche can occur, e.g. in girls with Turner syndrome [13]. The dichotomy whether DHEAS levels can serve as a measure of disease control or as a target for suppression by glucocorticoid therapy can be neglected, since in clinical practice, DHEAS levels are not used to measure disease control [1, 2]. CAH children with poor metabolic control have for example high serum 17-hydroxyprogesterone (17-OHP) and urine pregnanetriol (PT) levels but normal or low serum DHEAS levels.

Only a few studies on adrenarche in children with classic CAH have found low serum DHEAS levels in well-controlled patients [14–17]. Despite blunted adrenarche, the onset of pubarche (PH2) in children with classic CAH was found to be earlier in females and males with both clinical forms than in controls with a significantly earlier onset in SV-CAH than in SW-CAH [10].

Besides the known hormonal effects as an androgen, recent studies show positive effects of DHEA on well-being and sexuality in adult females with adrenal insufficiency [18]. Binder et al. [19] reported that atrichia pubis vanishes and psychological well-being improves significantly by daily replacement with 25 mg DHEA orally in adolescent girls with central adrenal insufficiency.

Hence, the aim of this retrospective, single-center, longitudinal study was to measure serum DHEAS levels in a large cohort of patients with classic CAH and to correlate

the data with Tanner stages of puberty. We studied potential contributing factors like medication dosage, bone age, height, and various laboratory parameters such as serum 17-OHP levels and 24-hour urine PT excretion.

Methods

Patients

We included 98 Caucasian children and adolescents (46 males, 52 females), who presented regularly at our outpatient endocrine unit. All individuals had classic CAH with 21-hydroxylase deficiency (salt wasting, SW: $n = 79$; simple virilizing, SV: $n = 19$). The phenotype classification was based on clinical and hormonal criteria, and the diagnosis was confirmed in all patients with molecular genetic analyses by direct sequencing (after exclusion of deletions). The disease-causing mutations were divided into four mutation groups (0, A, B, and D) as previously described by Speiser et al. [20] (online suppl. material 1, for all online suppl. material, see www.karger.com/doi/10.1159/000333696). All patients received glucocorticoid substitution/androgen-suppressive therapy with hydrocortisone (HC), prednisone (PR) or dexamethasone (DX). HC was given three times daily (~50% of the daily dosage in the early morning, 25% at noon, and 25% in the evening); PR was given twice and DX once daily in the morning. 96 patients additionally received fludrocortisone (twice daily) due to mineralocorticoid insufficiency diagnosed based on elevated renin concentrations. The quality of therapy was monitored during follow-up visits every 3–6 months by clinical presentation, bone age (BA) acceleration and laboratory measurements according to current guidelines [21]. None of the patients included in this study showed signs of severe under- or overtreatment such as Cushing's syndrome, severe hirsutism, increasing clitoris length, or growth failure.

Study Design

The longitudinal data of all patients were retrospectively ascertained from patient charts and the digital in-house CAH database. Based on the 98 patients, data from a total of 1,047 patient-years were available (males 433 years, females 614 years). The average available follow-up interval was $10.7 \text{ years} \pm 5.05 \text{ SD}$, median 10 years, range 1–18 years (males $9.41 \pm 5.29 \text{ years}$, females $11.8 \pm 4.59 \text{ years}$). The study was approved by our institutional review board. All subjects and parents gave their written informed consent/assent.

Standardized physical examination in our department included the measurement of height (Harpender stadiometer), weight (without clothes, except underwear), and the assessment of pubertal status (Tanner stages). Height standard deviation scores (SDS) were calculated using German references [22]. Pubarche was defined as the appearance of pubic hair (PH2), whereas puberty was defined as Tanner stage B2 in girls (by inspection and palpation) and testicular sizes $>3 \text{ ml}$ (G2) in boys, respectively.

Equivalent HC dosages (eHC) were calculated for PR and DX (factors 4 and 30, respectively) [23]. BA was assessed by an experienced observer using the atlas method of Greulich & Pyle, which has been found to be reliable for Central European children [24]. For the evaluation of the current status of skeletal maturation, we calculated the difference between BA and chronological age (CA) (BA delay, $\Delta\text{BA} = \text{BA} - \text{CA}$ in years) [4, 23].

