CLINICAL STUDY

Continuum of phenotypes and sympathoadrenal function in premature adrenarche

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Abstract

Objectives: Premature adrenarche (PA), the early rise in adrenal androgen (AA) production, can manifest with different clinical signs of androgen effect. Premature pubarche defined as appearance of pubic hair before the age of 8/9 years in girls/boys, is the most prominent clinical sign of PA and often erroneously described as a synonym of PA. Our aim was to determine the association of circulating AA concentrations with different prepubertal signs of androgen action (SAA). Secondly, we tested whether adrenomedullary function is altered in children with SAA, as it is in congenital adrenal hyperplasia (CAH) also causing adrenal hyperandrogenism.

Design and methods: We examined 73 Finnish prepubertal children with any hyperandrogenic sign(s) having appeared before the age of 8/9 years (girls/boys) (35 with pubic and/or axillary hair = PAH; 38 without = nonPAH), and 98 age- and sex-matched controls. Circulating adrenal steroid and catecholamine concentrations were measured and correlated with clinical parameters.

Results: None of the children with SAA had CAH or virilizing tumor. Serum dehydroepiandrosterone, dehydroepiandrosterone sulfate, and androstenedione concentrations overlapped between the SAA and control children, and they were lower in the nonPAH than PAH group ($P < 0.01$). SAA children had similar plasma epinephrine but higher norepinephrine (NE) concentrations than their controls (mean (95% confidence interval) 1.61 (1.44, 1.77) versus 1.39 (1.30, 1.49) nmol/l, $P = 0.03$).

Conclusions: PA forms a continuum with more pronounced increase in circulating androgens in children with PAH than in those without. Some children show SAA with fairly low androgen concentrations. The clinical significance of elevated NE concentrations associated with SAA needs to be confirmed in further studies.

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Introduction

The term adrenarche introduced about 60 years ago (1) refers to the increasing production of adrenal androgens (AA) in mid-childhood (reviewed in (2, 3)). This maturational biochemical process in adrenocortical function begins normally at around 6–8 years of age, is associated with morphological development of the zona reticularis (4) and changes in the expression of some key enzymes involved in AA production (5–7). Clinical signs of androgen effect appear later and adrenarche is defined premature (PA) when these signs are evident before the age of 8 years in girls or 9 years in boys, and serum AA concentrations are high for chronological age (8). Originally, premature pubarche (PP), the appearance of pubic hair before the above-mentioned age limits, was considered the main or only clinical manifestation of PA (9). This may be one reason why PP is still often erroneously described as a synonym of PA. Several later studies have revealed that

in addition to PP, other signs of androgen effect are quite common in PA; these include axillary hair, adult type body odor, oily hair or skin, comedones, acne, and slightly increased height SDS compared with prepubertal peers (10–13).

The triggering mechanisms of normal or PA are not completely understood. Cytochrome P450c17 (CYP17) catalyzes steroid 17 α -hydroxylation and 17,20-lyase reactions leading to the synthesis of dehydroepiandrosterone (DHEA), its sulfate (DHEAS), and androstenedione ($\Delta 4$ A). In adrenarche, enhancers (P450 oxidoreductase, cytochrome b5) and post-translational modifications (serine phosphorylation) of CYP17 leading to separate augmentation of 17,20-lyase activity have been suggested to be involved (3, 7, 14). Decreasing reticular zone activity of another key steroidogenic enzyme, 3 β -hydroxysteroid dehydrogenase (3 β -HSD) also has a role in the initiation of adrenarche (3, 6, 7). DHEA(S) and androstenedione are weak androgens (or androgen precursors) which are

converted to biologically more active androgens in peripheral tissues. In addition to the circulating AA concentrations and their peripheral conversion, the clinical signs of PA depend also on individual peripheral sensitivity to androgens determined by transactivational activity of the androgen receptor; some PP subjects actually show normal circulating prepubertal AA concentrations (8, 15). Few studies have reported highly elevated androgen concentrations in PP girls, referred as 'exaggerated adrenarche' or 'exaggerated adrenarcheal response' (16, 17), while in other reports serum androgen concentrations in PA children have usually been as expected for the pubic hair stage (10, 11). Before the clinical diagnosis of PA can be accepted on the basis of PP or other androgenic signs, central puberty, congenital adrenal hyperplasia (CAH), and virilizing tumors have to be excluded. The prevalence of mild steroidogenic enzyme defects, including the non-classical 21-hydroxylase deficiency (21-OHD), differs between populations (18, 19), and their occurrence among PP subjects has been variable in previous studies (11, 12, 20–25).

