A Case of Female Pseudohermaphroditism Caused by Aromatase Deficiency

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Abstract. Female pseudohermaphroditism is caused by several etiologies. Here we report a case of aromatase deficiency who showed ambiguous genitalia and maternal virilization during pregnancy. The mother had noticed her own virilization from 16 wk of gestation without androgen exposure and had low urinary estriol levels (5~10 µg/ml at 35 wk of gestation). At birth, the patient presented severe virilization (Prader V), and was assigned as a male with a micropenis and unpalpable testes but the patient had a normal female karyotype and a uterus and cystic ovaries found by magnetic resonance imaging. The patient had an increase in serum 17α-hydroxy progesterone levels (basal 4.9 → 37 ng/ml after a single 0.25 mg/m² infusion of ACTH), but the increase in adrenal androgen was not sufficient to virilize the external genitalia. Dehydroepiandrosterone, 17α-hydroxy pregnenolone and deoxycorticosterone were within the normal ranges. These findings suggested a diagnosis of nonadrenal female pseudohermaphroditism. From the clinical features and biochemical data, we endocrinologically diagnosed her as having an aromatase deficiency. The aromatase gene is now under investigation for definite diagnosis. We finally agreed that aromatase deficiency should be suspected when both the mother and the newborn have been virilized.

Key words: aromatase deficiency, female pseudohermaphroditism, maternal virilization, ambiguous genitalia

Introduction

Some newborn and older children are seen with ambiguous genitalia and it may be very difficult to assign them to either male or female gender. In the newborn, rapid gender assignment is essential to minimize emotional trauma in the family and also to diagnose and promptly treat potential life threatening situations. Female
pseudohermaphroditism is ambiguous genitalia with a normal karyotype (46,XX), normal ovarian, uterine and vaginal anatomy and virilized external genitalia believed to be due to exposure to excessive androgens in the first trimester of pregnancy. Most of the conditions are associated with congenital adrenal hyperplasia (CAH), but a small proportion of 46,XX females have an aromatase (CYP19) deficiency. Aromatase is expressed in a tissue-specific manner and a placental deficiency abolishes its function in protecting the female fetus from masculinization and the mother from virilization due to an excess of androgens.

Here we report a case of both the mother and the newborn having been virilized. From the clinical features and biochemical data, we endocrinologically diagnosed her as having an aromatase deficiency.

**Case Report**

The patient is the first child of a 31-yr-old mother. The disease history of this Japanese family was unremarkable. There is no evidence of consanguinity. The affected baby was 2.5-kg product of a full term pregnancy. The mother denied receiving any medication during the pregnancy. From 16 wk of gestation, the mother and her relatives had noticed her voice became lower, development of mild acne and coarsening of facial features, although the obstetrician had not noticed these changes. These symptoms became progressively worse until delivery. At 35 wk and 5 d of gestation, maternal urinary estriol was low at $5 \sim 10 \, \mu g/ml$. Ultrasonographic examination demonstrated a normal growth rate and no abnormal findings in the fetus. At 39 wk, she was born with a spontaneous normal vaginal delivery. After delivery, the maternal manifestations of virilization disappeared gradually except the lowered voice.

At birth, the baby had ambiguous genitalia. The baby had an enlarged clitoris (1.5 cm long; Prader V), complete fusion of posterior scrotolabial folds, and a single meatus on the top of the clitoris. At a local hospital the baby was assigned as a male with a micropenis and unpalpable testes. At 7 wk of age, the baby’s karyotype was examined for the ambiguous genitalia and revealed to be 46, XX and fluorescence in situ hybridization (FISH) analysis revealed negative SRY. At 4 mo of age, this infant was referred to our hospital for further examination.

At that time, the infant’s length was 59.2 cm ($-1.04 \, \text{SD}$) and weight was 5.5 kg ($-1.3 \, \text{SD}$). The blood pressure was normal. The infant had ambiguous genitalia; there was 1 cm long 0.6 cm...
wide phallus-like structure, complete posterior labial fusion, and a single meatus opening at the top of a phallus-like clitoris. No gonads were palpable (Fig. 1).

Serum electrolytes and lipid profile were within the normal range; other serum chemistry and blood cell count were also normal. ACTH and cortisol were within the normal range (data not shown). T was below 5 ng/dl, and the serum E2 level was 22.7 pg/ml. After a single bolus intravenous ACTH administration, serum 17α-hydroxy progesterone (17OHP) and progesterone levels increased from 4.9 to 37 ng/ml, and from 12 to 110 ng/ml (Table 1). Dehydroepiandrosterone (DHEA), 17α-hydroxy pregnenolone (17OHPreg) and deoxycorticosterone (DOC) were within the normal ranges (Table 1). On a human CG stimulation test (4000 IU/m² intramuscularly for three consecutive days), peak serum T was below 5 ng/dl. Both basal LH and FSH levels were within the normal range and LH showed a slightly hyper-response to a LHRH loading Test (Table 2). The patient’s urinary steroids profile measured by gas chromatography/mass spectrometry (GC/MS) is shown in Table 3. Although pregnanetriolone (Pt3) was higher than the control, it did not increase as much as in 21-OH deficiency. Sixteen α-hydroxy dehydroepiandrosterone (16αHD), 17α-hydroxy pregnenolone (17HP), 5β-tetrahydro-11-deoxy cortisol (5bTHS) and pregnenetriol (PT5) did not increase. An ultrasonographic examination revealed enlarged ovaries (right 3.8 x 2.4 cm, left 2.5 x 1.7 cm). Abdominal magnetic resonance imaging (MRI) showed that she had a uterus and cystic ovaries (Fig. 2).

