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Case Report

Aromatase Deficiency in Two Siblings with 46, XX Karyotype Raised as Different Genders: A Novel Mutation (p.R115X) in *CYP19A1* Gene

Short title: Aromatase Deficiency in a 46, XX DSD patient

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What is already known in this topic?
The *CYP19A1* gene encodes the aromatase enzyme which catalyses the conversion of androgens to oestrogens. In cases with 46, XX karyotype, mutations in the *CYP19A1* gene can lead to disorders of sex development.

What this study adds?
In this study, two 46, XX sibling having the p.R115X (c.343 C>T) novel pathogenic variant in the *CYP19A1* gene and raised as different genders are presented.

Abstract
Aromatase deficiency rarely causes a 46, XX sexual differentiation disorder. The *CYP19A1* gene encodes the aromatase enzyme which catalyses the conversion of androgens to oestrogens. In cases with 46, XX karyotype, mutations in the *CYP19A1* gene can lead to disorders of sex development. Clinical findings in aromatase deficiency vary depending on the degree of deficiency. Due to the effect of increased androgens; acne, cliteromegaly and hirsutism can be observed in mothers with placental aromatase deficiency. A decrease in the maternal virilisation symptoms is observable in the postpartum period. It is rarely reported that there is no virilization in pregnancy. In this study, two 46, XX sibling having the p.R115X (c.343 C>T) novel pathogenic variant in the *CYP19A1* gene and raised as different genders and no maternal virilisation in pregnancy are presented. In conclusion, 46, XX virilised females should be examined in terms of aromatase deficiency once congenital adrenal hyperplasia has been excluded, even if no history of maternal virilisation during pregnancy is present.
Keywords: 46, XX disorder of sex development, aromatase deficiency, *CYP19A1* gene

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Introduction

Aromatase deficiency is a rare autosomal recessive disorder caused by mutations in the *CYP19A1* gene (1). The *CYP19A1* gene encodes the aromatase enzyme which catalyses the conversion of androgens to oestrogens. In the affected 46, XX cases, clinical findings in the neonatal period are between mild cliteromegaly to complete labioscrotal fusion due to differ from exposure due to increased androgen exposure in the intrauterine phase. An increase in virilisation at puberty or the non-appearance of secondary sex characteristics, are the main clinical features in the late period. Affected 46, XY cases have normal prepubertal growth. Delayed epiphyseal closure, onychoid body structure, and a decrease in bone mineral density can be observed in both sexes (2). This study presents a novel pathogenic variant in the *CYP19A1* gene in two siblings raised as different genders.

Case 1: A 14-year-old patient who had been raised as a male was brought to the pediatric endocrinology clinic for undescended testis and hypospadias. Although parental consanguinity was not reported to be present in the family, the family history revealed that they were living in a village of 500 inhabitants. Patient's mother who has 1 gravity and 1 parity had no symptoms of excessive androgen production such as hair loss, virilisation, or acne during pregnancy. On physical examination, height, weight, and phallus were measured to be 154.9 cm (SDS: - 2.5), 57 kg (SDS: -0,6), and 2 cm respectively. Breast tissue and palpabl gonads were not detected. Prader stage 3, two urogenital openings and stage 2 pubic pilosity were also noted. On laboratory examination, bone age was 11 years. Follicle stimulating hormone (FSH) level was 70 mIU/l (1.5-12.8mIU/l), Luteinizing hormone (LH) 30 mIU/l (0.1-12 mIU/l), free testosterone 0.9 pg/ml (0.8-1.4 pg/ml), Estradiol 22.9 pg/ml(7-60 ng/ml). Adrenocorticotropic hormone (ACTH), cortisol and 17-hydroxyprogesterone (17-OHP) were both found to be normal. Pelvic ultrasonography revealed 19 x 14 mm right ovary and 15 x 12 mm left ovary and an absence of uterus. Karyotype was 46, XX and SRY was negative following FISH analysis. On laparoscopic examination a normal-looking bilateral ovaries and a small uterus were observed. The biopsy findings of the right gonad were consistent with ovarian tissue and ovarian follicle cysts were observed.Sequence analysis of *SOX9* gene revealed no mutation. Clinical and laboratory findings of the patient aromatase deficiency was considered and a novel homozygotes nonsense p.R115X

(c.343C>T) pathogenic variant was found in Sanger sequencing of *CYP19A1* gene (figure 1a). The parents were heterozygous for the same mutation (figure 1c and 1d). There were no clinical findings in the parents. The mutation found in the cases was predicted to be pathogenic by *in silico* analysis.