Adrenal medulla and cortex are now known to interact by autocrine, paracrine, and endocrine mechanisms (26). Small birth size has been associated with elevated circulating DHEAS and epinephrine concentrations in childhood (27). On the other hand, CAH patients with 21-OHD have reduced adrenomedullary activity, and the degree of medullary hypofunction associates with the severity of cortisol deficiency (28). Whether adrenomedullary function is altered also in adrenal hyperandrogenic conditions without cortisol excess or deficiency has not been systematically studied.

It has been suggested that both girls (2) and boys (29) with PP have an increased risk for metabolic disturbances and should thus be followed up, but it remains to be determined whether this applies to all prepubertal children with androgenic signs. In order to shed more light on the phenotypic variability of PA, a non-selected group of prepubertal children with androgenic signs was examined. Our objectives were i) to confirm the rareness of non-classical CAH (NC-CAH) in our population, ii) to determine the association of circulating AA concentrations with different signs of androgen action (SAA), and iii) to analyze adrenomedullary function in these children.

Subjects and methods

Subjects

The inclusion criteria of the SAA group were the appearance of any of the following clinical sign(s) before the age of 8 years in girls and 9 years in boys: pubic/axillary hair, acne, comedones, adult type body odor, and oily hair. All children meeting these criteria between October 2004 and January 2006 in Northern

Savo, a region in Eastern Finland with a population of 250 000, were invited to participate in the study. Study subjects were collected among the patients admitted to the pediatric outpatient clinic of Kuopio University Hospital due to the hyperandrogenic symptoms. In addition, information letters were sent through the health care centers to the well baby and school clinics. Announcements were published annually in main local newspapers. Seventy-six eligible children were found and 73 (96.1%) of them were willing to participate. The control group consisted of 98 healthy children born between 1995 and 1999 and living in the same area. A list of a random sample of child citizens was obtained from Finland's population register. Invitation letters were sent to the families of the children selected from the list by order sampling in each age and gender group, in order to yield a matched control group for the SAA subjects. Approximately, 20% of the control child population invited was willing to participate. Children with central puberty (based on clinical examination), any known endocrine disorder or long-term medication (including oral/inhaled corticosteroids) were excluded. In both groups, the current evaluation was performed before the age of 9 and 10 years in girls and boys respectively. The prevalence of metabolic syndrome and its components in the PA girls of this study population was recently reported (30). The study protocol was approved by the Research Ethics Committee of the Kuopio University Hospital. An informed written consent was obtained from the parents and an approval from the children.

Clinical evaluation

The occurrence and appearance time of androgenic signs were obtained by a structured interview of the parents and children. Adult-type body odor was recorded if the parents or the child reported a clear change in the type of axillary odor and/or a need for antiperspirant. In addition, axillary odor and wetness in the clinical examination was recorded. Similarly, if the parents or the child complained about greasiness of hair and/or need for daily hair-wash, 'oily hair' was recorded. Tanner pubertal stage, axillary hair, acne, and comedones were evaluated in a systematic physical examination performed by a single investigator (P U). Height was measured with a calibrated Harpenden stadiometer (Holtain Ltd, Crymych, UK) and recorded to the nearest 0.1 cm as the mean of three repeated measurements. Weight was measured after an overnight fast and recorded to the nearest 0.1 kg. Body mass index (BMI; weight (kg)/height²(m)) was calculated, and BMI SDS determined by British reference values (31). Birth weight (BW) data was obtained from the hospital and well-baby clinic records and converted to SDS according to Finnish growth charts adjusted for duration of gestation and gender (32).

Laboratory analyses

Sampling and ACTH-test After an overnight fast, a serum sample for basal cortisol (F), DHEA, DHEAS, $\Delta 4A$, 11-deoxycortisol (11DOF), 17-hydroxyprogesterone (17OHP), estradiol and testosterone measurements, and a plasma sample for ACTH analysis were drawn between 0900 and 1000 h from all subjects. Then a low-dose ACTH test was performed by administering $1 \mu\text{g}/1.73 \text{ m}^2$ of synthetic ACTH (Synacthen, Novartis Pharma GmbH, Munich, Germany) intravenously to each subject. A serum sample for F, DHEA, $\Delta 4A$, 11DOF, and 17OHP measurements was taken after 30 min. Low-dose ACTH tests have been validated for evaluating hypothalamo-pituitary-adrenal axis function including DHEA and 17OHP responses (33–35). In early afternoon, after one-hour rest in bed, a plasma sample for nor-epinephrine (NE) and epinephrine (E) analyses was drawn into a specific tube. All subjects underwent a GnRH-test (Relefact $3.5 \mu\text{g}/\text{kg}$ intravenously, Hoechst, Frankfurt am Main, Germany) with LH and FSH sampling at 30, 60, and 90 min. Serum samples were separated within an hour of sampling, immediately frozen and stored at -80°C until assayed. All plasma samples were taken into chilled tubes, separated within 15 min, and stored at -80°C until assayed.

