0031-3998/95/3706-0820$03.00/0
PEDIATRIC RESEARCH

Vol. 37, No. 6, 1995
Printed in U.S.A.

Absence of Molecular Defect in the Type II 3β-Hydroxysteroid Dehydrogenase (3β-HSD) Gene in Premature Pubarche Children and Hirsute Female Patients with Moderately Decreased Adrenal 3β-HSD Activity

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ABSTRACT

To date the molecular basis and hormonal criteria for inherited mild late-onset 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency congenital adrenal hyperplasia (CAH) have not been defined. We have thus investigated the presence or absence of mutation in the type II 3β-HSD gene encoding adrenal/gonadal 3β-HSD in each of five premature pubarche children and hirsute female patients manifesting moderately decreased adrenal 3β-HSD activity. ACTH-stimulated hormonal levels in all patients compared with mean levels in pubertal stage-matched normal subjects were between 2.5 and 6.5 SD for 17-hydroxypregnenolone levels, and between 2.5 and 7 SD for dehydroepiandrosterone levels in all except one patient. 17-Hydroxypregnenolone to cortisol ratios were between 2.5 and 4.3 SD, and dehydroepiandrosterone to androstenedione ratios were between 3 and 8.6 SD. The type II 3β-HSD gene regions of a putative promoter, exons I, II, III, and IV, and exon-intron boundaries in all subjects were amplified by polymerase chain reaction and then sequenced. All patients had normal sequences of the type II 3β-HSD gene in both alleles. Three female patients heterozygotic for severe 3β-HSD deficiency CAH with one allele mutation of the gene demonstrated normal ACTH-stimulated hormone profiles. These data indicate that moderately decreased adrenal 3β-HSD activity resulting in modestly increased Δ5 precursor steroid levels and Δ5 to Δ4 steroid ratios in premature pubarche and hirsute patients is not caused by a mutation in the type II 3β-HSD gene. This suggests that the moderately decreased adrenal 3β-HSD activity in the patient is not due to mild late-onset variants of inherited 3β-HSD deficiency CAH, whereas the carriers for true 3β-HSD deficiency CAH do not express decreased adrenal 3β-HSD activity. (***Pediatr Res* 37: 820–824, 1995**)

Abbreviations
3β-HSD, 3β-hydroxysteroid dehydrogenase
CAH, congenital adrenal hyperplasia
PCR, polymerase chain reaction

Severe 3β-HSD deficiency in the adrenal cortex results in the deficiency of cortisol and aldosterone, leading to CAH and salt-wasting adrenal crisis in early postnatal life (1). Concomitant testicular 3β-HSD deficiency results in sexual ambiguity in genetic males. In genetic females mildly virilized genitalia may be present due to excess production of weak adrenal androgens during fetal life (1). The clinical and biochemical spectrum of severe 3β-HSD deficiency CAH, however, is heterogeneous from non-salt-wasting to salt-wasting forms (1–5). In addition, 1.5–13% of children presenting with premature pubarche (6, 7) and 3–60% of older female patients presenting with hirsutism and menstrual disorders (8–10) were reported to have mild late-onset 3β-HSD deficiency based on ACTH-stimulated hormonal findings. However, diagnosis of

Received January 17, 1995; accepted March 22, 1995.
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Supported by a Genentech Foundation grant, by a grant from the U.S. Public Health Service (HD24360), and by a biomedical research grant from the University of Illinois, College of Medicine, Chicago.

mild 3β-HSD deficiency based on the published hormonal criteria in past reports (7–12) is questionable due to lack of any definite genotypic proof.

Molecular studies in patients with salt-wasting and non-salt-wasting forms of severe 3β-HSD deficiency CAH have recently demonstrated a deleterious point mutation in the coding region or in exon-intron boundaries of the type II 3β-HSD gene only (11–15). These findings suggest that 3β-HSD deficiency CAH is caused by a mutation in the type II 3β-HSD gene. If mild variants of 3β-HSD deficiency CAH exist, it is likely to result from a less deleterious mutation in the type II 3β-HSD gene. To determine the molecular basis of mild adrenal 3β-HSD deficiency in children with premature pubarche and in young women with hirsutism, we investigated the presence or absence of the type II 3β-HSD gene mutation in those patients whose ACTH-stimulated hormonal profiles indicated decreased adrenal 3β-HSD activity.

