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**Case Report**

# Aromatase Deficiency due to a Novel Mutation in *CYP19A1* Gene

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**What is already known on this topic?**
Aromatase deficiency is an autosomal recessive genetic disorder that is rarely reported in the literature. Aromatase enzyme converts androgens into estrogen in many tissues. Aromatase deficiency causes ambiguous genitalia in the female fetus and maternal virilization during the pregnancy due to increased concentration of androgens. For girls with aromatase deficiency, ovaries are usually large and polycystic.

**What this study adds?**
We identified a novel mutation in the CYP19A1 gene in a patient who presented with ambiguous genitalia and maternal virilization during pregnancy. In our patient, the ovaries were hypoplasic despite increased gonadotropin levels.

**Abstract**
**Background:** Aromatase deficiency is a rare autosomal recessive genetic disorder with an unknown incidence. Aromatase converts androgens into estrogen in the gonadal and extra-gonadal tissues. Aromatase deficiency causes ambiguous genitalia in the female fetus and maternal virilization (hirsutism, acne, cliteromegaly, deep voice) during the pregnancy due to increased concentration of androgens.
**Methods and results**: The-nineteen-month-old girl was assessed due to ambiguous genitalia There were findings of maternal virilization during pregnancy. The karyotype was 46, XX. Congenital adrenal hyperplasia (CAH) was not considered since adrenocorticotropic hormone (ACTH), cortisol, and 17-hydroxyprogesterone (17-OHP) levels were within normal ranges. follicle-stimulating hormone (FSH) and total testosterone were elevated and estradiol was low when she was two months old. Depending on these findings, aromatase deficiency was considered. A novel homozygous mutation (IVS7-2A>G[c.744-2A>G]) was identified in the *CYP19A1* gene. Pelvic ultrasound showed hypoplasic ovaries rather than large and cystic ovaries.
**Conclusion:** We identified a novel mutation in the *CYP19A1* gene in a patient who presented with ambiguous genitalia and maternal virilization during pregnancy. Presence of large and cystic ovaries is not essential in aromatase deficiency.

**Key words:** aromatase deficiency, CYP19A1 gene, maternal virilization, ambiguous genitalia

## Introduction

Aromatase is a member of the cytochrome P450 superfamily and encoded by the *CYP19A1* gene located on chromosome 15q21.1. Aromatase is the key enzyme for estrogen biosynthesis in all vertebrates. *CYP19A1* gene and aromatase are expressed in numerous tissues including ovaries, testicles, placenta, adipose tissue, skin, and brain. Aromatase catalyzes the three precursors including androstenedione, testosterone and 16-α-hydroxydehydroepiandrosterone sulfate (after conversion to 16-α-hydroxyandrostenedione) into estrone, estradiol and estriol, respectively (1,2,3). Aromatase deficiency leads to increased androgen levels both in the mother and the fetus. Aromatase deficiency causes specific signs of maternal virilization including cystic acne,

hirsutism, cliteromegaly, and deep voice while resulting in significant masculinization in the external genitalia of the female fetus (4).

In this study, we present a case with a novel homozygous IVS7-2A>G (c.744-2A>G) mutation in the *CYP19A1* gene causing significant virilization both in the mother and the female fetus.

**Case Report**

The-nineteen-month-old girl presented with ambiguous genitalia. She was born at term in another hospital via spontaneous vaginal delivery with a birth weight of 3500 g. The parents of the patient were first-degree cousins. Ambiguous genitalia was recognized at birth and the signs of maternal virilization (hirsutism, acne, cliteromegaly, and deep voice) were noted at approximately 20 weeks of gestation. The patient presented to another hospital at the fifteenth day after birth. CAH was not considered since ACTH, cortisol, and 17-OHP levels were within normal ranges. The other parameters were as follows: FSH 66 mIU/ml (0.24-14.2), luteinising hormone (LH) 9.7 mIU/ml (0.02-7), total testosterone 0.9 ng/ml (0.2-0.64), estradiol 5 pg/ml (<15). The karyotype was 46, XX and pelvic ultrasonography revealed the uterus as 5x8x13 mm (normal range 33.1 ± 4.1 mm), the right ovary as 5x3x3 mm (0.02 ml; normal range 0.2-0.9 ml), and the left ovary as 5x3x3 mm (0.02 ml; normal range 0.2-0.9 ml). The patient presented to our hospital at the age of 19 months with a body weight of 11 kg (SD score + 0.07) and a length of 82 cm (SD score + 0.48). Genital examination showed bilateral impalpable gonads, a penis-like phallus of 1.5 cm, single penoscrotal urethral opening, and laboscrotal fusion defect (Prader stage IV). Hormonal analyses were unremarkable except for significantly elevated FSH level (Table 1). Aromatase deficiency was considered due to the presence of maternal virilization, detection of hypergonadotropic hypogonadism during mini-puberty, and low estradiol levels despite elevated total testosterone levels. *CYP19A1* gene mutation analysis was performed by sequencing the coding exons and the exon-intron boundaries of the genes. Genomic DNA was isolated from peripheral blood cells with QIAGEN DNA Blood Mini Kit according to the protocol provided with the kit. To amplify the exons of *CYP19A1* gene, primers were used as listed in Table 2. Sequencing was performed with Miseq V2 chemistry on MiSeq instrument (Illumina California, USA) and the analysis was performed with IGV software. A novel homozygous (IVS7-2A>G(c.744-2A>G) mutation was found in the *CYP19A1* gene (Figure 1). To our knowledge, this mutation has not been identified to date. The mutation was interpreted as a "disease-causing" mutation by the MutationTaster and Splice Site Finder modeling program. The parents were heterozygous carriers for the same mutation (Figure 2).