The Council of Disorders of Sex Development decided that the case should be raised male on the ground of more distincted male sexual identity. Salpingo-oophorectomy, hysterectomy and genitoplasty were performed. Intramuscular testosterone propionate and testosterone phenylpropionate treatments were administered with a 100mg / month starting dose and gradually increased every 6 months. Oral estradiol hemihydrate treatment of 0.25 mg / day was initiated in the follow-up. At the age of 21, bilateral testicular prosthesis was surgically implanted. During follow-up bone mineral densitometry showed early onset osteoporosis (L1-L4 Z score: -2,2) and oral calcium supplementation was given. Calcium, phosphorus, PTH and vitamin D levels were within normal limit. At the age of 22, weight, height, and phallus were measured to be 86.6 kg (SDS: 1.29), 173.5 cm (SDS:-0.43), and 7 cm respectively.

Case 2: 8 year-old sibling who had been raised as a female. On physical examination, height, and weight were 125.5 cm (SDS: -0,3), 22.3 kg (SDS: -0,9) respectively. Phallus was measured to be 1cm. There were no palpable gonads, two urogenital openings and stage 1 pubic pilosity were also noted. Pubertal development was found to be stage 2 according to Prader score. Similar to the other sibling, bone age of the case was found to be retarded (5 years 9 months). FSH level was 22 mIU/l (1.0-4.2 mIU/l), LH: 30 mIU/l (0.1-0.3 mIU/l), free testosterone 0,2 pg/ml (0.15-0.6 pg/ml), Estradiol: 5 pg/ml (N<15). The patient had normal ACTH, Cortisol and 17 OHP. Uterus and left ovary were not visualized on pelvik USG; whereas there was a 12-mm right ovary. Karyotype was found to be 46, XX. FISH analysis showed that SRY was negative. The same homozygous pathogenic variant in the *CYP19A1* gene was also detected in this sibling (figure 1b). The Council of Disorders of Sex Development recommended the case to be raised as a female on the grounds that the female sexual identity was more distinct. L1-L4 Z score was found to be -2,4 in bone mineral densitometry during follow-up period. Calcium, phosphorus, PTH and vitamin D levels were within normal limits but oral intake of calcium was increased. At the age of 11, oral estradiol hemihydrate treatment was carried out with 0.25 mg / day starting dose and was gradually increased every 6 months. At the age of 16, physical examination showed a weight of 58.7 kg (SDS: 0.28), height 160 cm (SDS:-0.44), and stage 5 puberty and control pelvik USG was showed utesus which diameter of 62x35 mm and the patient was treated with a combination of oestrogen and progesterone. After this treatment she had menarche and regular menstural cycles.

The parents of the patients were informed about the diagnosis and consents for laboratory analyses and publication were obtained.

Discussion

Aromatase is a member of the cytochrome P450 superfamily that catalyses a reaction in which an oxygen atom is attached to an organic molecule-also known as hydroxylation (3). The human aromatase enzyme (P450C19) is the product of *CYP19A1* gene that converts androgens (P19) to oestrogens (P18) and is a microsomal enzyme responsible for oestrogen synthesis in all vertebrates (3, 4). The enzyme-encoding gene is composed of 10 exons (5). Mutations in the *CYP19A1* gene lead to loss of enzyme function and decrease in oestrogen synthesis. Most of the reported mutations contain single base changes in exons (6, 7). In the study, the *CYP19A1* gene sequence analysis detected homozygous novel nonsense p.R115X pathogenic variant in both siblings (figure 1a and 1b). This nonsense mutation is predicted to be pathogenic using *in silico* analysis (MutationTaster) (8) and minor allele frequency data in several public databases (NCBI dbSNPbuild141 (http://www.ncbi.nlm.nih.gov/SNP/), 1000 Genomes Project (http://www.1000genomes.org/), Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/)).

