

CASE REPORT

Puberty in a case with novel 17-hydroxylase mutation and the putative role of estrogen in development of pubic hair

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Abstract

Objective: 17-Hydroxylase/17,20-lyase deficiency (17OHD) results from mutations in the *CYP17A1* gene, leading to failure to synthesize cortisol, adrenal androgens, and gonadal steroids. Adrenarche is a consequence of the increased production of adrenal androgens. Here, we report a case carrying novel R239Q mutation causing complete functional loss of *CYP17A1*, and thus absence of adrenal and gonadal sex hormone production. The patient has had unexpected pubic hair development and insufficient breast development with estrogen replacement therapy. Possible mechanisms leading to pubic hair development and breast underdevelopment are discussed.

Patient and methods: A 15-year-old female born to consanguineous parents presented with the lack of full breast development and irregular menses after the age of 14 years. She had Tanner III breast development on one side, Tanner I on the other side and Tanner I pubic hair and, no axillary hair development. The serum levels of FSH, LH, and progesterone were high and, estradiol was low. The measurement of basal and ACTH-stimulated steroids was consistent with the diagnosis of 17OHD. Genetic analysis revealed novel homozygous mutation R239Q in *CYP17A1* gene. Therapy with hydrocortisone was initiated and followed by the addition of conjugated estrogen. Her breast development did not improve considerably, however, pubic hair development started after estrogen treatment in spite of undetectable serum levels of androgens.

Conclusion: This case study suggests that estrogen exerts a permissive effect on pubic hair development in girls, even in the presence of very low-circulating androgens, and impaired breast development might be due to estrogen/progesterone imbalance in breast tissue.

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Introduction

17-Hydroxylase/17,20-lyase deficiency (17OHD), a rare autosomal recessive defect in adrenal and gonadal steroidogenesis, results from mutations in the *CYP17A1* gene, leading to the insufficiency and failure to synthesize cortisol, adrenal androgens, and gonadal steroids (1, 2). Complete loss of function mutations in *CYP17A1* cause absence of secondary sex characteristics in affected cases (3). However, presenting secondary sexual characteristics are variable amongst patients carrying different mutations (4, 5).

Adrenarche is a consequence of the maturation of the zona reticularis of the adrenal cortex, resulting in increased synthesis and secretion of adrenal androgens, namely the 19 carbon steroids (C19) DHEA and DHEA-sulfate (DHEAS). Since DHEA and DHEAS are weak or inactive as androgens, peripheral conversion of these steroids to testosterone and, subsequently dihydrotestosterone (DHT) is necessary to promote

androgen-dependent hair growth in girls (6). It is apparent that adrenal androgens, independent of gonadal androgens, may drive axillary and pubic hair development, which is proven by a series of cases demonstrating the growth of androgen-dependent hair in the absence of gonadal steroids and conversely demonstrating the absence of pubarche in girls with adrenal insufficiency (7–9). However, delayed pubarche following gonadarche observed in some female patients with adrenal insufficiency suggests that gonadal steroids also play a role in pubic hair growth (8).

Here, we report our experience in a 15-year-old patient with 17-hydroxylase deficiency carrying a novel R239Q mutation causing complete loss of the function of *CYP17A1*. We suggest that lack of full breast development in our patient is related to estrogen/progesterone imbalance and, pubarche occurring after the estrogen replacement therapy supports the notion that estrogen somehow effect the process of pubic hair growth. Possible mechanisms of these interactions are discussed.

Patient and methods

A 15-year-old female born to consanguineous parents presented with the lack of full breast development. History revealed that menarche had occurred at 14 years of age and was followed by irregular menstrual cycles. On physical examination, breast development was Tanner III on one side and Tanner I on the other side, axillary hair was absent and pubic hair was Tanner stage I with some vellus type hair. Blood pressure was 120/70 mmHg (75–90 and 50–75 percentile, systolic and diastolic respectively). Pelvic ultrasound showed normal sized uterus (44 × 14 mm) and enlarged ovaries (53 × 58 and 70 × 32 mm) with multiple immature cysts. The serum levels of Na, K, BUN, and creatinine were normal. The serum levels of FSH and LH were high, and E2 was low. The measurement of basal and ACTH-stimulated steroids revealed elevated 11-deoxycorticosterone (DOC) and progesterone levels with normal 11-deoxycortisol and decreased plasma renin activity (PRA), which was consistent with the diagnosis of 17OHD (Tables 1 and 2).