An informed consent was obtained from the parents.

**Discussion**

Aromatase deficiency is a rare disease caused by *CYP19A1* gene mutation and characterized by a decrease in estrogen synthesis. Aromatase deficiency is an autosomal recessive disorder and was first described by Shozu et al. (5). To date, a total of 36 cases from various ethnic origins have been reported in the literature (1,2, 6-14). In patients with aromatase deficiency, more than 30 distinct mutations have been identified in the *CYP19A1* gene including missense, nonsense, small deletions and insertions, splice-site mutations, and one large intragenic deletion (1,2, 6-16). Most of these mutations have been found to be located in exon 9 and 10 (9). The mutations identified at cases in Turkey have been reported in different exons (exon 5, 10, 11)(16,20,24). In our patient, the mutation was located in intron 7 of *CYP19A1* gene.

Clinical characteristics of patients with aromatase deficiency vary depending on the gender, age and the enzymatic activity (1). Aromatase deficiency leads to an increase in intrauterine androgen concentration, thereby result in varying degrees of postnatal virilization in the external genitalia in girls and no change in the external genitalia in boys at birth. Our patient had a karyotype of 46, XX and was born with ambiguous genitalia (Prader stage IV). During infancy and childhood there are either no symptoms of aromatase deficiency (particularly in boys). Although girls may represent with abdominal symptoms of ovarian cysts because of mild changes in the hypothalamic–pituitary–gonadal axis due to lacking feedback regulation of low levels of estrogens that are clearly required troughout prepubertal period (3). Aromatase deficiency may lead to a number of clinical conditions in adolescent girls such as delayed puberty, hypergonadotropic hypogonadism, multicystic ovaries, and primary amenorrhea in accordance with estrogen deficiency and may also lead to signs of virilization such as acne, hirsutism, and cliteromegaly in accordance with androgen excess (1,2,17,18). Estrogen deficiency, on the other hand, causes delayed epiphyseal closure, eunuchoid body habitus, osteopenia, and osteoporosis that develop in both genders (19). A previous study reported a 27-year-old patient with bone pain and recurrent bone fractures secondary to minor trauma who had open epiphyses and also developed lumbar osteoporosis. Aromatase deficiency had detected at the patient and the study concluded that estrogen has a key role in maintaining bone mineral density (20).

In most of the fetuses with aromatase deficiency, maternal virilization can be early onset (12 weeks) or late onset (up to 30 weeks) (18, 21). The non-aromatized fetoplacental and maternal androgen precursors are converted to testosterone in the placenta and also to peripheral maternal tissues, thereby resulting in maternal virilization. After the birth, the signs of virilization disappear gradually and the androgen levels return to normal (1). In our patient, the signs of maternal virilization (hirsutism, acne, and deep voice) developed at approximately 20 weeks

of gestation. Although hirsutism and acne resolved after the birth, deep voice interestingly persisted, which was consistent with the literature (13).

Both basal and GnRH-stimulated FSH levels have been shown to be higher in in girls with aromatase deficiency during the first two years of life compared to normal subjects (50-75 and 200-255 mIU/ml, respectively). However, the estradiol and estrone levels tend to be remarkably low during the same period (21, 22). Moreover, basal LH is often within normal limits or slightly elevated during infancy (5-10 mIU/ml). A previous study showed that in a girl with aromatase deficiency, the FSH and LH levels persistently increased and multicystic ovaries developed between the ages of three and four (21). However, Belgorosky et al. (23) reported that the basal FSH and LH levels in a girl with aromatase deficiency were found to be increased during mini-puberty and decreased dramatically within two and five months. In our patient, gonadotropin (FSH, LH) levels have been elevated since birth.