Table 1. Detailed clinical and laboratory cohort data of the children with classic CAH

	Males, n = 46	Females, n = 52	p male vs. female
	mean $\pm$ SD median [quartiles]	mean $\pm$ SD median [quartiles]	
Age years	8.68 $\pm$ 5.15 8.0 [4.0, 13]	8.58 $\pm$ 5.02 8.0 [4.0, 12]	ns
Height SDS	0.32 $\pm$ 1.72 0.20 [-0.80, 1.3]	-0.58 $\pm$ 1.21 -0.60 [-1.4, 0.30]	<0.0001
BMI SDS	1.02 $\pm$ 1.31 1.07 [0.19, 1.8]	0.78 $\pm$ 1.29 0.92 [-0.12, 1.7]	ns
eHC mg/m ² /day	16.5 $\pm$ 5.22 15.4 [12.8, 19.4]	14.7 $\pm$ 5.15 13.7 [11.2, 16.8]	0.0024
Δ BA years	1.73 $\pm$ 2.19 1.0 [0.0, 3.5]	0.358 $\pm$ 1.37 0.10 [-0.60, 1.1]	<0.0001
DHEAS _a ng/ml	268 $\pm$ 393 100 [29.6, 349]*	135 $\pm$ 236 65 [38.8, 135]*	0.0105
DHEAS _r ng/ml	265 $\pm$ 444 100 [17.0, 338]*	146 $\pm$ 421 62 [33, 129]*	0.0053
17-OHP ng/ml	10.9 $\pm$ 19.3 3.50 [1.39, 12.8]	11.8 $\pm$ 35.8 4.10 [1.45, 10.4]	ns
PT μ g/day	4,120 $\pm$ 8,266 1,572 [478, 4,239]	1,765 $\pm$ 1,953 1,140 [374, 2,392]	0.0002

* DHEAS_a vs. DHEAS_r: not significantly different for both sexes for $p < 0.05$.

eHC = Equivalent hydrocortisone dosage; BA = bone age; DHEAS = dehydroepiandrosterone sulfate; index a = averaged; r = raw data; 17-OHP = serum 17-hydroxyprogesterone; PT = 24-hour urine pregnanetriol; ns = not significant for $p < 0.05$. Conversion factors: DHEAS: ng/ml to nmol/l, 2.714; 17-OHP: ng/ml to nmol/l, 3.03.

Blood sampling in the morning between 8 and 12 a.m. (approx. 10–14 h after the last dose of HC/PR) was performed for monitoring the therapy. Serum or plasma was then separated by centrifugation and stored at -20°C until assay. In addition, the day before, patients had collected samples of 24-hour urine at home according to our in-house protocol.

Laboratory Methods

DHEAS was measured with a Cobas e411 analyzer using system reagents (Roche, Mannheim, Germany). Intra- and interassay coefficients of variation (CV) were both below 7.0%. The conversion factor of DHEAS from ng/ml to nmol/l is 2.714.

Most of the serum 17-OHP levels (about 90%) were determined with a commercial RIA (Diagnostics Systems Laboratories, Sinsheim, Germany). Intra- and interassay CV were below 8%; sensitivity was 0.02 $\mu\text{g/l}$. About 10% of all serum probes were

measured with the new established LC-MS/MS method. Intra- and interassay CV were below 10%; the limit of detection was 0.05 ng/ml. The reliability of both assays was investigated and published elsewhere [25]. The conversion factor from ng/ml to nmol/l is 3.03. Levels of PT in specimens of urine collected during 24 h were simultaneously determined by isotope dilution/gas chromatography-mass spectrometry procedure. Intra- and interassay CV were $<10.0\%$.

Statistics

In order to minimize normal variations of DHEAS and an overestimation of a single patient, values of 1 year of life of a single patient were averaged (DHEAS_a) for comparison with the clinical parameters age, height, weight, medication, and BA. For correlation analysis of DHEAS with 17-OHP and PT, the raw data of all variables were used (DHEAS_r).