METHODS

The study was approved by the IRB of the institution and was conducted after appropriate informed consent was obtained from the subjects.

Patients. Five children with premature pubarche occurring at ages 4–6 y demonstrated mild growth acceleration after pubarche based on growth percentile changes and/or advanced bone age, to chronologic age (Table 1). Five female patients, ages varying from 14 to 38 y, presented with complaints of peripubertal or postpubertal onset of slowly progressive hirsutism and menstrual disorders (Table 1). Two hirsute patients had a history of early pubarche. Menarche was appropriate in all. All patients with premature pubarche or hirsutism had normal genitalia both at birth and at evaluation, and had no history of salt-wasting. The height and weight of all patients at the time of evaluation, and ethnic or racial backgrounds of the patients are shown in Table 1. All patients underwent an ACTH stimulation test.

Control normal subjects. Eighteen healthy prepubertal children (10 girls and eight boys) from ages 4 to 9 y, and 22 children from ages 8.5 to 14 y with Tanner II–III stage pubic hair development (12 girls and 10 boys) had ACTH stimulation tests. Thirty-one healthy female subjects, ages 14–43 y (mean age 26 y), with normal menstrual histories and no evidence of hirsutism or acne had ACTH stimulation tests at various phases of the menstrual cycle. Three mothers of patients with proven 3β-HSD deficiency CAH due to type II 3β-HSD gene mutation (13, 15) (our unpublished finding) also underwent ACTH stimulation tests.

ACTH stimulation test and hormonal assays. The test was performed by a bolus administration of synthetic ACTH (Cortrosyn; Organon, West Orange, NJ), 0.25 mg, intravenously at 0830–1100 h. Blood samples were obtained at 0 min before and 60 min after ACTH administration, and sera were kept at −20°C until hormonal assay. Serum 17-hydroxypregnenolone, dehydroepiandrosterone, androstenedione, and cortisol levels were determined by a RIA after a purification procedure with celite chromatogram as described elsewhere (16). The inter- and intraassay variations of these assays were 10–15% and less than 5–10%, respectively.

PCR and sequencing of the type II 3β-HSD gene. The genomic DNA from all subjects were prepared from peripheral white blood cells. A putative promoter region, exons I, II, III, and IV, and exon-intron boundary regions of the type II 3β-HSD gene were amplified by PCR using primers designed based on the type II 3β-HSD gene structure (17) as depicted in Fig. 1. Direct sequencing of the PCR products was performed according to femtomoles of DNA sequencing system (Promega, Madison, WI). The primers for PCR and seven additional internal primers were used for the sequencing (Fig. 1).

RESULTS

ACTH-stimulated hormonal profiles and type II 3β-HSD gene sequence findings in the patient and in proven carriers for severe 3β-HSD deficiency CAH (Fig. 2). Compared with mean values of the normal Tanner II–III children, the five children with premature pubarche had ACTH-stimulated hormonal levels between 3 and 6.5 SD for 17-hydroxypregnonelone and between 2.6 and 6 SD for dehydroepiandrosterone in all except one child, 17-hydroxypregnenolone to cortisol ratios of 2.7 to 4.3 SD, and dehydroepiandrosterone to androstenedione ratios of 4 to 8.6 SD (Fig. 2), indicating moderately decreased adrenal 3β-HSD activity. The type II 3β-HSD gene sequences in all five children with premature pubarche were normal in the regions of the putative promoter, exons I, II, III, and IV, and all exon and intron boundaries.

ACTH-stimulated hormonal levels in all five hirsute female patients compared with mean values of the normal female subjects were between 2.5 and 4 SD for 17-hydroxypregnenolone, 2.5 and 7 SD for dehydroepiandrosterone, 2.5 and 4.3 SD for 17-hydroxypregnenolone to cortisol ratios, and 3 and 5 SD for dehydroepiandrosterone to androstenedione ratios in all except one patient (Fig. 2), indicating moderately decreased adrenal 3β-HSD activity. Sequences of the type II 3β-HSD gene in all five female patients with hirsutism and menstrual disorders also did not reveal any mutation, although a polymorphism at codons 142 and 298 in exon IV was found in one hirsute female patient.