Assays Serum DHEAS and $\Delta 4A$ concentrations were determined with specific Coat-A-Count RIAs (Diagnostic Products Corporation, Los Angeles, CA, USA). In the DHEAS assay, the intra-assay coefficient of variation (CV) was 3.8–5.3% and the inter-assay CV was 6.3–11%. For the $\Delta 4A$ assay, the intra-assay CV was 3.2–9.4% and inter-assay CV 4.1–15.6%. Serum DHEA concentrations were measured with an in-house RIA derived from a previously described method (36). Serum 11DOF concentrations were determined with liquid chromatography–mass spectrometry (LC–MS; PE Sciex, Foster City, CA, USA) and serum 17OHP concentrations with a previously described LC–MS method (37), both with a detection limit of 1 nmol/l. Serum F concentrations were measured with the Immulite 2000 Cortisol chemiluminescence immunoassay (Diagnostic Products Corporation), with intra-assay CV of 5.2–7.4% and total variation of 6.8–9.4%. Specific RIAs for serum testosterone (Diagnostic Products Corporation) and estradiol (DiaSorin, Sluggia, Italy) measurements had detection limits of 0.35 and 0.02 nmol/l respectively. Serum LH and FSH concentrations were determined with time-resolved fluoroimmunoassays by AutoDelfia (PerkinElmer Lifesciences, Turku, Finland). Total CV of the LH assay was 2.5% and that of the FSH assay 2.8%. For the first 125 subjects, plasma ACTH concentrations were analyzed by an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) with inter-assay CV of 6.4–8.4%. For the remaining 46 subjects, Immulite chemiluminescent immunoassay (Diagnostic Products Corporation) was used.

The intra-assay CV for the Immulite ACTH assay was 3.1–9.6% and inter-assay CV 5.1–9.4%. Plasma E and NE concentrations were analyzed with HPLC (Chromsystems GmbH, Munich, Germany). The intra- and inter-assay CV for E were 6.0 and 8.8% and those for NE measurements 3.5 and 8.4% respectively.

Data and statistical analyses

To assess steroidogenic enzyme activities, the following serum steroid concentration ratios were calculated: DHEAS/DHEA (SULT2A1=DHEA sulfotransferase activity), $\Delta 4A$ /DHEA (3β -HSD activity), ACTH-stimulated (st) (11DOF+F)/17OHP (21-hydroxylase activity), and stF/11DOF (11β -hydroxylase activity). ACTH-stimulated values were used for some steroid ratios due to the immeasurably low baseline concentrations of some intermediate steroids. SPSS 14.0 software statistical package (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. All continuous parameters were tested for normality prior to other statistical analyses. The independent samples t-test was performed to analyze the differences between the study groups, in the case of the non-normally distributed parameters after logarithmic (ln) transformation. For parameters remaining non-normally distributed after ln-transformation or being non-continuous, the non-parametric Mann–Whitney test was used. To evaluate the differences independently of confounding factors, an analysis of covariance was performed by incorporating BMI SDS or age as covariate. When comparing AA concentrations between all three subgroups, one-way ANOVA with Bonferroni correction was used. The possible associations between parameters were analyzed by Spearman correlation tests. Frequencies of non-continuous variables between the study groups were compared with the χ^2 test. A significance level $P < 0.05$ was used for all analyses. Values are presented as mean (95% confidence interval) or as median (interquartile range) if not otherwise specified.

Results

Clinical findings

The characteristics of the study groups are presented in Table 1. In the SAA group, the median appearance age of the first androgenic sign was 6.4 (range 2.0–8.8) years. Adult-type body odor was the most common sign, and there were eight SAA subjects in whom it was the only androgenic symptom (Table 2). Pubic and/or axillary hair (PAH) was usually accompanied by other symptoms that had appeared earlier. By clinical findings, the SAA subjects were further divided into two subgroups: those with 35/73 PAH and those with other symptoms only (38/73 nonPAH). Virilizing tumors were not found in any of the PAH subjects

Table 1 Characteristics of the prepubertal children with signs of androgen action (SAA) and control children. The SAA group is further divided into those with pubic and/or axillary hair (PAH) and those with other symptoms only (nonPAH). Values are presented as median (interquartile range).