In girls with aromatase deficiency, the ovaries are usually large and polycystic in every stage of life (newborn, childhood, and puberty) due to the chronic stimulation by gonadotropins that cannot be suppressed owing to estrogen deficiency or androgen excess (1,2). In our patient, no cystic formation was observed in the ovaries despite high gonadotropin levels and also the ovarian volumes were below the age-matched limits. To date, hypoplasic ovaries have been reported in a total five cases (two studies) whose characteristics were similar to those of our patient (9,16,24).

Literature reviews indicate that there is little documentation on the effects of estrogen replacement to prevent estrogen deficiency in women with aromatase deficiency. Moreover, there is no consensus for estrogen replacement treatmen's dosage and age of initiation. On the other hand, data regarding early initiation of the treatment and the long-term follow-up of the patients are considerably limited. To our knowledge, there has been only one study investigating the effects of estrogen replacement therapy on longitudinal growth, bone age maturation, multicystic ovaries, bone density, and regulation of the pituitary gonadotropin feedback in a girl with aromatase deficiency who was started on low-dose estrogen therapy at the age of 3.5 years and continued the therapy until the age of 15. The study revealed that estrogen is required for normal growth, pituitary-gonadal development and bone maturation not only in puberty but also in early childhood (3). In a review on treatment of the aromatase deficiency reported that estrogen replacement therapy can be initiated at as early as the age of two years. The study also noted that this treatment should be initiated and sustained with the lowest dose of estrogen possible to prevent the development of ovarian cysts and to avoid early development of breasts and acceleration of bone age. The study suggested that oral conjugated estrogens (0.15 mg/day or every other day) or micronized estradiol (0.25 mg/day or every other day) can be used and the dose may be titrated to maintain the suppression of FSH and LH (4). In light of these findings, low-dose estrogen replacement therapy was planned to start at the age of two for the development of uterus and ovaries, normal growth, bone maturation and normalization of bone mineral density.

In conclusion, the case reported in this study was present with ambiguous genitalia and developed aromatase deficiency due to a novel mutation in CYP19A1 gene. Presence of large and cystic ovaries is not essential in aromatase deficiency. On the contrary, the ovaries can even be hypoplasic. Aromatase deficiency should be kept in mind in patients with 46, XX karyotype presenting with ambiguous genitalia along with the signs of maternal virilization.

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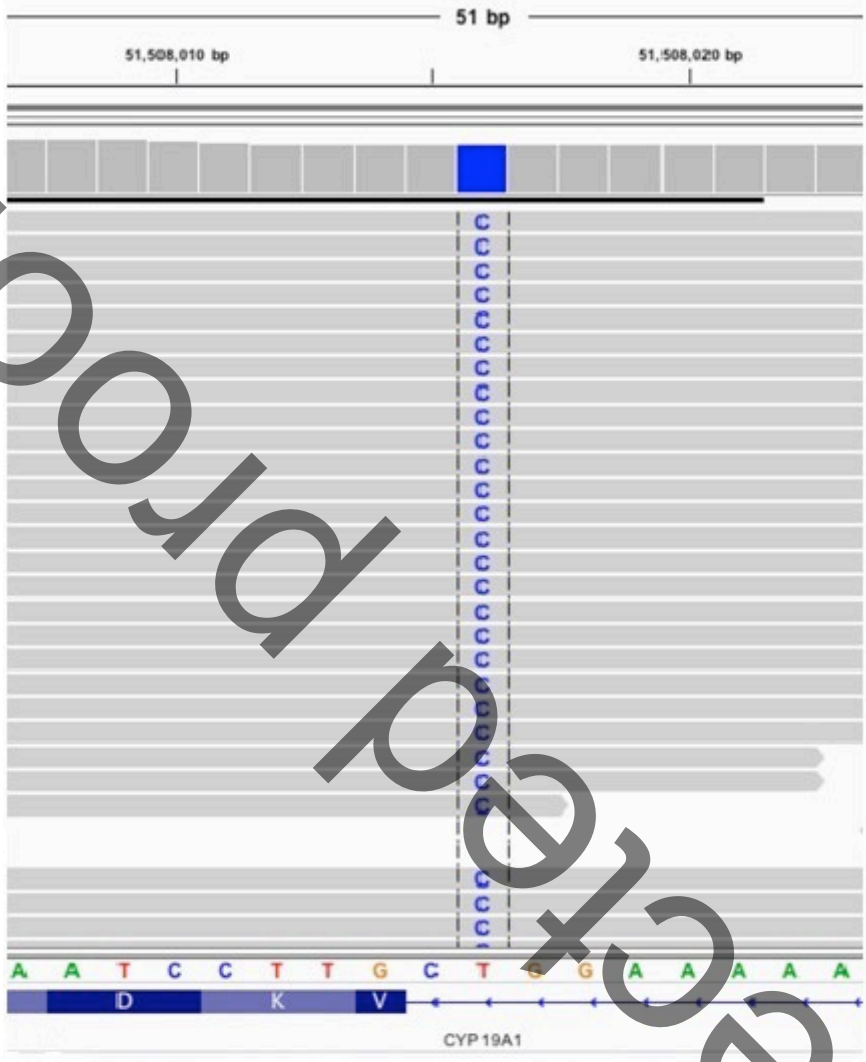