Therapy with hydrocortisone was initiated and followed by the addition of conjugated estrogens (Premarin) with initial dose of 0.625 mg and increased to 1.875 mg in 2 years. Cyclic treatment with addition of progesterone to estrogen had then been continued

for an additional 3 years. Her breast development became Tanner stage III in Tanner I side and Tanner III at the other side with some asymmetry between two breasts without remarkable improvement in the volume. However, pubic hair development became evident starting from the 4th month of estrogen treatment and with slow progression became Tanner stage V with relatively light pigmentation at the end of the 3rd year of treatment. Interestingly, serum levels of DHEAS, testosterone, and DHT were still below the detection limits of the assay (<15 µg/dl, <0.02 ng/ml, and <1 ng/l respectively) at that time while she was developing pubarche. Axillary hair had not developed in the following 5 years despite Tanner stage V pubic hair development.

Plasma ACTH, serum FSH, LH, estradiol, DHEAS, progesterone, cortisol, and testosterone levels were analyzed by commercial kits based on solid-phase, two-site sequential or competitive chemiluminescent immunometric assay or electrochemiluminescence immunoassay. Serum levels of DHT, 17OH progesterone, PRA, and aldosterone were determined by RIA, and DOC and 11-deoxycortisol by HPLC.

After obtaining informed consent, genomic DNA from the patient, parents, and unaffected sister was extracted from peripheral blood leukocytes and used to perform PCR exonic amplification of the *CYP17A1* gene as described previously (10). Genetic analysis revealed novel homozygous mutation R239Q in the *CYP17A1* gene in the patient (CGA-CAA, g.3461, GenBank accession number NC_000010), and the parents and unaffected sister were heterozygous for the same mutation. The cDNA for *CYP17A1* mutation R239Q was generated by overlapping PCR and subcloned into pcDNA3. HEK-293 cells were seeded in 12-well plates and transfected with 0.5 µg pcDNA3-*CYP17A1* plasmids using FuGENE6 as described (mock, wild-type *CYP17A1*, and mutation R239Q) (11, 12). Cells were incubated with 0.5 ml complete medium containing 0.1 µmol/l pregnenolone with 150 000 c.p.m. [³H]-pregnenolone (PerkinElmer NEN Life Sciences, Shelton, CT, USA). Aliquots (1 ml) were removed after 1 and 4 h, extracted, and chromatographed on plastic silica gel plates using 3:1 chloroform/ethyl acetate as described (11, 12). The plates were dried, and sandwiched against a BAS-TR2040 tritium phosphorimaging screen (Fuji Medical Systems, Stamford, CT, USA). The plate was imaged using a Molecular Dynamics (Sunnyvale, CA, USA) Storm 860 phosphorimager, and steroids were quantitated with ImageQuant V5.0 software. Under conditions where 100% of pregnenolone was reproducibly metabolized to 17-hydroxypregnenolone and DHEA by the wild-type enzyme, pregnenolone metabolism by mutation R239Q was indistinguishable from mock in quadruplicate and duplicate incubations from two different experiments (Fig. 1).

Table 1 Serum hormone levels of the patient with 17-hydroxylase/17,20-lyase deficiency (17OHD).

		Reference ranges
FSH, mIU/ml (IU/l)	15.2 and 13.4 (15.2 and 13.4)	2–12 (2–12)
LH, mIU/ml (IU/l)	18.2 and 11.6 (18.2 and 11.6)	2–15 (2–15)
E2, pg/ml (pmol/l)	27.4 and <20 (101.5 and <74)	27–246 (100–911)
DHEAS, µg/dl (µmol/l)	<30 and <30 (<0.8 and <0.8)	35–430 (0.95–11.6)
Progesterone, ng/ml (nmol/l)	38.4 and 4.3 (122.1 and 13.7)	0.95–21 (3–66.8) 0.48–1.72 (1.5–5.5) ^a
17-OH P, ng/ml (nmol/l)	1.74 (5.3)	0.2–2.6 (0.6–7.88)
Cortisol, µg/dl (nmol/l)	3.06 (84.4)	6–25 (165.5–689.7)
Deoxycorticosterone, ng/dl (nmol/l)	265 (8)	2–34 (0.06–1.02)
11-Deoxycortisol, ng/ml (nmol/l)	2.8 (8.09)	<8 (<23.1)
Aldosterone, pg/ml (nmol/l)	120 (33.3)	20–240 (5.55–66.6)
ACTH, pg/ml (pmol/l)	110 (24.4)	<100 (<22.2)
Plasma renin activity, ng/ml per hour (ng/l per second)	0.5 (0.14)	1.5–5.7 (0.42–1.58)

SI units were given in parentheses.