	Control (n=98)	SAA (n=73)	P	nonPAH (n=38)	PAH (n=35)	P
Girls/boys (n)	80/18	63/10	0.53 ^a	31/7	32/3	0.22 ^a
Age (years)	7.6 (6.9, 8.2)	7.5 (7.0, 8.0)	0.73 ^b	7.2 (6.7, 7.7)	7.9 (7.2, 8.3)	0.002 ^b
BW (g)	3570 (3290, 3940)	3500 (3030, 3750)	0.042 ^b	3540 (3100, 3880)	3410 (2870, 3720)	0.55 ^b
BW SDS	-0.01 (-0.47, 0.77)	-0.05 (-0.90, 0.64)	0.24 ^b	0.06 (-0.98, 0.87)	-0.19 (-0.88, 0.42)	0.37 ^b
BMI (kg/m ²)	16.3 (15.1, 18.0)	17.6 (15.8, 20.9)	0.001 ^c	17.2 (15.4, 19.3)	17.8 (16.4, 22.4)	0.050 ^b
BMI SDS	0.26 (-0.46, 1.17)	0.79 (0.00, 2.26)	0.001 ^c	0.68 (-0.28, 1.84)	0.88 (0.31, 2.28)	0.11 ^b
AN (n)	4 (3/1)	9 (5/4)	0.020 ^a	5 (2/3)	4 (3/1)	0.82 ^a

BW, birth weight; BW SDS, birth weight standard deviation score; BMI, body mass index; BMI SDS, body mass index standard deviation score; AN, acanthosis nigricans. Italics indicates $P \leq 0.05$.

^a χ^2 test.

^bIndependent samples t-test.

^cMann-Whitney test.

when examined by adrenal ultrasonography and biochemistry. All boys had testicular volumes ≤ 3 ml and none of the girls presented with palpable breast tissue.

Serum hormone concentrations

All study subjects were considered prepubertal on the basis of the GnRH tests and/or serum estradiol/testosterone concentrations. The mean baseline serum testosterone concentration was higher in the SAA than control girls (Table 3), concordantly with some previous studies (10, 11). Low-dose ACTH stimulation caused a significant increase in F, $\Delta 4A$, DHEA, 11DOF,

and 17OHP concentrations ($P < 0.001$ for all). Despite the higher median st17OHP concentration in the SAA than control children, none of them met the criteria of NC-CAH (38); the basal and st17OHP concentrations were < 3.0 and < 30.3 nmol/l respectively, in all subjects. The serum adrenal steroid profiles of the SAA and control children are shown in Table 4. The mean baseline and stF concentrations did not differ between the SAA and control groups. Despite overlapping, the baseline serum $\Delta 4A$, DHEA, and DHEAS concentrations were the highest in the PAH subjects but also significantly higher in the nonPAH than control subjects, the differences between the subgroups remaining significant ($P < 0.02$ for all) after adjusting for BMI SDS. In the PAH group, serum androgen concentrations were comparable between those with pubic hair ($n = 28$) and those with axillary hair only ($n = 7$).

There were nine SAA girls (three PAH and six nonPAH) with serum DHEAS concentration below 1 $\mu\text{mol/l}$ (~ 40 $\mu\text{g/dl}$) which has been presented as a cut-off level for biochemical adrenarche (8). None of these nine SAA children had either $\Delta 4A$ or DHEA above the 75th percentile of the controls. Only one SAA subject exhibited 'exaggerated adrenarche' with DHEAS exceeding 6 $\mu\text{mol/l}$ (16). Notably, all the SAA children in whom axillary odor was the only androgenic sign had a DHEAS concentration of at least 1 $\mu\text{mol/l}$. Among the controls without any adrenarcheal signs, there were 36 children with the evidence of biochemical adrenarche at the time of evaluation (DHEAS ≥ 1 $\mu\text{mol/l}$). These control children were older than those with lower DHEAS ($P = 0.002$).

The SAA children had higher mean serum DHEAS/DHEA and a trend towards lower st (11DOF + F)/17OHP ratio than the controls (Table 4). Serum baseline DHEAS, DHEA, and $\Delta 4A$ concentrations correlated expectedly with age in the SAA ($r = 0.31$ – 0.47 ; $P < 0.01$ for all) and control children ($r = 0.22$ – 0.33 , $P < 0.03$ for all), and they were strongly intercorrelated among the SAA children ($r = 0.61$ – 0.80 , $P < 0.001$ for all). Baseline and st $\Delta 4A$, but not DHEAS or DHEA concentrations correlated positively with BMI SDS in the

Table 2 Clinical signs of androgen action in the 73 prepubertal children.