In three mothers of patients with severe 3β-HSD deficiency CAH, ACTH-stimulated 17-hydroxypregnenolone and dehydroepiandrosterone levels, as well as 17-hydroxypregnenolone to cortisol ratios and dehydroepiandrosterone to androstenedione ratios, were within 2 SD of mean levels for the normal female subjects, with the exception of one mother who had a dehydroepiandrosterone to androstenedione ratio at 2.2 SD (Fig. 2). One mother had a frameshift mutation in exon IV of one allele of the type II 3β-HSD gene (13), whereas another mother had intron III mutation in one allele of the gene (15). A third mother had a small deletion (AAA → A at codon 273) resulting in a frameshift mutation in exon IV of one allele of the gene (our unpublished finding).

DISCUSSION

The children with premature pubarche and female patients with hirsutism with moderately decreased adrenal 3β-HSD activity did not demonstrate any allele mutation in the putative promoter, coding, and apparent RNA splicing regions of the

Table 1. *Clinical data for patients with premature pubarche or hirsutism with hormonal evidence of decreased adrenal 3β-HSD activity*

Patient	Sex	At initial evaluation of ACTH stimulation test: Age (y)	Ht: cm (Ht age)	Wt: kg (wt age)	Ethnicity/Race	Pubarchal age (y)	Childhood growth acceleration beginning at	BA* to CA†	Age at gonadarche‡ (y)	Age at menarche (y)	Hirsutism onset and progress	Menstrual problems
Premature pubarche												
1	F	8.5	140.7 (10.1)	29 (9.5)	Caucasian	6	6 y	BA 2 y > CA 8 y	ND§	ND	ND	n/a¶
2	F	9	132.4 (9)	26.5 (8.5)	Puerto Rican	4	5.5 y	BA 1 y > CA 9 y	9	ND	ND	n/a
3	F	7	112.7 (5.8)	18.5 (5)	Caucasian	5		BA 2 y > CA 7 y	ND	ND	ND	n/a
4	F	4	104.5 (4)	22.7 (7)	African-American	4		BA 2 y > CA 4 y	ND	ND	ND	n/a
5	M	6	121.6 (7)	23.4 (7.5)	Caucasian	5	6–7 y		ND	n/a	ND	n/a
Hirsute												
1	F	19	166	118.5	Caucasian	7	Unknown		8	9	9 y, slow	Initial irregularity → 2° amenorrhea
2	F	14	162.9	91.9	Caucasian	7	Tall		10	14	10 y, slow	Menometrorrhagia
3	F	29	162.5	109.5	Caucasian	10	Unknown		10	12	12 y, slow	Irregular, every 3 mo
4	F	38	168.2	72.3	Caucasian	10	Unknown		13	14	25 y, slow	Irregular, since age 28 y
5	F	22	157.2	73.4	Caucasian	10	Unknown		10	12	16 y, slow	Irregular, every 2 wk to 2 mo

* BA, bone age.

† CA, chronological age.

‡ Gonadarche in female, thelarche; gonadarche in male, testicular enlargement.

§ ND, not yet developed.

¶ n/a, not applicable.

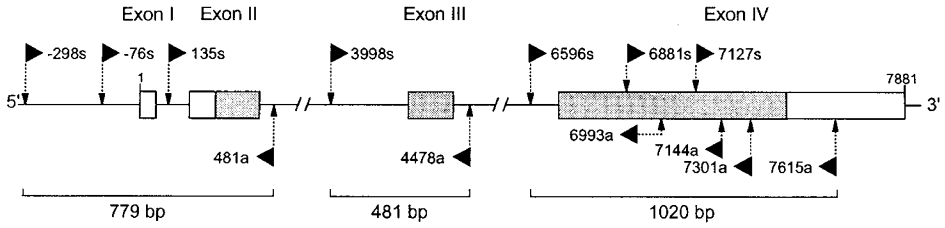

Figure 1. Schematic of primer (► and ◄) design for PCR and sequencing of the type II 3β-HSD gene. Primers 76s, 135s, 6881s, 7127s, 6993a, 7144a, and 7301a were used for sequencing only. The numbers indicate the nucleotide. "s" and "a" indicate "sense" and "antisense" strands. *Shaded area*, translated regions of the exons; *open boxes*, untranslated regions of the exon.