Clinical findings in aromatase deficiency vary depending on the enzyme levels. Due to the effect of increased androgens caused by placental aromatase deficiency; acne, cliteromegaly and hirsutism can be observed in mothers carrying affected fetus. A decrease in the maternal virilisation symptoms is observed in the postpartum period (9). The placental aromatase activity of 1-2% is reported to be protective against maternal virilisation during pregnancy (10). In the family presented here, the mother had no symptoms of excessive androgen production such as hair loss, virilisation, or acne during pregnancy. Enzyme activities could not be studied in the patients and their mother. Marino et al reported that maternal virilisation was also not observed in their three cases with *CYP19A1* mutations. During the follow-up period, phenotypic variability was determined among the affected patients. Two patients had a new mutation (c.574C> T). They found c.628G> A mutation in four of the six unrelated patients (11).

It has been reported that of 24 (12 males, 12 females) patients with proven *CYP19A1* deficiency, 70% of the females having *CYP19A1* mutation show virilisation compatible with Prader stage 4 – 5, while males are usually presented with metabolic problems and short stature (7, 12). For affected female cases, variable phenotype, such as cliteromegaly due to increased androgen levels in the intrauterine phase or complete labioscrotal fusion can be observed. Aromatase deficiency has been has been speculated that in aromatase-deficient prepubertal girls, an amplification of follicle-stimulating hormone (FSH) signaling might occur in the presence of high intraovarian androgen production and be responsible for the development of ovarian follicular cysts (3). On the other hand , hypoplastic ovaries rather than enlarged ovaries in aromatase-deficient females have rarely been reported. Lin et al (13) and Akçurin et al (14) reported a few cases of aromatase deficiency with hypoplastic ovaries and uterus. Lin et al (13) suggested that the streak ovaries may be an inherent manifestation of CYP19A1 deficiency. Also, polycystic ovaries may appear in later periods depending on human chorionic gonadotropin (hCG) stimulation. Cliteromegaly, hirsutism and acne can be seen in affected individuals with the non-appearance of secondary sex characteristics in the adolescence period (3). The studies have showed that loss of function mutations in the gene may result in various phenotypic changes, especially appearing in the pre-pubertal and pubertal period (11). In the study of Li et al., (13) aromatase mutations have shown that in humans, they can produce variable or "non-classic" phenotypes. They reported that low residual aromatase activity may be sufficient for the development of breast and uterus in adolescence despite significant androgenization in the uterus.Such phenotypic variability can be further influenced by modifying factors such as non-classical pathways of estrogen synthesis, variability in the core modifiers, or differences in androgen responses.

Siblings presented in this study had been raised as different genders due to the appearance of their external genitalia and virilisation levels.

In aromatase deficiency, oestrogen replacement treatment regulates gonadotropin secretion, glucose metabolism and liver functions while reducing lipid and insulin levels (14, 15). In our cases, lipid levels and glucose metabolism were found to be normal. However, decreased FSH and LH levels were observed with the oestrogen replacement treatment. Bone mineralisation and maturation are adversely affected in patients with aromatase deficiency. Oestrogen has positive effects on bone density by prolonging the life cycles of osteoblasts and osteocytes while reducing bone resorption (4). Osteoporosis was detected in both of our patients. Hormone replacement therapy was initiated and oral intake of calcium was increased as they are followed up.

Conclusion

In conclusion; 46, XX virilised cases should be examined in terms of aromatase deficiency after congenital adrenal hyperplasia was excluded even if there was no maternal history of virilisation during pregnancy and the *CYP19A1* mutation analysis should be performed. Early diagnosis of this disorder is of vital importance for gender selection and hormone replacement therapy.

