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Case Report

Aromatase deficiency in a male patient - Case report and review of the literature

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ABSTRACT

Objective: Aromatase, or CYP19A1, is a type II cytochrome CYP450 enzyme that catalyzes the conversion of C19 androgens to C18 estrogens. Its crucial role in both female and male physiology has been deduced from human and animal studies using aromatase inhibitors, genetically altered mice, and patients with aromatase deficiency. The latter is an extremely rare disorder. Its diagnosis is particularly difficult in males, who go through puberty normally and therefore usually present as adults with elevated testosterone, bone abnormalities (e.g., delayed bone age and low bone mass), and metabolic syndrome. In this report, we describe a new case of a male patient with aromatase deficiency harboring a known mutation who presented with less severe clinical and biochemical features.

Case report: The patient presented with low bone mass and delayed bone age after a finger fracture at age 25 years. FSH, LH and testosterone levels were normal, but estradiol and estrone levels were absent or barely detectable, raising suspicion for aromatase deficiency. A homozygous c.628G>A mutation in exon 5 was confirmed by direct sequencing. Unlike previously reported cases of aromatase deficiency, he did not display biochemical features of insulin resistance, dyslipidemia, or overweight/obese status. Therapy with estradiol led to the closure of growth plates and a dramatic increase in bone mass.

Conclusions: Here we explore genotype/phenotype associations of this new case compared to cases reported previously. We conclude that the specific nature of mutation c.628G>A, which can potentially result in several different forms of the aromatase enzyme, may lend an explanation to the variable phenotypes associated with this particular genotype.

1. Introduction

Aromatase, or CYP19A1, is a type II cytochrome P450 enzyme located in the endoplasmic reticulum [1]. It catalyzes the conversion of C19 steroids testosterone, 16alpha-hydroxytestosterone, and androstenedione to C18 steroids 17beta-estradiol, estriol, and estrone respectively [1]. The CYP19A1 gene is located on chromosome 15q21.2 and encodes a roughly 500 amino acid long protein that is expressed throughout the body, including the placenta, gonads, adipose tissue, bone, cartilage, skin, brain and vascular smooth muscle [1,2]. The distinct phenotypes of patients with aromatase deficiency as well as aromatase knockout mice have confirmed a crucial role of aromatase in both female but also male physiology. While the first publications of aromatase expression and its regulation date back to the 1970s, the first case of a patient with aromatase deficiency was only reported in 1991 in a female infant who exhibited signs of a 46, XX disorder of sexual development. In addition, the patient's mother showed increased signs of virilization, elevated androgen levels, and low serum and urinary estrogen levels during the third trimester of her pregnancy [3].

By now, we know that female patients with aromatase deficiency have a dramatic phenotype that includes ambiguous genitalia at birth, failure to enter puberty, a propensity to develop ovarian cysts, and pronounced virilization [4]. Likewise, female aromatase knockout mice develop a male body habitus, have small or polycystic ovaries with no corpora lutei, small uteri, and are infertile [5].

In male mice and humans however, the phenotypic features of aromatase deficiency are more subtle and are mostly related to estrogen actions in bone [4–6]. Male patients with aromatase deficiency are normal at birth. Furthermore, they normally undergo puberty, and only become symptomatic during early adulthood when they begin exhibiting problems with tall stature and bone pain, accompanied by low bone density, unfused growth plates, genu valgum, and increased fractures [6]. The diagnosis is rarely made early. One exception was an asymptomatic boy whose mother had significant virilization during pregnancy [7]. In two other male cases, the suspicion of aromatase deficiency was raised by an earlier diagnosis in their older female siblings [8,9]. The data on male fertility in the presence of aromatase deficiency are

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inconsistent in humans with both normal [10] and abnormal findings reported [11–13]. Similar to humans, male aromatase knockout mice have low bone density. They are fertile at first, but show decreased fertility at advanced age associated with impaired spermatogenesis [14,15]. Interestingly, sexual behavior, measured as mounting frequency, was significantly compromised in male aromatase knockout mice prior to apparent infertility [16]. In humans, the data on sexual behavior is limited, though overall normal libido and erections have been reported [6].

