

A novel compound heterozygous variant in *CYP19A1* resulting in aromatase deficiency with normal ovarian tissue

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ABSTRACT

Background. Aromatase deficiency leading to virilization in mother and female fetuses during pregnancy is a rare disease. It is characterized by impaired estrogen production, increased gonadotropins, and ovarian cysts.

Case. Herein, we report a clinical phenotype of the virilized female due to a novel compound heterozygous variant in *CYP19A1* [IVS10 + 1 G> A; c.344 G> A (p.R115Q)], with normal gonadotropin levels at the time of admission and histologically normal ovarian tissues.

Conclusion. Aromatase deficiency should also be considered even if the initial follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels are normal, and ovarian cysts are lacking.

Key words: disorders of sex development, p450 oxidoreductase deficiency, clitoromegaly, hirsutism, ambiguous genitalia.

Aromatase is a type II cytochrome P450 enzyme located in the endoplasmic reticulum, which catalyzes the conversion of C19 steroids (testosterone, 16-alpha-hydroxytestosterone, and androstenedione) into C18 steroids (17 beta-estradiol, estriol, and estrone). It is encoded by the *CYP19A1* gene located on chromosome 15q21.1.¹ Aromatase deficiency is a rare autosomal recessive disorder that leads to increased androgen levels in both the fetus and the mother. The latter condition results in specific signs of maternal virilization including cystic acne, hirsutism, clitoromegaly, and deep voice.²

It was first described by Shozu et al.³ in 1991. Subsequent experimental studies showed that aromatase-knockout female mice developed a male body habitus, had small or polycystic ovaries with no corpora lutei, small uteri, and they were infertile.⁴ Until now, nearly 40 cases with varying clinical presentations from various ethnic groups were reported.

Female cases with aromatase deficiency have ambiguous genitalia at birth, failure to enter puberty, a propensity to develop ovarian cysts, increased gonadotropins and pronounced virilization.¹ During childhood, both serum basal or gonadotropin-releasing hormone (GnRH)-induced follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels are expected to be elevated. Thereby, high concentrations of circulating gonadotropins and increased intra-ovarian androgen levels usually cause large and polycystic ovaries from the infancy period.¹

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Received 27th August 2019, revised 7th May 2020,
accepted 21st May 2020.

Herein, we report a virilized female infant with normal ovarian morphology and initially normal gonadotropin levels associated with a novel compound heterozygous variant [IVS10 + 1 G> A; p.R115Q (c.344 G> A)] in *CYP19A1*.

Case Report

A 4-month-old girl was referred to our outpatient clinic due to clitoromegaly, which was decreasing in size since birth. She was born with a birth weight of 2710 grams at 35th week of gestation. Her parents were unrelated. Her mother had developed acne, hair loss, voice change, and hirsutism during pregnancy. There was no similar case in family history.

Physical examination revealed normal weight [6.8 kg; 0.4 standard deviation score (SDS), 50-75p] and length (64 cm; 0.5 SDS, 50-75p). Clitoris was 1 cm long and labia minora were fused posteriorly. There were no gonad-like structures in the inguinal region. The remaining systemic examination was normal.

A number of laboratory tests already performed before admission to our unit were interpreted as normal [16th day of life, adrenocorticotropic hormone 29.6 pg/ml (0-46), cortisol 1.57 µg/dl (0.55-19.8 µg/dl), 17-OH progesterone 9.89 ng/ml (<20 ng/ml), androstenedione 1.15 ng/ml (0.3-3.3 ng/ml), anti-Mullerian hormone 0.04 ng/ml (<4.7 ng/ml for females)]. Gonadotropin levels were normal at the time of admission (Table I). Ultrasonography revealed a normal uterus and but no ovarian tissue, confirming previous findings. Karyotype was identified as 46,XX. Fluorescence in situ hybridization (FISH) analysis demonstrated no Y chromosome among 200 nuclei. The testis-determining gene

(*SRY*) was not detected via the polymerase chain reaction (PCR). At 6th month of age, vaginoscopy and laparoscopy were performed in order to plan surgery and vagen, uterus, and ovaries were considered normal. In addition, gonadal biopsies were performed during the laparoscopy due to investigate the gonadal karyotype and for differential diagnosis of ovotesticular syndrome. The biopsy specimens from both gonads were histologically consistent with normal ovarian tissue (Fig. 1). The karyotype analyses of those specimens revealed 46,XX in both gonads.