Our own data on healthy Caucasian children ($n = 425$, males $n = 227$) served as control group [25]. The detailed cohort data are (age, n , DHEAS serum levels ng/ml: median (quartiles)): Boys: 1 year, $n = 26$, 357 (129, 667), 2–4 years, $n = 27$, 66 (1, 145), 5–6 years, $n = 22$, 132 (46, 187), 7–8 years, $n = 28$, 133 (50, 233), 9–10 years, $n = 22$, 464 (207, 865), 11–12 years, $n = 20$, 860 (416, 1,575), 13–14 years, $n = 27$, 1,510 (1,160, 1,870), 15–16 years, $n = 31$, 1,460 (1,068, 2,070), and 17–18 years, $n = 24$, 1,430 (1,188, 2,548). Girls: 1 year, $n = 24$, 246 (92, 500), 2–4 years, $n = 19$, 3.6 (1, 73.5), 5–6 years, $n = 21$, 57 (10, 163), 7–8 years, $n = 20$, 343 (205, 436), 9–10 years, $n = 21$, 444 (240, 602), 11–12 years, $n = 28$, 463 (260, 1,248), 13–14 years, $n = 27$, 1,020 (628, 1,630), 15–16 years, $n = 20$, 1,610 (553, 2,635), and 17–18 years, $n = 18$, 1,435 (925, 2,090) [26] (online suppl. material 2).

The onset of Tanner stages and menarche was compared with published data from normal Caucasian children [27, 28], as performed elsewhere [13]. Gaussian distribution of the parameters was tested using the D'Agostino-Pearson omnibus normality test ($p > 0.05$). In order to compare each variable between genders, clinical forms (SV, SW), and other subgroups, the Mann-Whitney U test was used where appropriate. For the assessment of significant deviations from a hypothetical value, we employed the Wilcoxon signed rank test. In addition to linear regression analysis, Spearman (r_s) correlation coefficients were assessed, since some variables were not distributed normally. All tests were performed two-tailed and a $p < 0.05$ was considered to be significant. For calculation and presentation, we used GraphPad PrismTM software version 5.03.

Results

Patient Group

The clinical data of the patients are given in table 1. The detailed genetic groups were among males: 0: $n = 17$ ($n = 16$ salt wasting, SW), A: $n = 17$ ($n = 16$ SW), B: $n = 8$ ($n = 6$ simple virilizing, SV), and D: $n = 4$ (presented with SW, $n = 3$, or SV, $n = 1$, with no detectable mutation on the second allele), and among females: 0: $n = 22$ ($n = 21$ SW), A: $n = 18$ ($n = 18$ SW), B: $n = 10$ ($n = 8$ simple virilizing), and D: $n = 2$ (SW, $n = 1$; SV, $n = 1$).

Fig. 1. Onset of Tanner stages. Upper panels: boys, pubic hair (PH) and genital stage (G). Lower panels: girls, PH and breast stages (B) with menarche. Shown are means with SD. Significance level is indicated by asterisks (***) $p < 0.001$; ** $p < 0.01$; * $p < 0.05$).

Pubarche

All Tanner stages of pubic hair (PH2 to PH4) occurred significantly earlier in CAH children than in controls (mean $\pm$ SD, CAH vs. controls; males: PH2, 7.2 ± 2.9 vs. 13.2 ± 1.1 years ($p < 0.001$); PH3, 9.7 ± 2.5 vs. 13.7 ± 1.0 years ($p < 0.001$); PH4, 12.1 ± 1.4 vs. 14.3 ± 0.9 years ($p < 0.001$); PH5 14.3 ± 2.4 years (no controls); females: PH2, 9.6 ± 2.1 vs. 11.8 ± 1.0 years ($p < 0.001$); PH3, 10.3 ± 2.1 vs. 12.5 ± 0.9 years ($p < 0.001$); PH4, 11.7 ± 1.5 vs. 13.2 ± 0.9 years ($p < 0.001$); PH5 14.1 ± 2.3 years (no controls); fig. 1).

A subgroup analysis between male SV and SW clinical forms is described below.

Puberty

Tanner stages G2 and G3 in boys occurred significantly earlier in CAH patients than in controls (mean $\pm$ SD,

CAH vs. controls; G2: 9.3 ± 2.5 vs. 12.4 ± 0.92 years ($p < 0.001$); G3: 12.8 ± 1.5 vs. 13.6 ± 1.0 years ($p < 0.01$), but G4 occurred significantly later (G4: 15.2 ± 1.6 vs. 14.3 ± 0.88 years ($p < 0.001$); fig. 1).