In line with the noted ubiquitous extragonadal expression of aromatase, increased adiposity, glucose intolerance and insulin resistance have been observed both in mice and humans with aromatase deficiency, though variably [10–12].

Herein, we describe a new case of a male patient with aromatase deficiency and a homozygous mutation in CYP19A1 with the typical findings of low bone mass and unfused growth plates yet overall absent metabolic features. We shall review this case in comparison with the current literature focusing on male pathology as well as clinical and biochemical features of the disease before and after therapy with estradiol. Finally, we hypothesize that mutation c.628G>A, reported here, could be the reason for the phenotypic variability of aromatase deficiency seen in patients with this particular genotype.

2. Patient and methods

2.1. Case report

The reported Caucasian patient came to medical attention at the age of 25 years after he suffered a low-trauma finger fracture and radiographic analysis revealed unfused growth plates and delayed bone age (15 years, Fig. 1). Magnetic resonance tomography ruled out a pituitary pathology. A formal DXA scan revealed low bone mass, initiating a referral to Endocrinology.

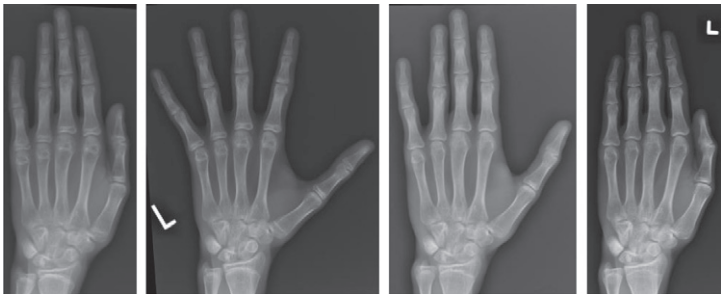
He is the second son (and third child) of consanguineous parents of Middle Eastern descent. Interestingly, the patient stated that his older sister likely was started on estrogen therapy in her 20s for unknown reasons. However, no further workup was done until after the patient's diagnosis, at which point sequencing revealed the same aromatase gene mutation. His older brother is presumably healthy, although minimal information is known.

Of note, during his mother's pregnancy with him, she had signs of increased virilization and elevated androgen levels that resolved postpartum. Immediately postpartum, the infant boy suffered a spontaneous right pneumothorax, requiring temporary chest tube placement and intubation. A subsequently noted left pneumothorax resolved without additional therapy. No sequelae were noted upon follow-up.

The patient normally underwent puberty between age 13 and 14. At the time of his first visit to the Endocrinology clinic (age 25), he remarked regular morning erections and a normal libido. He had had no prior surgeries or fractures, and his past medical history was negative except for recurrent dental cavities during childhood and diffuse alopecia between age 13 and 18 years. The latter was diagnosed as telogen effluvium. He continued to grow and was the tallest in his family significantly exceeding the heights of both of his parents at the time of his presentation. In addition, he complained of bilateral knee pain since age 20 years. He did not smoke, drink alcohol or use illegal drugs. Family history at the time of presentation was negative for gonadal, endocrine or bone diseases. On physical examination, he appeared tall, very slender and with long fingers, reminiscent of arachnodactyly. Height at his first presentation was 1.80 m, weight 56.65 kg (BMI 17.5), blood pressure was 146/72, and heart rate 87/min. There was no gynecomastia, both testes were descended and about 20 mls volume bilaterally. The patient's legs were normal, with no evidence of significant genu valgum. Moderate acanthosis nigricans was noted in both axillae. Of note, HbA1c and an oral glucose tolerance test, obtained prior to presentation in our clinic returned normal results (HbA1c 4.8%, glucose at 0 min was 91 mg/dl, at 60 min was 131 mg/dl, and at 120 min was 97 mg/dl).