^aNormal adult female values luteal and midfollicular phases respectively.

Table 2 Basal and peak serum steroid hormone levels after two consecutive ACTH stimulation tests performed 2 weeks apart.

	Reference ranges	1st Test		2nd Test	
		0 min	60 min	0 min	60 min
Cortisol, µg/dl (nmol/l)	Pre: 6–25 (166–690) Post: 18–42 (497–1159)	3.3 (91)	5.5 (151.7)	4.9 (135.2)	6.6 (182.1)
17-OH P, ng/ml (nmol/l)	Pre: 0.2–2.6 (0.6–7.88) Post: 2–10 (6–30.3)	7.8 (23.6)	6.3 (19.1)	6.1 (18.5)	9.1 (27.5)
Progesterone, ng/ml (nmol/l)		38.4 (122.1)	33.6 (106.8)	4.3 (13.6)	14.7 (46.7)

SI units are given in parentheses.

Discussion

Here, we presented a normotensive patient carrying a novel R239Q mutation with high DOC and suppressed PRA. In 17OHD, impairment of cortisol production leads to absence of negative feedback by cortisol causing overproduction of ACTH, and resultant abundance of the weak glucocorticoid corticosterone provides adequate systemic glucocorticoid action and feedback on ACTH secretion. The hypothalamic–pituitary–adrenal axis then reaches a steady state at a higher set point; however, the drive to overproduce corticosterone allows the accumulation of the potent mineralocorticoid DOC, and high DOC production stimulates salt and water retention, which suppresses renin secretion. Thus, aldosterone production is low, but hypertension and hypokalemia develop because of DOC excess (3). However, our patient did not have hypertension despite decreased PRA and normal serum levels of aldosterone and electrolytes. Similarly, most of the cases with 17OHD have variations in the blood pressure, serum potassium, and the aldosterone secretion rate even in the patients carrying same mutations (3, 5). Furthermore, normal plasma renin levels in hypertensive patients and low renin levels in normotensive patients were also described (13, 14). This heterogeneity has not been completely understood, but many factors, including the severity of *CYP17A1* mutation, dietary sodium intake, environment, and other genetic factors regulating electrolyte metabolism might have a role in these

mechanisms. Furthermore, capacity for menstruation, gonadal histology, and morphology are also variable without completely explained mechanisms in 17OHD (3). High serum levels of 17OH progesterone as well as spontaneous breast development and menstrual cycles in our patient were discordant with *in vitro* finding of the mutation showing almost complete functional loss of *CYP17A1*. However, it is not an uncommon condition that *in vitro* experiments do not reflect completely the true *in vivo* conditions (5).

In the present case, novel R239Q mutation causing almost complete loss of function of *CYP17A1* lead to very low adrenal and gonadal androgen production; consequently, pubarche had not occurred despite menarche in the patient. However, after the initiation of estrogen replacement, pubarche was observed despite very low serum levels of androgens suggesting that estrogen might have some role in pubarche development independent of circulating DHEAS.

In normal puberty, increase in adrenal androgen production can be detected around 6 years of age, and the phenotypic outcome consists of the development of axillary and pubic hair that occurs at ~8 years of age after the increment of serum DHEAS levels over 40 ng/dl (15–17). Although the increase in adrenal androgen production is one of the hallmarks of pubertal development and normally precedes gonadarche (18), the initiation and progression of adrenarche are believed to be independent of maturation of the hypothalamic–pituitary–gonadal axis and gonadal steroidogenesis (19–21). Patients with adrenal insufficiency undergo gonadarche in the absence of adrenarche (8, 20), indicating that adrenarche is not a requirement for activation of gonadarche. However, delayed pubarche following gonadarche is observed in some girls with adrenal insufficiency as a result of production of gonadal steroids and androgens (8). Absence of adrenarche in some adrenal insufficiency cases might be related to probable coexistence of adrenal and gonadal failure at the same time as in the cases with 17OHD.