Breast stages and menarche in girls showed a different pattern, i.e. thelarche (B2) and Tanner stage B4 appeared significantly earlier in CAH girls, whereas B3 and menarche appeared at the same time as in the reference population (mean $\pm$ SD, CAH vs. controls; B2: 10.3 ± 1.6 vs. 11.1 ± 0.94 years ($p < 0.001$); B3: 11.8 ± 1.6 vs. 12.1 ± 0.95 years ($p > 0.05$); B4: 12.4 ± 1.5 vs. 13.0 ± 0.95 years ($p < 0.05$); B5: 14.2 ± 2.0 years (no controls); menarche: 13.4 ± 1.5 vs. 13.4 ± 1.1 years ($p > 0.05$); fig. 1). In order to exclude that an earlier onset of B4 in CAH children was due to higher body weight and, accordingly, higher content of fat tissue of the breasts, we divided the B4 group into 'early' (≤ 12.5

Table 2. Correlations of serum DEHAS concentrations with different variables

	Males			Females		
	r_s (p) vs. DHEAS	r_s (p) vs. age	r_s (p) vs. 17-OHP	r_s (p) vs. DHEAS	r_s (p) vs. age	r_s (p) vs. 17-OHP
DHEAS vs.						
Age, years	0.613 (<0.0001)	1		0.378 (<0.0001)	1	
Height, SDS	ns	ns		ns	ns	
eHC, mg/m ² /day	0.319 (<0.0001)	0.346 (0.0001)		ns	ns	
Δ BA, years	0.311 (0.0003)	0.357 (<0.0001)		0.210 (0.0001)	0.186 (0.0008)	
17-OHP, ng/ml	0.411 (<0.0001)	na		0.308 (<0.0001)	na	
PT, μ g/day	0.504 (<0.0001)	na		0.300 (<0.0001)	na	
17-OHP vs.						
PT, μ g/day			0.636 (<0.0001)			0.527 (<0.0001)

r_s = Spearman correlation coefficient; eHC = equivalent hydrocortisone dosage; BA = bone age; DHEAS = dehydroepiandrosterone sulfate; 17-OHP = serum 17-hydroxyprogesterone; PT = 24-hour urine pregnanetriol; ns = not significant for $p < 0.05$; na = not assessed. Conversion factors: DHEAS: ng/ml to nmol/l, 2.714; 17-OHP: ng/ml to nmol/l, 3.03.

years, $n = 12$) and 'late' (>12.5 years, $n = 7$) onset; however, the weight of both groups did not differ significantly (early onset 53 ± 14 vs. 56 ± 13 kg; BMI 1.24 ± 0.34 SDS vs. 0.98 ± 0.22 SDS).

A subgroup analysis between male SV and SW clinical forms revealed that Tanner stages PH2 (SV vs. SW, mean $\pm$ SD; 4.7 ± 2.3 vs. 8.3 ± 2.5 years, $p = 0.009$), PH3 (SV vs. SW, mean $\pm$ SD; 7.5 ± 1.8 vs. 10.6 ± 2.2 years, $p = 0.0177$), and PH4 (SV vs. SW, mean $\pm$ SD, 10.0 ± 0.71 vs. 12.5 ± 1.1 years, $p = 0.0425$) occurred significantly earlier in male SV than in SW-CAH. There was no significant difference for PH4 and PH5. In terms of genital stages, a similar pattern was observed: G2 occurred earlier in SV than in SW-CAH (SV vs. SW, mean $\pm$ SD 7.9 ± 1.7 vs. 10.0 ± 2.6 years, $p = 0.0425$), but there was no significant difference for stages G3 and G4, respectively. Median age of diagnosis was within the first year of life, except for boys with SV-CAH (6.5 years).

In girls, the subgroup analysis between SV and SW clinical forms showed no significant difference for the age at different Tanner stages of pubic hair, breast development or menarche.

Serum Dehydroepiandrosterone Sulfate (DHEAS)

Serum DHEAS levels are shown in figure 2. Overall, boys and girls showed significantly lower DHEAS levels than the controls (boys from the CA of 5 years onwards and girls from the age of 7 years onwards, respectively). DHEAS levels during the first year of life were also significantly lower, i.e., the high neonatal levels of the con-

trols were not found in (treated) CAH children. During infancy when zona reticularis was inactive, DHEAS levels did not differ from the controls.

In boys, an obvious increase in DHEAS levels was observed between ages 11 and 15 years, but thereafter DHEAS levels remained clearly below their healthy peers. Thus, correlation with age was significant ($r_s = 0.613$, $p < 0.0001$; table 2). In contrast, girls showed a slight increase with age with a significant correlation compared to the controls ($r_s = 0.378$, $p < 0.0001$; table 2), but adrenarche was not identified.

There was no significant correlation of DHEAS levels with height (table 2).