2.2. Laboratory analysis

LH, FSH, estradiol, SHBG and prolactin were measured by electrochemiluminescence immunoassay (Cobas e601 analyzer, Roche



Date: (month/year)	5/2011	1/2012	4/2013	3/2014
Estrogen dose: (μg/d)	0	25	50	25
Patient age: (years, months)	25, 2	25, 11	27, 2	28, 1
Bone age: (years, months)	15	15, 6	17	17, 6
Patient height: (m)	1.80	1.83	1.83	1.85
Patient weight: (kg)	56.65	61.95	59.6	59.5
BMI: (kg/m^2)	17.5	18.6	17.9	17.3

Fig. 1. Bone age improved over three years of treatment with estradiol. X-ray images depicting bone maturation before and after treatment with estradiol. The first image, dated 5/2011, was taken just before starting transdermal estradiol. Patient's chronologic age, bone age, weight, and BMI are also included.

Table 1
Time course of FSH, LH, estradiol, estrone, testosterone, SHBG and prolactin levels before and after therapy with estradiol. Normal ranges are shown in parenthesis in the left column. Transdermal estradiol doses are shown in the upper row.

Date	5/7/11	1/31/12	4/30/13	12/24/13	3/17/14	5/6/14	1/10/15
Medication dose (μg/d)	0	25	50	75	25	50	50
FSH (1.5–12.4 mIU/ml)	4.8		1.6	0.9			2.1[a]
LH (1.7–8.6 mIU/ml)	5.5	3.1	3.2	2.8			3.3[a]
Estradiol (8–43 pg/ml)	7	30	41	90	9		31[a]
Estrone (9–36 pg/ml)	<1	9.3	8.2		2.7		<10[a]
Testosterone (240–950 ng/dl)	869	400	348	300	611		353[a]
SHBG (10–57 nmol/l)	36	35	34		34		35[a]
Prolactin (4.0–15.2 ng/ml)	7.4				17.8	22	8.5[a]

[a] Performed in out of state laboratory.

Diagnostics, Indianapolis, IN), while testosterone and estrone were determined by liquid chromatography mass spectrometry (Applied Biosystems API 5000TM Tandem Mass Spectrometer, ARUP, Salt Lake City, UT). ALT, AST, alkaline phosphatase, bilirubin, cholesterol, triglycerides, HDL and LDL, fasting glucose were measured using the Modular P Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN).

2.3. Genetic analysis

DNA was extracted from leucocytes and all coding exons 2–10 were sequenced bi-directionally including exon/intron splice junctions (carried out by GeneDx, Gaithersburg, MD).

2.4. Bone age and density analysis

Bone age was assessed by standard XRay of the left hand using the Greulich and Pyle atlas as a reference. Bone density was assessed at both lumbar spine and both femurs using dual XRay absorptiometry (Lunar Prodigy, GE Healthcare, Madison, WI).

2.5. Statistical analysis

Data analysis was carried out using Microsoft Excel (version 2016). A p-value of <0.05 was considered statistically significant.

2.6. Ethics

The patient consented to the publication of his clinical, biochemical and imaging data.

3. Results

Based on the patient's clinical presentation with a tall stature, severely delayed bone age, and a bone mineral density below the expected range for his age, sex steroid deficiency or resistance were the major differential diagnoses pursued. His initial gonadal profile revealed mid-normal FSH and LH, high-normal testosterone, and barely detectable estradiol and estrone levels (Table 1). These steroid hormone levels raised the suspicion of aromatase deficiency. Bidirectional sequencing of leucocyte DNA confirmed the presence of a homozygous mutation of G>A in nucleotide 628 (c.628G>A). This mutation most likely results in an inactive protein with a premature stop codon after exon 5, although other mutated proteins are possible ([17], see discussion). Following confirmation of the diagnosis of aromatase deficiency, the patient was started on transdermal estradiol. Of note, mild vitamin D deficiency was noted prior to the diagnosis of aromatase deficiency and had resolved after vitamin D supplementation before start of estrogen therapy. On therapy, estradiol and estrone levels increased, while LH, FSH and testosterone levels decreased (Table 1). As expected, a strong positive correlation was observed between administered estradiol doses and serum levels ($n = 7$, $r = 0.84$, $p < 0.01$, Fig. 2, top panel). A significant negative correlation was noted between estradiol doses and serum testosterone levels ($n = 7$, $r = -0.87$, $p < 0.01$, Fig. 2, bottom panel).