Number of subjects with each combination of signs	n
<i>Children with pubic or axillary hair (PAH group)</i>	35
Pubic hair, body odor, oily hair, acne/comedones	8
Pubic and axillary hair, body odor, oily hair, acne/comedones	6
Pubic hair, body odor, oily hair	3
Pubic and axillary hair, body odor, oily hair	2
Axillary hair, body odor, oily hair, acne/comedones	2
Pubic and axillary hair, body odor	2
Axillary hair, body odor, acne/comedones	2
Pubic hair, oily hair, acne/comedones	2
Pubic hair, body odor	2
Pubic hair, oily hair	2
Pubic hair, body odor, acne/comedones	1
Axillary hair, body odor, oily hair	1
Axillary hair, oily hair	1
Axillary hair, acne/comedones	1
<i>Children without pubic or axillary hair (nonPAH group)</i>	38
Body odor, oily hair, acne/comedones	12
Body odor, oily hair	11
Body odor	8
Body odor, acne/comedones	5
Oily hair, acne/comedones	1
Acne/comedones	1

Designation: body odor, adult type body odor.

Table 3 Gonadotropins and gonadal sex steroids in the prepubertal children with signs of androgen action (SAA) and in control children according to sex. Values are presented as median (range).

	Girls SAA (n=63)	Control (n=80)	Boys SAA (n=10)	Control (n=18)
S-LH basal (IU/l)	<0.10 (<0.10, 0.30)	<0.10 (<0.10, 0.29)	<0.10 (<0.10, <0.10)	<0.10 (<0.10, 0.18)
S-FSH basal (IU/l)	1.0 (0.2, 3.2)	1.0 (0.2, 2.7)	0.4 (0.2, 0.9)	0.6 (0.2, 1.3)
Peak GnRH stimulated S-LH (IU/l)	2.1 (0.3, 7.3)	2.1 (0.6, 6.5)	1.4 (0.6, 2.8)	1.9 (0.8, 5.1)
Peak GnRH stimulated S-FSH (IU/l)	9.9 (4.6, 35)	12 (3.1, 27)	3.2 (2.0, 4.7)*	5.0 (1.6, 9.6)
Peak S-LH/FSH ratio ^a	0.20 (0.05, 0.60)	0.19 (0.07, 0.42)	0.47 (0.21, 1.40)	0.44 (0.27, 0.90)
S-Estradiol (nmol/l)	0.03 (<0.02, 0.04)	0.03 (<0.02, 0.04)	NM	NM
S-Testosterone (nmol/l)	0.43 (<0.35, 1.17) [†]	<0.35 (<0.35, 1.37)	0.36 (<0.35, 0.45)	<0.35 (<0.35, 0.44)

Mann-Whitney test. * $P=0.006$ between SAA and control boys; [†] $P=0.001$ between SAA and control girls; all other differences between SAA and control groups non-significant. S, serum; NM, not measured.

^aS-LH/FSH ratio <1 is regarded as prepubertal; one SAA boy with prepubertal testis volumes (2.5/2.0 ml) and S-testosterone (<0.35 nmol/l) had LH/FSH ratio of 1.4 suggesting imminent puberty (peak stimulated FSH 2.0, LH 2.8 IU/l).

SAA ($r=0.39$ and $r=0.42$, $P<0.001$ for both) and control group ($r=0.21$, $P=0.04$ and $r=0.25$, $P=0.01$). BW SDS correlated inversely with DHEAS ($r=-0.34$, $P=0.004$), DHEA ($r=-0.27$, $P=0.02$) and $\Delta 4A$ ($r=-0.30$, $P=0.01$) concentration only in the SAA subjects. Serum F concentrations did not correlate with age or BMI SDS in either study group.

Plasma catecholamines

The SAA children had higher mean plasma NE concentration than their controls (1.61 (1.44, 1.77) versus 1.39 (1.30, 1.49) nmol/l, $P=0.03$, also after adjustment for BMI SDS), while there was no difference

in the mean plasma E concentration between the study groups (0.22 vs 0.23 nmol/l, $P=0.83$; Fig. 1). When only children with SAA and biochemical adrenarche (serum DHEAS concentration ≥ 1 $\mu\text{mol/l}$) (8) (PA, $n=64$) were compared with controls without biochemical evidence of adrenarche (DHEAS <1 $\mu\text{mol/l}$, $n=60$), the difference in the mean NE concentration was of almost similar magnitude but statistically not quite significant (1.56 vs 1.42, $P=0.12$). The difference in the mean NE concentrations was also similar between the SAA and control boys and girls separately (0.25 and 0.20 nmol/l respectively) but statistically significant only when analyzed together. The mean NE concentrations did not differ between the PAH and

Table 4 Plasma ACTH and adrenal steroid concentrations in the children with signs of androgen action (SAA) and in control children. The SAA group is further divided into those with pubic or axillary hair (PAH) and those with other symptoms only (nonPAH). Values are presented as median (interquartile range) and also (range) for adrenal androgens.