DHEAS and Clinical Forms (Salt Wasting, SW, vs. Simple Virilizing, SV)

In boys, we found significantly higher DHEAS levels in SV than in SW. This is most probably due to the later age of diagnosis in boys with SV (median 6.5 years) compared to boys with SW, who were diagnosed during the first year of life (fig. 2). Additional analyses of different age groups in boys showed that DHEAS levels of SV boys were significantly higher from 3 to 14 years of age than in their SW peers: age group 3–6 years (median with quartiles, ng/ml): SV 230 (29.5, 336) vs. SW 30 (10, 100), $p = 0.0082$; age group 7–10 years: SV 104 (58.5, 710) vs. SW 50 (10.8, 100), $p < 0.0001$; age group 11–14 years: SV 349 (105, 543) vs. SW 178 (63.5, 288), $p = 0.0308$; age group 15–18 years: SV 557 (367, 1,134) vs. SW 695 (499, 852), $p = 0.8911$ (not significant).

Fig. 2. Serum DHEAS levels in CAH (grey bars) boys (upper panel) and girls (lower panel) compared to controls (clear bars). Small panels show DHEAS levels among simple virilizing (clear bars) and salt-wasting (grey bars) subgroups of CAH children. Shown are medians with interquartile ranges. Significance level is indicated by asterisks (***) $p < 0.001$; ** $p < 0.01$; * $p < 0.05$).

Fig. 3. Correlations of serum DHEAS levels with age, eHC, and Δ BA (= BA – CA) in boys (left panels) and girls (right panels). Shown are results of regression analyses with 95% CI.

In contrast, there was no difference between both groups in girls, since the diagnosis was made early with in both groups. SV girls were usually diagnosed due to their ambiguous genitalia (fig. 2).

DHEAS and Metabolic Control

Standard eHC dosages ranged between 6.1 and 32.6 mg/m²/day, the quartiles were 11.9 and 17.8 mg/m²/day, respectively (table 1). DHEAS levels were positively correlated with eHC in boys ($r_s = 0.319$, $p < 0.0001$), but not in girls (table 2; fig. 3). Additionally, eHC levels were significantly correlated with age in boys ($r_s = 0.346$, $p < 0.0001$) and girls ($r_s = 0.210$, $p < 0.0001$).

BA, expressed as Δ BA, i.e. BA – CA, was significantly more advanced in boys than in girls (table 1). DHEAS was positively correlated with Δ BA (boys: $r_s = 0.311$, $p = 0.0003$; girls: $r_s = 0.210$, $p < 0.0001$) and also with CA (boys: $r_s = 0.357$, $p < 0.0001$; girls: $r_s = 0.200$, $p < 0.0001$; table 2; fig. 3).

Serum levels of 17-OHP were not significantly different between boys and girls (table 1). There was a positive correlation of DHEAS with 17-OHP levels among boys and girls; urinary levels of PT showed a similar pattern (table 2). Additionally, 17-OHP was positively correlated with PT in boys and girls (table 2).

Discussion

Our results from this retrospective, longitudinal single-center study provide evidence that pubarche and the following development of pubic hair arise earlier in girls and boys with CAH, although serum levels of DHEAS are remarkably lower than controls in boys after adrenarche and tremendously reduced in girls, who do not show adrenarche at all.

There are some, mostly fragmentary, data on the onset and course of puberty in CAH patients in the literature, mainly within studies having another main topic, e.g. growth. Van der Kamp et al. [7] reported in CAH girls with salt-wasting Tanner stage B2 at ages 10.6 ± 0.84 years ($n = 12$) and 10.4 ± 1.3 years ($n = 12$) in 'non-salt wasters'; in CAH boys (SW) Tanner stage G2 (defined as testes volume >3 ml) at age 11.8 ± 1.5 years ($n = 20$) and 11.2 ± 1.5 years ($n = 9$) in 'non-salt wasters', respectively. Data on molecular genetics, especially of the 'non-salt-wasting' group, were not available. Another retrospective analysis of patients with classic CAH of Canadian centers showed an average age of the onset of puberty defined as Tanner stage 2 of 10.4 ± 1.6 years ($n = 31$) in girls and 11.1