References

1. Baykan, E.K., et al., Aromatase deficiency, a rare syndrome: case report. J Clin Res Pediatr Endocrinol 2013; 5: p. 129-32.
2. Korach, K.S., Insights from the study of animals lacking functional estrogen receptor. Science 1994; 266: p. 1524-7.
3. Belgorosky, A., et al., Genetic and clinical spectrum of aromatase deficiency in infancy, childhood and adolescence. Horm Res 2009; 72: p. 321-30.
4. Verma, N., et al., Growth and hormonal profile from birth to adolescence of a girl with aromatase deficiency. J Pediatr Endocrinol Metab 2012; 25: p. 1185-90.
5. Janner, M., C.E. Fluck, and P.E. Mullis, Impact of estrogen replacement throughout childhood on growth, pituitary-gonadal axis and bone in a 46,XX patient with CYP19A1 deficiency. Horm Res Paediatr 2012; 78: p. 261-8.
6. Harada, N., et al., Biochemical and molecular genetic analyses on placental aromatase (P-450AROM) deficiency. J Biol Chem 1992; 267: p. 4781-5.
7. Jones, M.E., et al., Of mice and men: the evolving phenotype of aromatase deficiency. Trends Endocrinol Metab 2006; 17: p. 55-64.
8. Schwarz, J.M., et al., MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 2014; 11: p. 361-2.
9. Zirilli, L., et al., Human models of aromatase deficiency. J Steroid Biochem Mol Biol 2008; 109: p. 212-8.
10. Deladoey, J., et al., Aromatase deficiency caused by a novel P450arom gene mutation: impact of absent estrogen production on serum gonadotropin concentration in a boy. J Clin Endocrinol Metab 1999; 84: p. 4050-4.
11. Marino, R., et al., Five new cases of 46,XX aromatase deficiency: clinical follow-up from birth to puberty, a novel mutation, and a founder effect. J Clin Endocrinol Metab 2015; 100: p. E301-7.
12. Rochira, V. and C. Carani, Aromatase deficiency in men: a clinical perspective. Nat Rev Endocrinol 2009; 5: p. 559-68.
13. Lin L, Ercan O, Raza J, Burren CP, Creighton SM, Auchus RJ, Dattani MT, Achermann JC. Variable Phenotypes Associated with Aromatase (*CYP19*) Insufficiency in Humans, The Journal of Clinical Endocrinology & Metabolism: 92(3).p: 982–990.
14. Akçurin S, Türkkahraman D, Kim WY, Durmaz E, Shin JG, Lee SJ. A Novel Null Mutation in P450 Aromatase Gene (CYP19A1) Associated with Development of Hypoplastic Ovaries in Humans. J Clin Res Pediatr Endocrinol. 2016 Jun 5;8(2):205-210.
15. Belgorosky, A., et al., Hypothalamic-pituitary-ovarian axis during infancy, early and late prepuberty in an aromatase-deficient girl who is a compound heterocygote for two new point mutations of the CYP19 gene. J Clin Endocrinol Metab 2003; 88: p. 5127-31.
16. Maffei, L., et al., Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. J Clin Endocrinol Metab 2004; 89: p. 61-70.

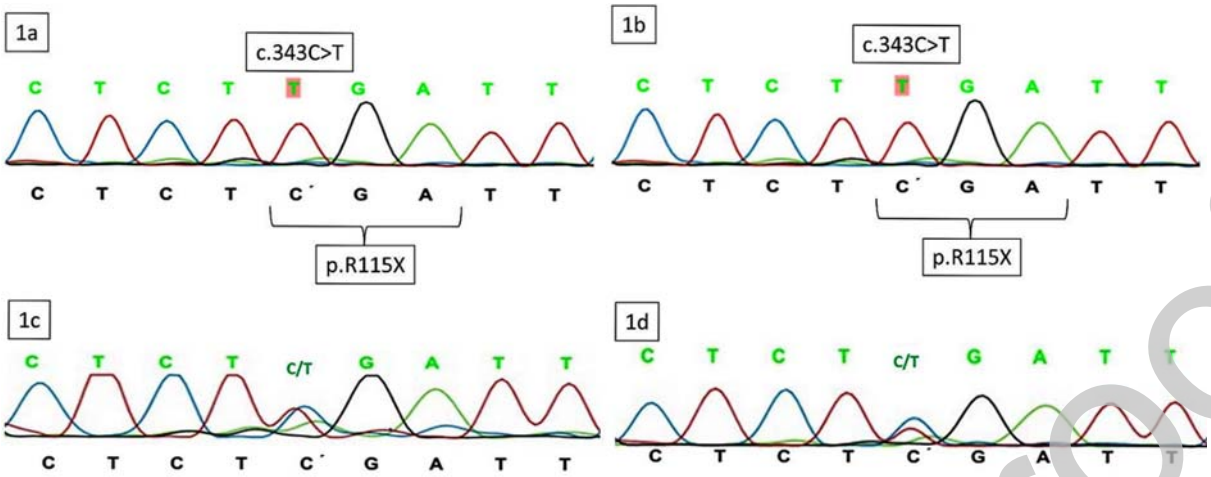

Figure 1. Figure 1a(Case 1) and 1b (Case 2): a novel homozygous nonsense pathogenic variant p.R115X (c.343 C>T) was detected in the *CYP19A1* gene sequence analysis. Figure 1c (Mother) and 1d (Father): The parents were heterozygous for the same mutation.