The diagnosis of aromatase deficiency was considered according to these findings. Cytochrome p450 oxidoreductase deficiency can also be thought in similar circumstances, however, normal 17-OH progesterone levels are uncommon in this situation. Genetic analysis of the case revealed a compound heterozygous variant in *CYP19A1* [novel IVS10 + 1 G> A; novel p.R115Q (c.344 G> A)] (Fig. 2). *In silico* analyzes categorized the variants to be pathogenic. The mother and the father were heterozygous

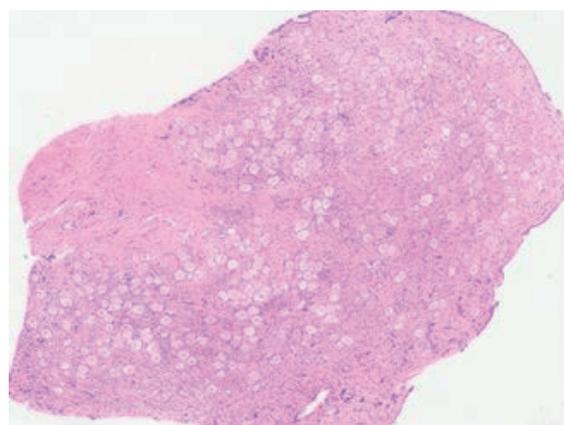


Fig. 1. Normal ovarian tissue histology from the biopsy specimens of both gonads.

Table I. Hormone levels of the case at different time points.

	16 th day	4 th month	6 th month	8 th month	11 th month	17 th month
FSH (mIU/ml)	6.4	7.02	36.3	18.7	27.9	75.1
LH (mIU/ml)	0.53	0.97	4.36	1.27	0.77	15.64
Estradiol (pg/ml)	5.0	<20	<20	<20	<20	<20
Total testosterone (ng/dl)	78	<10	<10	-	-	-

FSH: follicle-stimulating hormone, LH: luteinizing hormone.

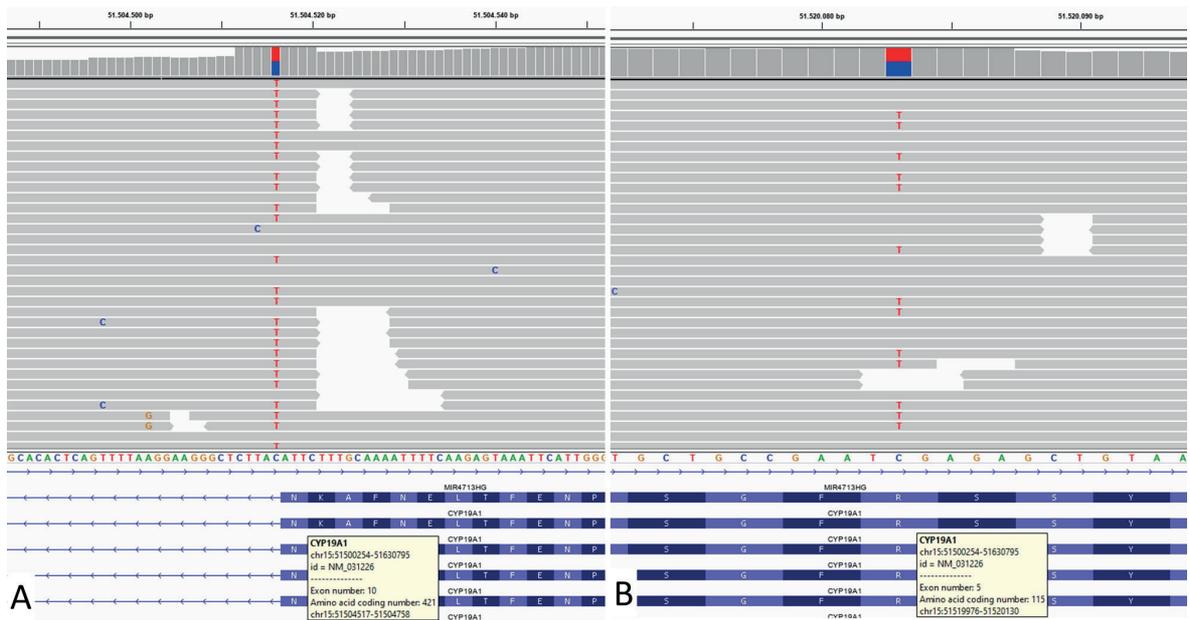


Fig. 2. Partial sequences of CYP19A1 of the patient demonstrating (a) heterozygous IVS10 + 1 G> A, (b) heterozygous c.344 G> A.

carriers of p.R115Q (c.344 G>A) and IVS10 + 1 G>A variant, respectively.