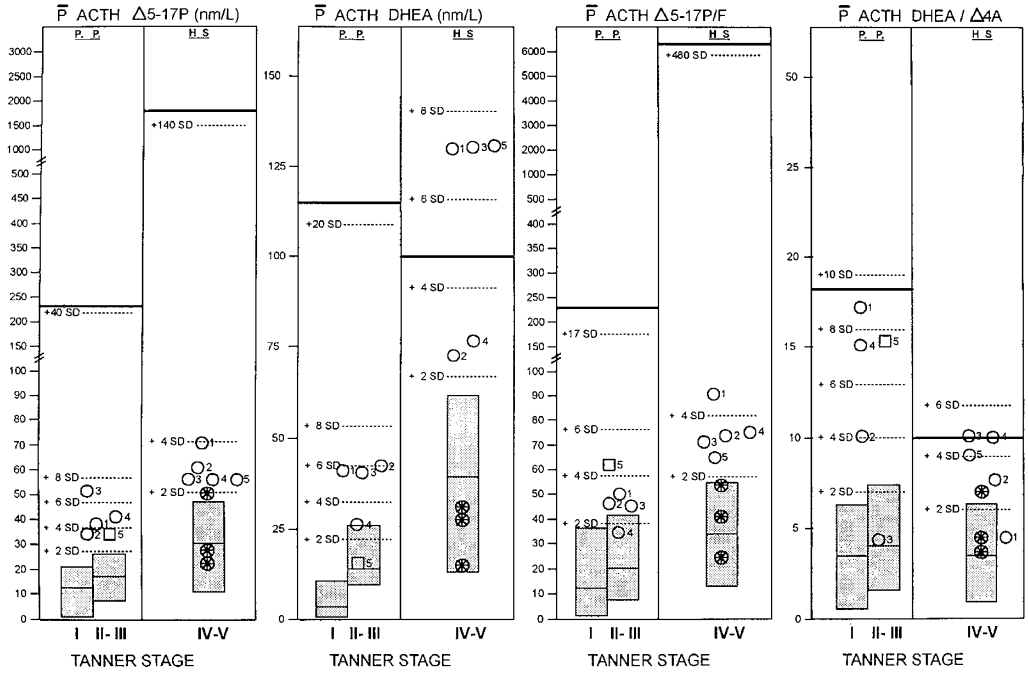

Figure 2. ACTH-stimulated serum 17-hydroxypregnenolone (Δ5–17P) and dehydroepiandrosterone (*DHEA*) levels and ratios of 17-hydroxypregnenolone/cortisol (*Δ5–17P/F*) and dehydroepiandrosterone/androstenedione (*DHEA/Δ4-A*) in children with premature pubarche (*PP*) and in female patients with hirsutism (*HS*) without type II 3β-HSD gene mutations (□, male; ○, female), and in three carrier mothers for severe 3β-HSD deficiency CAH (*) with one allele mutation in the type II 3β-HSD gene. The *shaded bars* are hormonal values (mean and range) of normal Tanners I and II–III children and Tanner IV–V female subjects. +2 to 480 SD followed by a *dotted line*: the level 2–480 SD above the pubertal stage matched normal subjects' mean values. The *number* identifies the same patient in Table 1 and Figure 2. Under PP, the *bold horizontal line* indicates the lowest hormonal value reported in the children with severe 3β-HSD deficiency CAH (2, 3). Under HS, the *bold horizontal line* indicates either the only value reported or the lowest value reported in the adolescent or adult patients with proven severe 3β-HSD deficiency CAH (4, 5). To convert nm/L to ng/dL, multiply by 33.3 for Δ5–17P and multiply by 28.6 for DHEA.