Initial bone age was 15 years which is similar to previously published studies with reported bone ages ranging from 14 [18] to 16 years [10]. Complete closure of all epiphyses was noted approximately 3 years after start of therapy (Fig. 1) with a steady progression of maturing bones over this time frame. In addition, his severely reduced bone density improved dramatically upon start of estradiol therapy. The maximum effect was noted during the first year of therapy with a gain of about 19% at the spine and between 16 and 20% at the femoral neck, his Z-scores improved by more than one standard deviation (at the spine from −2.6 to −1.3, at the right femoral neck from −2.8 to −1.7, see Table 2). Overall, these findings equal those of previous reports (for instance [11,19]).

Assessment of the patient's metabolic profile including lipids, fasting glucose, liver and renal function tests returned normal results (Table 3). Of note, a downward trend in ALT, AST as well as LDL was noted on estradiol therapy, while triglyceride levels increased. Fasting glucose levels were normal at all times during his follow-up with an insulin level in the lower range of normal before start of therapy. Together

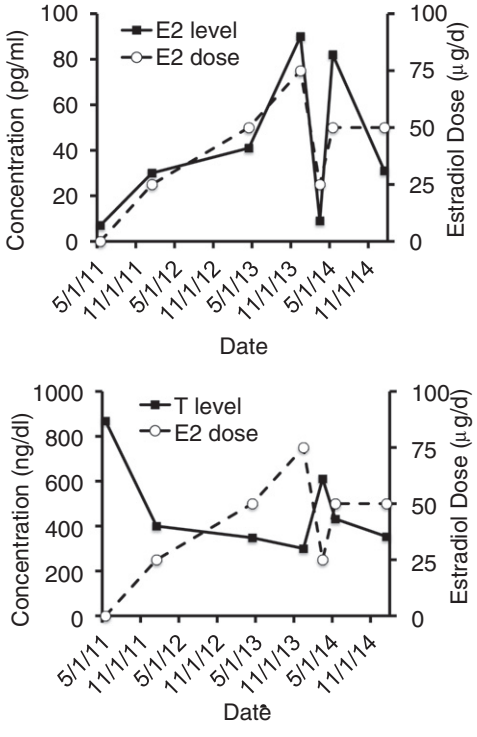


Fig. 2. Estradiol (E2) and testosterone (T) levels correlate closely with administered estrogen doses. Time course of E2 (top panel) and T levels (bottom panel) in relation to administered estrogen doses (correlation E2 level and dose: $r = 0.84$, $p < 0.01$; correlation T levels and E2 dose: $r = -0.87$, $p < 0.01$).

Table 2
The patient's bone density increased over time with transdermal estradiol therapy. Time course of bone mineral density and content, including Z-scores, of spine and both femurs before and after estrogen therapy are shown. Treatment was started after May 2011.