± 2.1 years ($n = 23$) in boys. The average age of menarche was 13.7 ± 1.5 years. No differentiation was made between clinical forms of CAH [8]. Balsamo et al. [6] studied a small number of CAH patients with SW (10 females, 8 males) and SV (10 females, 2 males). The occurrence of Tanner stages B2 and menarche was described at ages 11.9 ± 1.4 years (SW) and 9.6 ± 1.6 years (SV) and 13.6 ± 0.9 years (SW) and 12.7 ± 1.3 years (SV), respectively. Thelarche occurred significantly earlier in SV than in SW girls. In boys, Tanner stage G2 manifested itself at ages 10.5 ± 1.5 years (SW) and 12.2 ± 1.2 (SV). Recently, Bonfig et al. [10] added data on pubarche (PH2) in classic CAH patients: in girls 8.2 ± 1.2 years (SV, $n = 25$) and 9.6 ± 1.2 years (SW, $n = 32$). Thelarche (B2) occurred at ages 9.5 ± 2.0 years (SV) and 10.3 ± 1.4 years (SW). In boys, PH2 was reported at ages 7.4 ± 3.5 years (SV, $n = 13$) and 10.7 ± 1.4 years (SW). Gonadarche (G2) occurred at ages 9.6 ± 2.5 years (SV) and 11.0 ± 1.3 years (SW). Both parameters differed significantly between SW and SV.

Our results add to these data by a longitudinal perspective of all Tanner stages compared to normal ranges. We found that pubarche and all other stages of pubic hair occurred earlier in CAH patients than in controls in general, and pubarche was earlier in boys with SV than in SW, in particular. This is most likely due to a delayed diagnosis and start of therapy in SV boys, since the SV form in male neonates without newborn screening on CAH usually lacks obvious clinical signs. Therefore, the diagnosis of SV-CAH in boys within our cohort was made at a median age of 6.5 years. In agreement with Bonfig et al., this difference disappeared with higher PH stages. In contrast to Bonfig et al. [10], there was no difference in girls. Except for gonadarche (G2) in boys, there was no difference between Tanner stages B and G between SW and SV in girls and boys, respectively. Genital stages G2 and G3 occurred earlier, but G4 occurred later in boys, and stages B2 and B4 occurred later in girls, respectively. This is probably driven by an advanced BA as reported variously in CAH, and more pronounced so in boys than in girls [1, 4, 23, 29, 30]. We speculate that earlier pubarche before gonadarche in girls and SW boys might be due to an incomplete androgen suppression in order to avoid glucocorticoid overtreatment. Therefore, low serum testosterone levels might accelerate BA maturation, earlier pubarche and subsequently earlier pubertal stages. In line with previous reports, the age at menarche was normal, and there was no difference between SW and SV [3, 31].

More than three decades ago, Korth-Schutz et al. [14] reported suppressed DHEA levels in 17 treated boys and 19 girls with classic CAH. This result was confirmed lat-

er by two independent studies measuring DHEAS levels: Brunelli et al. [15] reported data of $n = 10$ SW and $n = 6$ 'non-classic' CAH girls, aged between 3 and 12 years. DHEAS levels were lower than in the controls at all ages. There was a positive correlation between serum DHEAS and 17-OHP concentrations, although some girls showed high 17-OHP and low DHEAS levels at the same time. In 'non-classic' girls, DHEAS levels were much higher than in the controls at the time of diagnosis, but fell below the references in the further course of treatment. There was also a positive correlation with 17-OHP. The second study of 23 SW-CAH girls and 7 boys aged 5–20 years also reported reduced DHEAS levels in girls. More detailed statistics in boys could not be performed due to the small patient numbers [17]. Recent data of Argentinean girls showed similar results [16]. Our data in girls add to the current knowledge on the first year of life in girls, since DHEAS did not show the neonatal elevation found in controls because glucocorticoid therapy had already started and adrenal DHEAS production was suppressed. There was no difference between SW and SV clinical forms. In boys, serum DHEAS levels were also suppressed neonatally, but, in contrast to girls, there was a significant increase in concentration between ages 11 and 14 years, indicating some form of adrenarche. In line with earlier pubarche (PH2), DHEAS levels in SV were higher than in SW-CAH.