	Controls (n=98)	SAA (n=73)	P	NonPAH (n=38)	PAH (n=35)	P
Hormone concentrations						
S-DHEAS ($\mu\text{mol/l}$)	0.7 (0.5, 1.3) (0.1, 4.4)	1.7 (1.2, 2.6) (0.4, 7.7)	<0.001	1.5 (1.0, 2.1) (0.4, 7.7)	2.2 (1.4, 3.3) (0.7, 5.9)	0.031 ^a
S-DHEA (nmol/l)	4.2 (2.6, 6.1) (1.2, 11.2)	7.1 (4.9, 10.0) (2.5, 22.4)	<0.001	5.7 (4.5, 8.1) (2.5, 18.4)	8.8 (6.2, 11.6) (4.0, 22.4)	0.007 ^a
S-stDHEA (nmol/l)	5.7 (4.2, 8.8) (2.1, 15.1)	10.1 (6.9, 14.4) (3.2, 26.4)	<0.001	8.0 (6.1, 11.3) (3.2, 26.4)	13.3 (9.4, 16.8) (4.9, 21.7)	0.004 ^a
S- $\Delta 4A$ (nmol/l)	1.5 (0.8, 2.2) (0.5, 5.0)	2.7 (1.8, 3.6) (0.6, 6.6)	<0.001	1.9 (1.2, 3.0) (0.6, 6.2)	3.3 (2.7, 4.0) (1.7, 6.6)	0.006 ^a
S-st $\Delta 4A$ (nmol/l)	2.0 (1.3, 2.8) (0.5, 6.0)	3.7 (2.3, 4.5) (1.0, 6.9)	<0.001	2.6 (1.9, 3.9) (1.0, 6.9)	4.1 (3.6, 4.7) (2.3, 6.6)	0.013 ^a
S-F (nmol/l)	220 (177, 293)	231 (159, 316)	0.71	221 (174, 276)	257 (150, 363)	0.73 ^a
S-stF (nmol/l)	599 (521, 688)	603 (529, 671)	0.78	570 (509, 667)	618 (549, 684)	0.33 ^a
S-st11DOF (nmol/l)	3.2 (1.8, 4.5)	1.9 (≤ 1.0 , 4.6)	0.071 ^b	2.4 (1.3, 4.7)	1.6 (≤ 1.0 , 3.8)	0.35 ^b
S-st17OHP (nmol/l)	3.3 (1.9, 4.7)	4.1 (2.9, 5.4)	0.034 ^b	4.3 (2.9, 5.6)	3.8 (2.6, 4.7)	0.32 ^b
P-ACTH (nmol/l)	3.9 (2.4, 5.4)	3.6 (2.3, 6.0)	0.68	3.8 (2.3, 6.3)	3.3 (2.2, 5.7)	0.76 ^a
Serum steroid ratios						
DHEAS/DHEA ¹	200 (151, 277)	250 (209, 299)	0.004	237 (209, 303)	262 (208, 289)	0.88 ^a
$\Delta 4A/DHEA$ ²	0.31 (0.23, 0.42)	0.35 (0.27, 0.50)	0.44	0.31 (0.24, 0.50)	0.38 (0.27, 0.50)	0.82 ^a
st(11DOF+F)/17OHP ³	176 (134, 279)	149 (118, 206)	0.051 ^b	143 (114, 208)	166 (132, 204)	0.19 ^b
stF/11DOF ⁴	201 (128, 303)	302 (135, 537)	0.032 ^b	272 (131, 467)	376 (153, 562)	0.18 ^b
F/ACTH ⁵	57 (43, 87)	51 (40, 101)	0.86	49 (39, 92)	68 (40, 112)	0.95 ^a

Independent samples t-test, ^acovariance analysis with age as covariant, ^bMann-Whitney test (non-normal distribution). P, plasma; S, serum; F, cortisol; DHEAS, dehydroepiandrosterone sulfate, DHEA, dehydroepiandrosterone; st, ACTH stimulated; $\Delta 4A$, androstenedione; 11DOF, 11-deoxycortisol; 17OHP, 17-hydroxyprogesterone. Steroid ratios as surrogate markers of: ¹SULT2A1, ² β -HSD, ³21-hydroxylase and ⁴11 β -hydroxylase enzyme activity and ⁵ACTH sensitivity. Italics indicates $P\leq 0.05$.