**Table 1** Baseline steroid levels and steroid responses to a 0.25 mg/m² bolus of ACTH

|                  | 17OHP (ng/ml) | 17OHPreg (ng/ml) | DHEA (ng/ml) | DOC (ng/ml) | Prog (ng/ml) |
|------------------|---------------|------------------|--------------|-------------|--------------|
| 0 min            | 4.9           | 4.0              | 0.7          | 0.32        | 12           |
| 60 min           | 37            | 9.0              | 1.1          | 1.92        | 110          |

0 min: 0.32 (0.13–1.06) 2.97 (0.62–8.28) 1.33 (0.32–5.85) 0.21 (0.07–0.52) 0.23 (0.05–0.53)

60 min: 1.42 (0.85–2.07) 16.10 (8.96–31.73) 3.89 (1.08–11.11) 0.65 (0.20–1.58) 1.13 (0.75–2.00)

17OHP; 17α-hydroxy progesterone, 17OHPreg; 17OHPregenolone, DHEA; dehydroepiandrosterone, DOC; deoxycorticosterone, Prog; progesterone. 17OHP, 17OHPreg, DHEA, DOC and Prog were measured by RIA. Normal values for girls aged < 1 yr.

**Table 2** GnRH loading test

LHRH infusion (100 µg/m²)

|                  | patient basal levels | patient peak levels | control basal levels | control peak levels |
|------------------|----------------------|---------------------|----------------------|---------------------|
| LH (mIU/ml)      | 0.92                 | 42.8                | 0.07 (<0.05–0.98) (3)a | 18.2 (8.5–40) (4)b  |
| FSH (mIU/ml)     | 3.9                  | 17.4                | 3.7 (1.12–17.3) (3)a  | 33.3 (13.5–58.5) (4)b |

Serum LH and FSH were measured by Time-resolved fluoroimmunoassay. Normal values for girls aged 3 mo. Data are given as medians (2.5–97.5 percentiles). Normal values for infant girls. Data are given as means (range).
In the present case, the clinical manifestations were characterized by female pseudohermaphroditism and the mother’s mild virilization during pregnancy. Her karyotype was 46, XX and the SRY gene were negative by FISH.

Differential diagnoses of female ambiguous genitalia were as follows: virilizing form of CAH; 21-hydroxylase deficiency, 11β-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase deficiency (3β-HSD), true hermaphroditism, aromatase deficiency, transplacental virilizing drugs (androgens and progesterone), maternal androgen production (6, 7), and others.

As for the 21-hydroxylase deficiency, serum 17OHP and urinary Ptl levels were relatively low for genital virilization, and urinary PT5 and 16αHD did not increase. Normal levels of urinary 5bTHS and serum DOC indicated that 11β-hydroxylase activity was normal. Furthermore, no increase in DHEA or 17-HP was observed, suggesting that 3β-HSD could be excluded. Usually, maternal virilization does not occur with fetal CAH during pregnancy. Upon human CG loading test, the lack of a rise in serum testosterone levels was incompatible with true hermaphroditism.

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Considering both fetal and maternal virilization leading to female pseudohermaphroditism, we can consider an aromatase deficiency, transplacental drugs, luteoma of pregnancy, adrenal tumors, and ovarian tumors. The mother denied receiving any medication during pregnancy and her virilization disappeared after delivery. Her

### Table 3 Urinary steroids profile

|      | 1 mo | 3 mo | control mean (min–max) | 21-OH deficiency | 3β-HSD deficiency |
|------|------|------|------------------------|------------------|-------------------|
|      |      |      |                        | 5-11 d old (n=14) | 15 d old (5)     |
| Ptl  | 1.529| 0.543| 0.017 (0.000–0.080)    | 8.774            | 0.629 (mg/gCre)  |
| 16αHD| 2.221| 0.077| 3.224 (0.326–20.520)   | 85.741           | 1133.638 (mg/gCre) |
| 17HP | 0.383| 0.058| 0.014 (0.000–0.100)    | 22.402           | 30.724 (mg/gCre)  |
| 5bTHS| 0.163| 0.057| 0.091 (0.000–0.320)    | 1.575            | 3.705 (mg/gCre)   |
| PT5  | 1.036| 0.505| 0.231 (0.030–0.880)    | 15.365           | 14.217 (mg/gCre)  |
| An   | 1.483| 0.434| 0.059 (0.017–0.123)    | 1.575            | 3.705 (mg/gCre)   |