Adrenarche, which is defined as the significant increase of DHEA(S) production between ages 6 and 8 years, has been shown to be independent of gonadotrophins, ACTH, or functional gonads [12, 32–34]. Morphologically, there is evidence that the third adrenal zone, the zona reticularis (ZR), produces DHEA(S), starting its development around age 3 years [12, 35, 36]. Enzymatic studies show that StAR, the enzyme performing the first step of steroid hormone synthesis, is equally expressed in all zones of the adrenal [37]. The increased conversion of 17α -hydroxypregnenolone to DHEAS results from an increase in 17,20-lyase activity, but also a decreased 3β -HSD activity, along with an increased activity of sulfo-transferase (SULT2A1) [38]. This important step is regulated by multiple post-translational events [12]. Cytochrome b5 (CYB5), most evident in ZR, is an allosteric effector that interacts primarily with the oxidoreductase complex of CYP17 complex to stimulate 17,20-lyase activity. Its activity becomes more marked after age 5 years [37, 38]. Another key enzyme of steroidogenesis, HSD3B2, competes with CYP17 for 17α -hydroxyprogesterone. Therefore, low activity of HSD3B2 is crucial for sufficient DHEA production within the ZR [37, 39]. 21-Hydroxylase

deficiency in CAH patients leads to increased C19 steroid production and androgen excess and decreased cortisol and aldosterone levels [1]. Under substitution therapy with HC, this androgen excess is aimed to decline to normal levels [23]. During episodes of undertreatment, 17-OH-progesterone and testosterone levels are elevated, whereas the elevation of DHEAS concentrations, if present, is marginal. An exception to this is at the time of diagnosis of SV patients, who usually had a significant period of undetected disease, unless screened as newborns [15]. Interestingly, expression levels and immunohistochemical studies do not show differences of CYP21 among the different adrenal zones [12, 39]. A histological study of adrenals in 3 CAH patients showed poorly defined zones of the cortex [40]. This might be evidence for insufficient genesis and functional development of the ZR in CAH. Since DHEAS levels are normal in patients with cortisol excess (Cushing's syndrome) [41] and DHEAS levels are high in untreated CAH patients [15], circulating cortisol seems to play no role within the context of the onset of adrenarche, as suggested several years ago [42].

In contrast, very recent data from cultured adrenal cells (COS-7) demonstrate that cortisol inhibits 3β -HSD2 activity in cells transfected with HSD3B2. Thus, it is possible that intra-adrenal cortisol may participate in the regulation of adrenal DHEA secretion through inhibition of 3β -HSD2 and contribute to the initiation of adrenarche [43].

Although it is clear that adrenarche is primarily associated with adrenocortical changes as described above, there is some evidence that hormones related to body mass, such as leptin, play a role in adrenal function. This might be a promising approach for future studies [44–47]. In addition, defining adrenarche in CAH children is not possible, since they have low serum DHEAS levels. Thus, the transition from childhood to juvenility cannot be assigned exactly in CAH children [48].

The effectiveness of glucocorticoid substitution therapy, i.e. the metabolic control, over a period of several years is not easy to assess. Monitoring serum and urinary laboratory parameters cover only a few days or weeks, while advanced skeletal maturation as an index of poor metabolic control might be more representative over a longer period of time [4, 23]. However, since multiple examiners ($n = 5$, all under supervision of H.G.D.) have performed pubertal staging, this might be a bias of our data. Interestingly, we found in our cohort a positive correlation of serum 17-OH-progesterone and urine PT levels with serum DHEAS levels, although DHEAS levels were

below the normal range in older children of both sexes. A correlation of glucocorticoid dosage with DHEAS was not found in girls, but was in boys whose DHEAS and eHC levels were also correlated more strongly with age than in girls. This goes along with previously published data [4, 49]. Therefore, externally administered steroids seem to play a minor dose-dependent role, which explains the lack of sufficient adrenarche in CAH. However, for ethical reasons, any existing baseline effect cannot be analyzed in detail based on a study with untreated patients with classic CAH. In addition, BA is more advanced and mean PT is higher in male CAH patients. We speculate that (1) boys might be less compliant than girls and/or the advanced BA in boys could be due to (2) the higher proportion of boys with late diagnosis of CAH (SV-CAH) in our cohort.

In conclusion, we were able to show that pubarche and adrenarche are dissociated in classic CAH: earlier

pubarche is found in treated children with classic CAH in both sexes, whereas typical adrenarche does not occur. There was no age-independent correlation of DHEAS levels with genotype, glucocorticoid dosage, auxological data or quality of metabolic control.

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Disclosure Statement

T.M.K.V., L.Ö., C.S. and M.R. have nothing to declare. H.G.D. is member of KIGS' Germany Advisory Board and KIGS' International Board (Pfizer, less than USD 2,000 per year).

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