Date	4/15/11	1/31/12	9/20/2012	3/25/14
Medication dose (μg/d)	0	25	50	25
Bone mineral density L1–L4 (g/cm^2)	0.806	0.872	0.957	0.997
Z-score L1–L4	−2.6	−2.2	−1.5	−1.3
Bone mineral density right total femur (g/cm^2)	0.667	0.722	0.774	0.777
Bone mineral content right total femur (g)	24.7	26.8	28.2	28.8
Z-score right total femur	−2.5	−2.2	−1.8	−1.8
Bone mineral density right femoral neck (g/cm^2)	0.656	0.717	0.764	0.801
Bone mineral content right femoral neck (g)	3.8	4.1	4.3	4.5
Z-score right femoral neck	−2.8	−2.4	−2.0	−1.7
Bone mineral density left total femur (g/cm^2)	0.678	0.705	0.756	0.772
Bone mineral content left total femur (g)	24.9	26.1	28.7	29.8
Z-score left total femur	−2.4	−2.3	−2.0	−1.9
Bone mineral density left femoral neck (g/cm^2)	0.610	0.682	0.730	0.758
Bone mineral content left femoral neck (g)	3.6	3.8	4.2	4.4
Z-score left femoral neck	−3.1	−2.6	−2.3	−2.1

with a BMI that always remained slightly below normal levels, low normal values of HbA1c and normal glucose tolerance prior to estrogen therapy, these results do not suggest significant insulin resistance at any time before and during therapy, although the nonspecific finding of axillary acanthosis nigricans was noted during the initial exam.

4. Discussion

During the last two decades, we have learned a great deal about the complex role of estrogen in both male and female physiology. Studies of patients with monogenic diseases and genetically altered mice are particularly valuable to further our understanding of estrogen actions. That said, aromatase, or in other words estrogen, deficiency, especially in males, is an extremely rare condition with 10 male cases reported so far [8–13,18–21]. With such a rare disease, the detailed description of each new case within the context of the available literature is therefore a valuable resource.

In this case report, we describe a new case of a male patient with aromatase deficiency caused by a known homozygous mutation in exon 5 of the aromatase gene. Notably, this patient had unusually subtle clinical and biochemical features. He had low, barely detectable estradiol levels, as well as normal FSH, LH and testosterone values at the time of diagnosis (see Table 1). In all except two [9,10] of the previously reported male patients with aromatase deficiency, elevated FSH concentrations were noted at the time of diagnosis [8,9,11–13,18–21]. Likewise, the classic phenotype in reported female patients also includes elevated gonadotropins [4]. Though initially normal, FSH, LH and testosterone levels dropped in our patient upon treatment with estradiol suggesting that pituitary feedback is largely executed by estradiol as previously shown [22,23]. The fact, that our patient's initial gonadotropin levels were normal, in particular FSH, argues for possible residual aromatase activity exerting some pituitary feedback, likely at a local level. Alternatively, high testosterone might have provided a negative feedback on gonadotropins even in the absence of estradiol. However, the majority of case reports of male patients with aromatase deficiency with noted high normal or frankly elevated testosterone levels had elevated FSH concentrations [12,13,18,19,21,24], arguing against testosterone having a significant primary effect on FSH suppression.

In addition to the relatively less affected gonadal axis, no biochemical signs of insulin resistance were noted in our patient (see Table 3), and his BMI was slightly below normal. The only potential sign of insulin resistance was the presence of mild acanthosis nigricans, which is not always specifically found with insulin resistance. Again, these results are in stark contrast to the previously published male cases. To our knowledge, all reported males with aromatase deficiency had a BMI > 25 as well as elevated insulin and/or HOMA-IR levels [8–13,18–21]. Two patients had frank diabetes mellitus [10,11] and another patient had hepatomegaly that was confirmed as steatohepatitis per liver biopsy [20].

Similar to the metabolic phenotypes seen in humans, both male and female aromatase knockout mice had increased adiposity, hepatic steatosis, and insulin resistance, although the latter was only noted at one year of age [25]. Of note, despite elevated leptin levels in aromatase knockout mice, neither hyperphagia nor decreased resting energy expenditure were responsible for increased adiposity. Instead, aromatase knockout mice had significantly decreased lean mass and were less physically active [25]. Treatment of female aromatase knockout mice with estrogen reversed the increased adiposity, suggesting direct causality [25].

