NOTE

Nonclassical 3β -Hydroxysteroid Dehydrogenase Deficiency in Young Girls with Hirsutism and Premature Pubarche

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Abstract

Two young girls with hirsutism and premature pubarche showed non-classical 3β -hydroxysteroid dehydrogenase (3β -HSD) deficiency. Post-ACTH increased serum Δ^5 -17-hydroxypregnenolone and increased ratio of Δ^5 -17-hydroxypregnenolone/17-hydroxyprogesterone are the most sensitive indicators of nonclassical 3β -HSD deficiency. Nonclassical 3β -HSD deficiency may not be uncommon, but most cases may have gone unrecognized. Routine assay of Δ^5 -17-hydroxypregnenolone should be made generally available.

It is well known that partial deficiency of adrenal 21- and 11-hydroxylase can cause hirsutism in prepubertal and pubertal women (Newmark *et al.*, 1977; Emans *et al.*, 1983; Chetkowski *et al.*, 1984). Moreover, it has recently been observed that partial deficiency of adrenal 3β -hydroxysteroid dehydrogenase (3β -HSD) is also an important cause of hirsutism (Pang *et al.*, 1985; Medina *et al.*, 1986; Temeck *et al.*, 1987). Pang *et al.* (1985) reported that late-onset nonclassical 3β -HSD deficiency was present in 14% of hirsute women. Temeck *et al.* (1987) also found that three of 23 children with premature pubarche (13%) had a nonclassical form of this enzyme deficiency. Nonclassical 3β -HSD

deficiency may not be uncommon and most of such cases may have gone unrecognized. A report is presented on two young girls with hirsutism due to nonclassical 3β -HSD deficiency.

Subjects

Patient 1. This patient is a 9 2/12-year-old girl. She was born at term after an uneventful gestation and delivery with a birth weight of 3,100 g and a length of 50 cm. Her father was 168 cm in height and mother 160 cm. Her mother's menarche occurred at 13 years of age. Her parents are not consanguineous. A 4-year-old younger sister has been healthy. As far as could be determined, none of her relatives was similarly affected. Her early milestones were normal. She has never received hormone therapy. At the age of about 6 years, moderate hirsutism was noted in the legs and upper arms, which slowly increased in amount. Breast development which appeared

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at 8 3/12 years of age progressed normally. Acne and public hair appeared at 9 years. Her growth changed from the -1.6 SD line at the age of 3 6/12 years to the $+0.5$ SD line at the age of 9 years. Psychomotor development was normal.

At 9 2/12 years of age, she was evaluated for hirsutism, accelerated growth and sexual development. Her height was 132.7cm ($+0.5$ SD) and weight 34.8 kg ($+1.7$ SD). She had moderate hirsutism, which was particularly evident over the lateral aspects of the upper and lower limbs, and she had acneiform rashes on both cheeks. Physical examination revealed Tanner stage 3 breasts, Tanner stage 2 pubic hair, and mild clitoromegaly, but there was neither axillary hair nor menarche.

Laboratory studies showed normal blood cell counts and urinalysis was normal. Liver and renal function tests and serum electrolytes were normal. In the ACTH test, basal rather high Δ^5 -17-hydroxypregnenolone (Δ^5 -17P, 1550 ng/dl) further increased to 1860 ng/dl (Table 1), and basal dehydroepiandrosterone (DHEA) and cortisol values were 360 ng/dl and 19.7 μ g/dl, respectively, which increased to 720 ng/dl and 35.0 μ g/dl, respectively, but other serum steroid levels did not show marked any increase as follows: 17-hydroxyprogesterone (17-OHP), 170 $\rightarrow$ 250 ng/dl; Δ^4 -androstenedione (Δ^4 -A), 50 $\rightarrow$ 70 ng/dl; DHEA sulfate (DS), 49.4 $\rightarrow$ 80.7 μ g/dl; and testosterone, 0.6 $\rightarrow$ 0.8 ng/ml. The ACTH-stimulated ratio of Δ^5 -17P/17-OHP and DHEA/ Δ^4 -A showed high values of 7.4 and 10.3, respectively, which were higher than those of the control subjects (Table 1). In the dexamethasone suppression test, high basal Δ^5 -17P (960 ng/dl) decreased to 60 ng/dl and other steroid levels were all dexamethasone suppressible. In the LH-RH test, serum LH increased from 33 mIU/ml to 300 mIU/ml at 30 min and was even maintained a high level of 110 mIU/ml at 120 min, while the serum FSH level increased from 11 mIU/ml to 39 mIU/ml at 30 min. Plasma aldosterone and renin activity in the supine posture were 5.0 ng/dl and 0.6 ng/ml/hr, respectively. Serum estrone (E_1) was 24.7 pg/ml, estradiol (E_2) 43.2 pg/ml and prolactin 21.0 ng/ml. Daily urinary excretion of 17 OHCS and 17 KS were 2.9 mg/day and 2.1 mg/day, respectively. Chromosomal analysis was normal (46, XX). Bone age (Greulich and Pyle) was 10 6/12 years. Adrenal computed tomography