Figure 1. Homozygous mutation (IVS7-2A>G(c.744-2A>G) in intron 7 of the *CYP19A1* gene

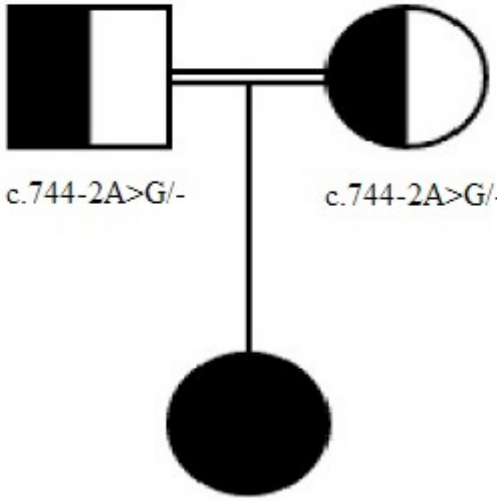


**Figure 2**. Pedigree of the patient's family. Solid black symbols depict affected individuals; half-filled symbols represent heterozygous carriers.

| | 15 days | 70 days | 19 months |
|---|---|---|---|
| FSH(mIU/ml) | 66 (0,24-14,2) | 52,4 (0,24-14,2) | 110 (1-4,2) |
| LH (mIU/ml) | 9,7(0,02-7) | 4,9 (0,02-7) | 13,7(0,02-0,3) |
| Testosterone(ng/ml) | 0,9 (0,2-0,64) | 0,39 (<0,1) | 0,03(<0,03-0,0,1) |
| Estradiol(pg/ml) | <5 (<15) | <5 (5-50) | <5 (5-20) |
| Androstenedione(ng/ml) | | | 0,2 (0,08-0,5) |
| 17 OH P(ng/ml) | | 1,67 (0,4-2) | 0,73 (0,03-0,9) |
| Cortisol (mcg/dl) | | 22,3 (2,8-23) | 18(3-21) |
| ACTH(pg/ml) | | 28,7(10-60) | 42 (10-60) |
| DHEA-S(ug/dl) | | 61 (5-111) | |
| Progesterone(ng/ml) | | 0,27 (0,07-0,52) | 0,2 (0,07-0,52) |

Table 1. Hormone test results of patient at different age time points.

ACTH, adrenocorticotropic hormone; DHEA-S, sulfated dehydroepiandrosterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; 17OHP, 17-hydroxyprogesterone

Table 2. Primers used fort he sequencing of the coding region of CYP19A1 gene

| | |
|---|---|
| CYP19A1-2F | TCTGAAGCAACAGGAGCTAT |
| CYP19A1-2R | CAGAGATCTTTCCAGGTTTG |
| CYP19A1-3F | GAAGTGAAGAGCCTCATGTT |
| CYP19A1-3R | TGTTAGATTTCTGGGGATTG |
| CYP19A1-4F | CAACATGCATTTGCTAAGAG |
| CYP19A1-4R | CTGGGTGATAGAGTCAGAGC |
| CYP19A1-5F | TTAGGAGACCACAGAAAAGC |
| CYP19A1-5R | GCAGAAACACTAGGGAAAAA |
| CYP19A1-6F | GAAGATGGAATCTTGCTGAG |
| CYP19A1-6R | TTAATCAACAGCTCCCTTGT |
| CYP19A1-7F | CACTTACTCATAAGCACCAAT |
| CYP19A1-7R | TTGGATTGGGATTACAGAAC |
| CYP19A1-8F | TCAATCACAGAGACATGTGG |
| CYP19A1-8R | TCTTTTCCGTCTATCTGGTG |
| CYP19A1-9F | GCTGGTGTGCATTAGAATTA |
| CYP19A1-9R | GCACAGGGAATGAGTAAGAA |
| CYP19A1-10F | AGGGCATTGTAGCTGATAAC |
| CYP19A1-10R | TGTTCACTGTGAGGATGACA |