type II 3β-HSD gene, although a mutation in some regulatory element or unidentified areas of intron cannot be excluded. To date, however, little evidence exists for such a mutation as the cause of human CAH disorder. Thus, it is unlikely that the clinical and hormonal abnormalities of these patients are caused by true mild 3β-HSD deficiency due to type II 3β-HSD gene mutations. Hormonally, these patients exhibited a significantly milder degree of decreased adrenal 3β-HSD activity compared with patients with proven severe adrenal/gonadal 3β-HSD deficiency (Fig. 2) (2–5). In addition, normal hormonal profiles in the carriers for severe 3β-HSD deficiency suggest that one allele mutation of the gene is not a cause for the milder degree of decreased adrenal 3β-HSD activity. These findings suggest the existence of some other etiologic factor(s) resulting in moderately decreased adrenal 3β-HSD activity in children and older female subjects with mild excess androgen symptoms. The pathogenesis of this milder degree of decreased adrenal 3β-HSD activity remains to be elucidated.

ACTH-stimulated dehydroepiandrosterone levels and dehydroepiandrosterone to androstenedione ratios in children with premature pubarche and without type II 3β-HSD gene mutation were markedly and slightly lower, respectively, than in age-matched children with severe 3β-HSD deficiency CAH (Fig. 2) (2). However, ACTH-stimulated dehydroepiandrosterone levels and dehydroepiandrosterone to androstenedione ratios in the hirsute female subjects without type II 3β-HSD gene mutations overlapped with levels reported in older patients with severe 3β-HSD deficiency CAH (Fig. 2) (4, 5). These findings indicate that hormonal parameters in the C21 glucocorticoid pathway are better indices than hormonal parameters in the C19 androgen pathway in diagnosing true adrenal 3β-HSD deficiency resulting from the type II 3β-HSD gene mutation.

The normal sequence of the type II 3β-HSD gene in patients with moderately decreased adrenal 3β-HSD activity indicates that the previously reported hormonal criteria for mild late-

onset 3β-HSD deficiency CAH (6–10) need to be revised. By comparison, phenotypically normal carriers for 21-hydroxylase deficiency express hormonal evidence of very mild 21-hydroxylase deficiency upon ACTH stimulation (18, 19). Moreover, ACTH-stimulated 17-hydroxyprogesterone levels in patients with mild late-onset 21-hydroxylase deficiency were >21 SD above mean values of homozygous normal relatives, and >6 SD above mean values of known carrier subjects (18, 19). The proven carriers for severe 3β-HSD deficiency in our study did not exhibit hormonal evidence of apparent mild 3β-HSD deficiency. Thus, it is uncertain whether comparable degrees of the precursor hormone abnormality found in patients with mild 21-hydroxylase deficiency will be expressed in patients with bonafide mild late-onset 3β-HSD deficiency resulting from mildly deleterious mutations in the type II 3β-HSD gene. Nevertheless, it is expected that patients with a homozygous or heterozygous mutation of a mildly deleterious nature in the type II 3β-HSD gene express a substantially greater degree of hormonal abnormality than do the patients described in this report who did not demonstrate any mutation in the type II 3β-HSD gene. Thus, the hormonal criteria for mild variants of true 3β-HSD deficiency CAH resulting from type II 3β-HSD gene mutations are yet to be defined. The previously published hormonal criteria for mild late onset 3β-HSD deficiency (7–12), therefore, should no longer be used for diagnosing so-called mild or late onset or non-classic 3β-HSD deficiency in children with premature pubarche and in female patients with hirsutism or menstrual disorders.

In conclusion, the moderately decreased adrenal 3β-HSD activity based on the ACTH-stimulated hormonal findings in children with premature pubarche and in female patients with hirsutism and menstrual disorders is not caused by an apparent mutation in the promoter, coding, or RNA splicing regions of the type II 3β-HSD gene. The pathogenesis for moderately decreased adrenal 3β-HSD activity of this undetermined etiology, as well as the molecular basis and hormonal criteria for bonafide mild late-onset 3β-HSD deficiency, if it exists, remain to be elucidated.

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