Table 1. Hormonal characteristics of patients with nonclassical 3β -hydroxysteroid dehydrogenase deficiency and of control subjects

Patient no	Sex	Age (yr)	Bone age (yr)	Height (SD) (cm)	Tanner Stage		60 min post-ACTH		
					breast	pubic hair	Δ^5 -17P* (ng/dl)	17-OHP** (ng/dl)	DHEA/ Δ^4 -A***
1	F	9.2	10.5	132.7 (+0.5)	III	II	1860	250	7.4
2	F	9.3	9.5	136.2 (+1.8)	I	III	1580	160	9.9
Controls									
1°	F	6.1	7.0	118.0 (+1.3)	II	I	500	350	1.4
2°	F	6.5	9.0	127.4 (+2.7)	III	I	100	60	1.6
3°	F	8.8	8.5	128.3 (+0.3)	I	I	630	480	1.3
Temeck <i>et al.</i> (1987)							1543 $\pm$ 277	<500	8.1 $\pm$ 2.6
									8.0-25.0

* Δ^5 -17 hydroxypregnenolone, ** 17-hydroxyprogesterone, *** dehydroepiandrosterone/ Δ^4 -androstenedione

° early central puberty, °° a girl whose two elder brothers have 21-hydroxylase deficiency (simple virilizing type)

two girls and one boy

(CT) showed mild bilateral hyperplasia. Pelvic ultrasonography done by high resolution real-time sector scanner was normal.

She was administered cyproterone acetate (100 mg daily, 87 mg/m² of body surface) for treatment of early activation of puberal gonadotropin secretion at the age of 9 4/12 years, and accelerated growth velocity became decreased from 9.5 cm/year to 4.5 cm/year and bone age of 10 6/12 years at the age of 9 2/12 years became 11 3/12 years at 10 years, but her hirsutism did not improve and serum Δ^5 -17P was still high (940 ng/dl). Paramethasone (1mg daily) was added from the age of 10 years. After 1 year of treatment with cyproterone acetate and paramethasone, serum Δ^5 -17P became normal (79 ng/dl), LH and FSH responses in the LH-RH test also became normal, bone age was 11 9/12 year, growth velocity was 3.8 cm/year, and hirsutism improved.

Patient 2. This 8 3/12-year-old girl was born at term after normal pregnancy and delivery. Birth weight was 3,750 g and length 51 cm. Her father was 173 cm in height and mother 161 cm. Both have been in good health. Her mother's menarche had occurred at 13 years of age. A maternal aunt has hair on the chest. None of the other relatives was similarly affected. There was no other sibling. Her parents are not consanguineous. Her milestones were normal. She has never received hormone therapy. She has been growing near the +2 SD line at a constant growth rate. At the age of 7 6/12 years, acne, pubic hair and moderate hirsutism on the legs and upper arms were noted. At 8 3/12 years she was referred to our hospital for evaluation of hirsutism. Her height was 136.2 cm (+1.8 SD), and weight 32.2 kg (+1.7 SD). Moderate hirsutism was noted over the areolae and the extensor surfaces of the upper and lower limbs. There was facial acne. Physical examination showed Tanner stage 3 pubic hair and mild clitoromegaly. No breast development or axillary hair were present. She has no menarche.

Routine laboratory data, including liver and renal function tests and serum electrolytes, were normal. Following the administration of ACTH, high basal Δ^5 -17P (940 ng/dl) rose to 1580 ng/dl (Table 1), and serum DHEA and cortisol increased from 220 ng/dl and 7.9 μ g/dl to 540 ng/dl and 25.7 μ g/dl, respectively. The ACTH-stimulated ratio of Δ^5 -17P/17-OHP and DHEA/

Δ^4 -A showed high values of 9.9 and 9.0, respectively, which were higher than those of the control subjects (Table 1), but no other serum steroid values were markedly increased as follows: 17-OHP, 60→160 ng/dl; Δ^4 -A, 40→60 ng/dl; DS, 33.9→56.9 μ g/dl; and testosterone, 0.2→0.4 ng/ml. The high basal Δ^5 -17P (750 ng/dl) fell to 30 ng/dl and other steroid levels were all suppressed by the administration of dexamethasone. In the LH-RH test, LH and FSH increased from 4.2 to 16 mIU/ml at 60 min and 3.2 to 22 mIU/ml at 60 min, respectively. Plasma aldosterone and renin activity in the supine posture were 4.2 ng/dl and 0.9 ng/ml/hr, respectively. Both serum E₁ and E₂ were less than 5.0 pg/ml. Daily urinary excretion of 17 OHCS and 17 KS were 2.3 mg/day and 2.5 mg/day, respectively. Chromosomal analysis was 46, XX. Bone age (Greulich and Pyle) was 9 6/12 years. Adrenal CT showed mild bilateral hyperplasia. Pelvic ultrasonography was normal. She was not treated, since she and her parents refused to cooperate.