Furthermore, it has recently been demonstrated that serum DHEAS levels were significantly higher in girls with Turner syndrome and primary ovarian failure than in Turner syndrome girls with spontaneous puberty onset (22). Despite that, pubarche was delayed in Turner syndrome girls with primary ovarian failure compared

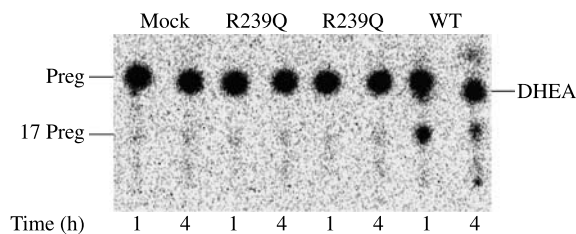


Figure 1 Phosphorimage of chromatographed steroids. Medium from HEK-293 cells mock-transfected (Mock) or transfected with pcDNA3-*CYP17A1* (mutation R239Q or wild-type as labeled) for indicated times. Under conditions where 100% of pregnenolone (Preg) were reproducibly metabolized to 17-hydroxypregnenolone (17 Preg) and DHEA by the wild-type enzyme, Preg metabolism by mutation R239Q was indistinguishable from mock in all incubations. Results from one out of the two experiments are shown.

with Turner syndrome girls with spontaneous pubertal development (13-year versus 11.9-year). These data demonstrate that normal timing of pubarche can be dependent on gonadal function as well as the DHEAS rise of adrenarche. It is conceivable that estrogen causes a sensitization of the hair follicles by increasing local androgen production or direct effect of estrogen on the hair follicles. Further supporting evidence of direct effect of estrogen on pubic hair development comes from case reports of infants that have been accidentally treated with estrogen containing creams. The use of dermal ointments containing estrogen resulted in the growth of pubic hair in both males and females ranging from 4 months to 2 years (23).

Additionally, in contrast to classical knowledge of absence of pubic and axillary hair development in complete androgen insensitivity (CAIS), almost all of the ungonadectomized patients with CAIS, having high serum levels of testosterone and normal or high normal male serum estradiol levels, develop Tanner stage II–IV sparse pubic hair (24–27). Furthermore, axillary hair develops only in one-third to two-third of the patients with CAIS and, it is proposed that only the absence of axillary hair, but not the pubic hair is the stronger evidence for complete absence of androgen action (25, 26). It is interesting that our patient did not have axillary hair development with Tanner stage V pubic hair development that demonstrated absence of androgen action consistent with this hypothesis. Additionally, she developed better pubic hair than the patients with CAIS after estrogen treatment which corresponded to normal female estrogen levels that were higher than normal or high normal male levels detected in CAIS patients. Although, these findings support the role of estrogen in pubic hair development, more complex interactions of adrenal and gonadal steroids and their receptors seem to play crucial roles in sexual hair as well as pubertal development.

Furthermore, sparse pubic hair development occurred in some CYP17A1 deficiency patients with spontaneous puberty (4, 28–32), pubic as well as axillary hair growth is noted after cyclic estrogen/gestagen treatment similar to our case in only one Turkish patient with 17OHD, living in Germany (13). It might be a coincidence that both of the patients are of Turkish origin, however, genetic factors related to sexual hair growth might be considered in that aspect.

Another issue that needs to be discussed in our case is that she was unable to achieve full breast development even with treatment of high doses of estrogen. Female patients and most of the male patients with 17OHD usually present to clinics due to absence of secondary sexual characteristics at pubertal ages. Furthermore, follow-up of these cases shows that those who have apparent gonadal failure and present with some or no breast development could not have full breast development (33–37). However, some rare patients with higher *in vitro* enzyme function presented with either

hypertension or infertility have full breast and normal to scanty pubic hair development (4, 29–31). It is speculated that synthesis of high amounts of progesterone in 17OHD leading to estrogen/progesterone imbalance during pubertal development might be a causative factor for impairment of breast tissue growth in patients presenting at pubertal ages. Supporting evidence of this is the normal serum levels of progesterone in cases with spontaneous full breast development (4, 29–31), and high serum progesterone levels in patients having breast underdevelopment even in the absence of gonadal failure (28, 38). In our patient, we suggest that the reason for insufficient breast development is her presentation at a quite late stage of puberty (1 year after menarche) after almost completed breast growth under the influence of high progesterone low estrogen environment. Additional reports with extensive experience about pubertal development in cases with 17OHD are essential to clarify breast underdevelopment, which might have an impact on treatment protocols. Furthermore, long-term high progesterone exposure in 17OHD is also suggested to cause irreversible endometrial immaturity and poor endometrial proliferative response to the estrogen treatment (28).

While these hypotheses remain to be tested, it appears that circulating DHEA and DHEAS alone are not obligatory requirement for pubic hair development. Gonadal steroids, particularly estrogen in girls, have a role most likely via some local mechanisms in pubic hair development. In addition, impaired breast development in patients with 17-hydroxylase deficiency might be due to estrogen/progesterone imbalance in breast tissue as the results of high progesterone and low estrogen biosynthesis.

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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