Ptl: pregnanetriolone, 16αHD; 16α-Hydroxy-dehydroepiandrosterone, 17-HP; 17α-hydroxy pregnenolone, 5bTHS; 5β-tetrahydro-11deoxycortisol, PT5; pregnenetriol, An: androsterone. *; healthy Japanese children aged 3–5 mo (n=14).
Table 4  Review of the clinical features of aromatase deficiency in genetic females

| case         | age  | gene defect                                      | pregnancy (mother) | aromatase activity |
|--------------|------|-------------------------------------------------|--------------------|--------------------|
| Kanazawa (8) | 0 yr | Homozygous splice junction defect exon 6         | Virilization (at 30 wk) | 0.30%              |
|              |      | (27 amino acid insertion)                       |                     |                    |
| Lyon (9)     | 0 yr | Homozygous for point mutation R457X (exon 10)   | Virilization (unknown) | unknown            |
| New York (10)| 28 yr| Homozygous for point mutation R357C (exon 9)    | Virilization (at 20 wk) | 0.20%              |
| Bonn (11)    | 18 mo| Homozygous for point mutation V370M (exon 9)    | Virilization (at 12 wk) | <1%                |
| San Francisco(12)| 14 yr| Compound heterozygote for two missense mutation | No virilization       | 0%                 |
|              |      | C437Y(exon 10), R435C (exon 10) and splice site  |                     | 1.1%               |
| Bern (13)    | 4 yr | Compound heterozygote base pair deletion (exon 3)| Virilization (at 12 wk) | 0%                 |

| case         | symptom                                                      | hormonal level            |
|--------------|--------------------------------------------------------------|---------------------------|
| Kanazawa (8) | Greatly enlarged phallus, complete fusion of scrobal fold,   | umbilical artery: E3 ↓ T ↑ |
|              | single meatus at base of phallus                              | E2 ↓, T ↑, FSH ↑, LH ↑    |
| Lyon (9)     | Ambiguous genitalia Prader IV                                | A ↑, 170HP ↑              |
| New York (10)| No breast development, progressive virilization, cystic ovaries, bone age delay, pseudohermaphroditism | E2 ↓, T →, FSH ↑, LH →, A ↑ |
| Bonn (11)    | Ambiguous genitalia Prader V                                 | DHEA ↑, 170HP →           |
| San Francisco(12)| No breast development, pseudohermaphroditism, progressive | E1 ↓, E2 ↓, T ↑, FSH ↑    |
|              | virilization, cystic ovaries, bone age delay                  | DHEA ↑, 170HP →           |
| Bern (13)    | Cystic ovaries, low bone density, pseudohermaphroditism      | E2 ↓, FSH ↑, A ↑          |

E3: estriol, A: androstenedione, 170HP: 17α-hydroxy progesterone, DHEA; dehydroepiandrosterone, E1: estrone.

urinary excretion of estriol at 35 wk of pregnancy was low. These phenotypes are known to be caused by a placental aromatase deficiency. From the clinical features and biochemical data, we endocrinologically diagnosed her as having an aromatase deficiency. The aromatase gene is now under investigation for definite diagnosis.

To the best of our knowledge, 6 affected females with an aromatase deficiency have been reported (Table 4) (8–13). These cases were severely virilized (Prader IV–V) and their mothers had virilization with facial acne, hirsutism, whole body pigmentation, and hoarseness. These symptoms progressed with advancement of gestation and all of them improved except for the lowered voice after delivery.

Three of the cases were found to have cystic ovaries. In the other 3 cases did the existence of cystic ovaries was not discussed. Low dose estrogen replacement therapy resulted in suppression of gonadotropin levels and resolution of the cystic ovaries (13). Therefore, the etiology of these ovarian changes seemed to be hypersecretion of gonadotropin due to positive feedback caused by low estrogen levels. Similarly, our patient had cystic ovaries discovered by ultrasonography and MRI, and hypersecretion of LH after LHRH loading. If enlargement of cystic ovaries is noted, estrogen supplement will then be taken into consideration.

Our patient had high serum 170HP levels. In past reports, the value was within the normal range except for one case (10). We suppose that obstruction of the adrenal and genital cascade in
steroidogenesis may occur due to an aromatase deficiency, resulting in high 17OHP and progesterone.

In summary, we report a patient with aromatase deficiency who had ambiguous genitalia and maternal virilization during pregnancy. From the clinical features and biochemical data, we endocrinologically diagnosed her as having an aromatase deficiency. We finally agreed that aromatase deficiency should be suspected when both the mother and the newborn have been virilized.

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