Control subjects. As it is not possible to conduct the ACTH test on healthy girls, the following three girls were considered to be control subjects: two girls aged 6 1/12 and 6 6/12 years with early central puberty and an 8 9/12-year-old girl whose two elder brothers showed 21-hydroxylase deficiency (simple virilizing type). None of the subjects have received hormonal therapy. The results of ACTH test are shown in Table 1. Basal Δ^5 -17P was not high and ACTH-stimulated Δ^5 -17P and the ratios of Δ^5 -17P/17-OHP were not as high as in patients 1 and 2.

Hormone Examination and Assay

ACTH stimulation test was performed on each subject with a standard 0.25 mg dose of ACTH-(1-24) given as an iv bolus at 0800 h. Blood samples were drawn before and 60 min after ACTH was given. All serum samples were stored at -40°C until the assay.

Dexamethasone suppression test was performed as follows. Patients received 2.0 mg/day dexamethasone in four divided doses for 4 days. Morning (0800 h) serum

steroid levels (Δ^5 -17P, 17-OHP, DHEA, DS, testosterone and cortisol) were measured before and after 4 days of dexamethasone administration.

Serum Δ^5 -17P was determined at Research Laboratories, Teikoku Hormone Mfg. Co., Ltd, Kawasaki, Japan, using a specific RIA procedure. Serum 17-OHP, Δ^4 -A, DHEA, DS, testosterone, cortisol, E_1 , E_2 and prolactin and plasma aldosterone and renin activity were determined in a single laboratory (Special Reference Laboratories, Tokyo, Japan) using a specific RIA procedure. Normal baseline steroids levels in healthy Japanese girls at Tanner stages 2-3 are as follows: Δ^5 -17P, less than 500 ng/dl; 17-OHP, 20-160 ng/dl; Δ^4 -A, 35-110 ng/dl; DHEA, 140-400 ng/dl, DS, 30-110 μ g/dl; testosterone 0.2-0.6 ng/ml; and cortisol 4-20 μ g/dl.

Discussion

3β -hydroxysteroid dehydrogenase (3β -HSD) deficiency now appears to be exhibiting heterogeneity (Pang *et al.*, 1985; Medina *et al.*, 1986; Temeck *et al.*, 1987). The classical form of 3β -HSD deficiency is present in females at birth with symptoms of mild virilization and salt wasting, while the allelic variant of the same defect may result in nonclassical 3β -HSD deficiency and symptoms of virilization later in life.

In recent years, nonclassical adrenal 3β -HSD deficiency in women with hirsutism has been reported (Pang *et al.*, 1985; Medina *et al.*, 1986; Temeck *et al.*, 1987). Pang *et al.* (1985) reported that 16 out of 116 women with hirsutism aged 13 6/12 to 43 years had nonclassical symptomatic 21 -hydroxylase deficiency and 17 had nonclassical adrenal 3β -HSD deficiency (14% or about 1 in 7 hirsute women) and that the increased growth rate suggested the possibility of excess androgen effect before the development of hirsutism.

On the other hand, Morris *et al.* (1989) could not observe any evidence of nonclassical congenital adrenal hyperplasia in 31 patients (28 girls and 3 boys), ranging in age from 3.2 to 7.9 years, with precocious adrenarche defined by the presence of early sexual hair development, but no signs of virilization, and bone age within +3 SD of the mean for chronological age. The reasons for the discrepancies between our findings and those of Pang *et al.* (1985) and those of Morris *et al.* (1989) may be due to discrepancies in the patients' conditions. Our patients and some subjects of Pang *et al.* (1985) had signs of precocious pubarche, including clitoral enlargement, hirsutism, marked growth velocity, and/or significantly advanced bone age, whereas those of Morris *et al.* (1989) had precocious adrenarche without signs of systemic virilization (clitoromegaly or phallic enlargement, hirsutism, acne, acceleration in growth velocity and markedly advanced bone age).

The most valuable hormonal tests in discriminating patients with 3β -HSD deficiency from patients with 21 -hydroxylase deficiency, normal men, and patients with hirsutism without an adrenal steroidogenic defect proved to be the relationship of Δ^5 to Δ^4 steroids and the high level of precursor of Δ^5 -steroids such as Δ^5 -17P after ACTH administration (Pang *et al.*, 1985; Medina *et al.*, 1986; Temeck *et al.*, 1987). Basal serum Δ^5 -17P, DHEA and DS and urinary 17KS are not always high in patients with nonclassical 3β -HSD deficiency (Pang *et al.*, 1985; Medina *et al.*, 1986; Temeck *et al.*, 1987). Our two patients were diagnosed as having nonclassical 3β -HSD deficiency based upon the increased response of serum Δ^5 -17P and the high ratios of Δ^5 -17P/17-OHP and DHEA/ Δ^4 -A to ACTH stimulation. Moreover, Δ^5 -17P was suppressed by dexamethasone suppression test. The data for the LH-RH test in patient 1 indicated early activation of

pubertal gonadotropin secretion (Pescovitz *et al.*, 1984 and 1988).

Only a few children have been reported to have nonclassical 3 β -HSD deficiency, but most cases may have gone unrecognized. When routine assay of Δ^5 -17P becomes more generally available, the recognition of this disorder will probably increase.

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