Figure 1 Mean (95% confidence interval) plasma norepinephrine (NE) and epinephrine (E) concentrations in prepubertal children with signs of androgen action (SAA; $n=73$) and in control children ($n=98$). Independent samples T-test: * $P=0.03$.

nonPAH subgroups (1.63 vs 1.58 nmol/l, $P=0.77$), or between the SAA girls and boys (1.62 vs 1.51 nmol/l, $P=0.92$). Neither did the NE concentrations correlate linearly with BMI SDS, age or any steroid concentration in either study group ($|r| \leq 0.12$, $P > 0.05$ for all). The SAA children with their NE concentrations in the highest quartile (≥ 1.86 nmol/l) had lower mean BW SDS (-0.32 vs 0.33 SDS, $P=0.04$) and higher mean $\text{st}\Delta 4\text{A}$ (4.2 vs 3.1 nmol/l, $P=0.02$) concentration than those with their NE levels in the lowest quartile (< 1.16 nmol/l).

Discussion

Our study confirms the rareness of androgen producing tumors, NC-21OHD and other steroidogenic enzyme defects in our Northern European population. Only about half of the children with SAA presented with pubic or axillary hair. Even in these subjects, adult-type body odor was the most common initial sign of androgen effect in their medical history, and it was also the most prevalent finding in the whole group of prepubertal children with SAA. Serum AA concentrations were higher in the PAH than nonPAH subjects suggesting that less androgen effect is needed for the appearance of adult-type body odor, oily hair, and comedones than for that of pubic or axillary hair. Few subjects with SAA (mostly nonPAH) had their serum AA concentrations within the normal prepubertal range. On the other hand, there were healthy children with no SAA, but presenting with biochemical adrenarche. The novel finding in the present study was the higher mean plasma NE concentration in the SAA children than in their controls.

Increasing adrenal reticular zone 17, 20-lyase and SULT2A1, and decreasing 3β -HSD activities are hallmarks of adrenarche. Circulating baseline or

ACTH-stimulated adrenal steroid concentrations and their ratios have traditionally been used to evaluate the development of adrenarche and to exclude/confirm congenital deficiencies in adrenal steroid synthesis, although peripheral conversion of some steroids may confound these interpretations. Although the mean $\text{st}17\text{OHP}$ concentrations were higher in our SAA than control children, none of the SAA subjects had their $\text{st}17\text{OHP}$ concentrations consistent with NC 21-OHD (38). However, as we did not screen for *CYP21A2* mutations, and as the low-dose ACTH test has not been validated for the detection of heterozygote carriers of *CYP21A2* mutations, heterozygous subjects were not identified with our study setting. Neither did the basal or ACTH-stimulated steroid concentrations suggest 11β -hydroxylase deficiency in any of the SAA subjects. On average, the SAA and control children had similar $\text{st}\Delta 4\text{A}/\text{DHEA}$ ratios, with no outliers among the SAA subjects, speaking against any 3β -HSD deficiency. Thus, none of our unselected prepubertal children with SAA exhibited even a mild steroidogenic enzyme defect. This finding disagrees with some previous reports (16, 23, 25), but is in line with the previously reported extremely low prevalence of NC 21-OHD in our population (19).

Serum androgen concentrations were the highest in the children with pubic or axillary hair but also significantly increased in the nonPAH group compared with the control children. The slightly younger mean age in our nonPAH than the PAH group suggests that some of the nonPAH subjects may also develop precocious pubic/axillary hair. Indeed, appearance of pubic and axillary hair were often preceded by other androgen induced symptoms in our PAH subjects. As the AA concentrations expectedly correlated with age, the slightly lower mean age of the nonPAH subjects compared with that of the PAH subjects may partly explain their lower androgen concentrations. Nevertheless, our findings indicate that PA is a continuum; some subjects present with only modestly increased AA concentrations and mild clinical signs and others with more premature and pronounced adrenarche. It is noteworthy that 9/73 children with SAA had their serum DHEAS concentration below the cut-off level for adrenarche ($1 \mu\text{mol/l}$) and a few children in the control group had no signs of androgen effect in the presence of higher DHEAS ($\geq 1 \mu\text{mol/l}$). This suggests that the rate of peripheral conversion of DHEAS to more potent androgens, and peripheral sensitivity via the androgen receptor affect the clinical presentation of PA.