During the follow-up, the fusion at the posterior of the labium minus was surgically corrected. No ovarian cyst was observed with pelvic ultrasonography until the last visit at the age of 3 years. Her weight was 17.2 kg (1.6 SDS, 85-95p), and height was 100 cm (0.9 SDS, 75-85p) on the last visit (BMI 17.5 kg/m², 1.19 SDS, 85-95p). Written informed consent was obtained from the parents for publication of the case.

Discussion

Aromatase deficiency is a rare disease characterized by a decrement in estrogen synthesis, due to reduced aromatase activity. Until today, more than 32 variants in the CYP19A1 gene, including missense, nonsense, small deletions and insertions, splice site variants, and one large intragenic deletion, have been described in patients with aromatase deficiency.^{1,5-8} The majority of variants were missense and located in exons 9 and 10, which encode the substrate (androgen)-binding site and haem-binding domains, respectively.⁵ In the

current study, we identified a novel compound heterozygous variant in CYP19A1 [IVS10 + 1 G> A; p.R115Q (c.344 G> A)]. In contrast to the previous cases reported from our country, the locations of variants detected in our case were different.⁶⁻⁸ The missense variant (c.344 G> A) is localized in exon 4 and IVS10 + 1 G> A is a splice site defect localized in 10th intron of the CYP19A1.

Due to placental aromatase deficiency, the conversion of androgen into estrogen decreases, and therefore high levels of androgen circulates in maternal blood.² The mother with a fetus, who has aromatase deficiency, develops progressive virilization symptoms during pregnancy such as an increase in acne, voice thickening, clitoromegaly, frontal baldness, and facial hirsutism. These findings in the mother regress after birth.^{2,9,10} Similarly, the mother of our patient developed virilization symptoms, including acne, hair loss, voice change, and hirsutism during pregnancy.

Aromatase deficiency results in varying degrees of virilization in the external genitalia in newborn girls due to high intrauterine androgen concentration. Gonads are non-palpable and

female internal genitalia differentiation was not affected. On the other hand, there is no change in the external genitalia in boys at birth. All infants born full-term with adequate weight for gestational age.^{1,3,11,12} The birthweight of our patient was normal for gestational age (2710 grams at 35th gestational week). She had clitoromegaly, posterior labia minora fusion, and but normal uterus and ovaries.

In female cases with aromatase deficiency, changes in the hypothalamic-pituitary-gonadal axis causesome clinical issues. During childhood, both serum basal and GnRH-induced FSH-LH levels are expected to be elevated, starting from 2nd month of age.¹ However, the estradiol and estrone levels tend to be remarkably low during this same period.^{9,11} In our case, gonadotropin levels were normal at the time of admission but started to increase by the age of 6 months. Excessive virilization and lack of the start of puberty are expected in affected females, with primary amenorrhea and also absence of breast development. The pubertal spurt is lacking and bone age delayed.¹⁰⁻¹² Increased intra-ovarian androgen levels and high concentrations of circulating gonadotropins usually cause large and polycystic ovaries from the infancy period.¹ However, in a total of six cases from four studies, hypoplastic or non-cystic ovaries have been reported. Gagliardi et al.⁵ reported streak ovaries in a case and commented that it was due to estrogen treatment before the diagnosis. Lin et al.¹⁰ reported a case with streak ovaries and suggested that the streak ovaries may be a constitutional presentation of *CYP19A1* deficiency. Other patients from two reports (age, 19 months-7 years of age) had not received estrogen treatment, and no cystic structure was noted.^{6,7} When the laparoscopy was required for our patient, it was considered to take a sample from the ovary in terms of the possibility of ovotestis.¹³ Although a normal gonadal appearance was seen, this would not always indicate a normal histology as a demarcation line was not remarkable in some patients with ovotestis described in literature.¹⁴ Unlikely to most of the patients with aromatase deficiency,

the biopsy specimens from both gonads of the present case were histologically consistent with normal ovarian tissue, and no cystic structure developed during the follow-up. Thinking retrospectively, we would not need to perform an ovarian biopsy if a genetic diagnosis could be made earlier.

Treatment in aromatase deficiency consists of estrogen replacement in girls; however, there is no concurrence on the dosage or age of beginning. Estrogen treatment normalizes bone maturation, gonadotropin secretion with a feedback mechanism.^{9,15,16} It is also useful for glucose and insulin metabolism and decreases lipid levels.^{9,16} We have not started estrogen replacement yet, as our case did not have any ovarian cysts or metabolic syndrome.

In conclusion, we presented a case with aromatase deficiency caused by a novel variant in the *CYP19A1* gene. Aromatase deficiency should be considered in children with ambiguous genitalia who have a history of maternal virilization during pregnancy and karyotype of 46,XX, even if the initial FSH and LH levels are normal and ovarian cysts are lacking.

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