In our non-selected group of children with SAA, only one girl had her DHEAS concentration above the normal pubertal range (39). This indicates that PA rarely manifests 'exaggerated' (defined as DHEAS exceeding the pubertal range $> 6.0 \mu\text{mol/l}$) (16) at prepubertal age in our population. Our findings differ from those of an Australian study reporting 9.1% prevalence of 'exaggerated adrenarche' among PA children (16). Ibanez and co-workers have shown that

girls with a history of PP and low BW have often adrenal hyperandrogenism in adolescence (40). We cannot judge by the present study whether the androgen concentrations in our PA children will remain elevated or further rise during puberty, but a previous study on Finnish PA subjects suggested that the AA levels are normal in adolescence (12). When comparing our findings with previous studies in other populations, it has to be taken into account that our study on PA differed from most previously published series: we recruited subjects from general population and had even the mildest signs of androgen effect as inclusion criteria, while others have mostly included only PP subjects. Thus, the variation in the long-term prognosis of PA in different studies may at least partly be explained by variable selection criteria and ethnic or genetic differences.

A novel and somewhat unexpected finding was that prepubertal children with SAA had slightly but significantly higher mean plasma NE concentrations than their age-matched controls. Instead, no difference was found in the plasma E concentrations between the study groups. Thus, the increased NE levels in our SAA children are most probably derived from sympathetic nerve endings. It is impossible to evaluate the ultimate clinical significance of the higher NE levels in SAA children based on this cross-sectional study alone. The difference in plasma NE concentrations between the study groups was rather small; the SAA children had on average 0.2 nmol/l higher serum NE concentrations than the controls, and the difference between the highest and the lowest NE quartiles among the SAA children was 1.5 nmol/l (mean 2.5 and 1.0 nmol/l respectively). On the other hand, even a relatively small difference in NE concentrations may be clinically significant as evidenced by recent studies by Flaa and co-workers (41, 42). In their studies on healthy young men, there was a 0.4 nmol/l difference in the mean baseline arterial NE concentrations between the subjects with the lowest and the highest blood pressure tertiles (41). NE stress response at entry correlated significantly with the resting systolic BP after an 18-year follow-up period (42). Although no stress response was evaluated in our study, it is possible that even the observed small difference in plasma NE concentrations may predict metabolic or cardiovascular changes becoming significant later in life. Among our SAA children, higher circulating NE concentrations associated with lower BW SDS. This is of special interest in the light of the previously reported association between low BW and adult cardiovascular morbidity (Barker's hypothesis).

Hyperinsulinemia can directly stimulate sympathetic nervous system activity even without hypoglycemia. This may be reflected in increased circulating NE concentrations (43). Our SAA subjects were more obese, had higher insulin concentrations and increased prevalence of childhood metabolic syndrome compared

with the controls (30). Thus, increased sympathetic tone associated with relative hyperinsulinemia could also explain the higher plasma NE concentrations in our SAA than control children. This possibility is supported by the observation that dietary weight loss in obese adult subjects improved all components of the metabolic syndrome and decreased sympathetic nervous system activity, reflected partly in decreasing NE spillover and concentrations during weight loss (44). Both hyperinsulinemia and increased sympathetic tone (45) are important risk factors for adult cardiovascular morbidity. Thus, higher NE concentrations might predict an increased risk of developing unfavorable metabolic features later in life in children with prepubertal SAA. However, as the NE difference between the study groups was marginal, our finding needs to be confirmed in further studies.

Untreated and under-treated subjects with 21-OHD have increased androgen concentrations in the adrenal medulla similarly as supposedly our SAA subjects compared with their age-matched prepubertal controls. However, the catecholamine secretion pattern differs between these two hyperandrogenic conditions; CAH patients have decreased E and normal NE concentrations (28), whereas our SAA children show normal E and increased NE concentrations. Instead, patients with isolated glucocorticoid deficiency also show low E concentrations (46). Thus, our study is concordant with earlier findings that the low intra-adrenal glucocorticoid but not the high androgen concentration leads to reduced E production.

In conclusion, steroidogenic enzyme defects are rarely seen in children with SAA in our Northern European population. There is a continuum of phenotypes in prepubertal children with SAA; subjects with pubic or axillary hair have more pronounced increase in circulating androgens than those without it. Owing to the wide variation of the PA phenotypes, we should be cautious in generalizing the predictions derived from studies on selected PP subjects to all children with prepubertal androgenic symptoms. Higher circulating NE concentrations in prepubertal children with SAA compared with controls suggest alterations in the sympathoadrenal system. The clinical significance of this unexpected finding needs to be further studied.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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