Interestingly, our patient had both dental cavities and transient diffuse hair loss (telogen effluvium) during childhood and towards the end of puberty respectively. In experimental mouse models of periodontal disease, ovariectomy led to increased alveolar bone loss and prevalence of pathogenic bacteria and gingivitis, with estrogen supplementation reversing these effects [26,27]. In humans, periodontal disease, as well as alveolar bone and tooth loss, have been associated with postmenopausal osteoporosis and estrogen deficiency [28–30]. Furthermore, postmenopausal breast cancer survivors treated with aromatase inhibitors for 2–11 months had significantly more dental plaques and showed increased attachment loss as well as gingival bleeding compared to an age- and sex-matched control group [31], suggesting a link of dental health to estrogen levels.

With regard to hair loss and estrogen deficiency, both estrogen receptors and aromatase are expressed in mammalian hair follicles. Estrogen is thought to induce catagen (the regression phase) and arrest hair follicles in telogen (the resting phase, reviewed in [32]). Topical estrogen has been used empirically for the therapy of both telogen effluvium and androgenetic alopecia (reviewed in [32]). Alternatively, treatment with aromatase inhibitors such as letrozole or exemestane can cause hair loss in 2–15% of female patients with breast cancer (per prescribing information).

Table 3
Time course of metabolic parameters including ALT, AST, alkaline phosphatase (AlkPhos), bilirubin, glucose, insulin, triglycerides, HDL, LDL and creatinine before and after therapy with transdermal estradiol. Normal ranges are in parentheses on the left column. Treatment was started after May, 2011.

Date	5/7/11	1/31/12	4/30/13	12/24/13	3/17/14	1/10/15
Medication dose (μg/d)	0	25	50	75	25	50
ALT (0–50 U/l)	23	28	14	16	17	24[a]
AST (0–50 U/l)	26	26	24	21	22	17[a]
AlkPhos (40–130 U/l)	166	222	119	80	109	81[a]
Bilirubin (0–1.2 mg/dl)	0.6	0.5	0.4	0.6	0.5	0.6[a]
Glucose, fasting (60–99 mg/dl)	80	87	76	77	95	76[a]
Insulin (3–25 μIU/ml)	8					
Cholesterol (<200 mg/dl)	168				147	
Triglycerides (<150 mg/dl)	58				92	
HDL (>40 mg/dl)	62				58	
LDL (<160 mg/dl)	94				71	
Creatinine (0.67–1.17 mg/dl)	1.04	0.90	1.02	0.94	1.14	1.08[a]

[a] Performed in out of state laboratory.

Thus, we speculate that the hair loss and dental caries in our patient could have been attributed to estrogen deficiency, and that such symptoms might be harbingers of aromatase deficiency. However, dental cavities and hair loss have not been noted in other aromatase-deficient male or female patients; thus, these observations in our patient could simply be coincidental rather than related to aromatase deficiency.

What could be the reason behind the less severe phenotype in our patient relative to others with the same enzyme deficiency? We posit that this more subtle presentation might be related to the nature of the mutation. The c.628G>A aromatase gene mutation in our patient was first reported in a male patient by Maffei et al. [11] and was then thought to abolish the 3′ donor splice site in exon 5, resulting in intron retention and the creation of a premature stop codon that leads to expression of a nonfunctional protein. Notably, however, since this mutation is in the exon portion of the splice donor consensus sequence, and the inviolate "GT" is still present in the adjacent intron after exon 5, it is possible that a small amount of splicing can still occur at this site. If a full length protein were formed from mRNA with this c.628G>A mutation also it would contain an amino acid replacement of glutamic acid 210 by lysine (E210K (11)). Interestingly, *in vitro* activity of this aromatase variant appears to be normal. Thus, if small amounts of this protein were still made in our patient, it could explain the more mild phenotype. Alternatively, based on mRNA and minigene expression studies of another female patient and her heterozygous father, Pepe et al. [17] showed that mutation c.628G>A results in an in-frame deletion protein simply lacking exon 5. When expressed in COS1 cells, the aromatase variant lacking exon 5 exhibited no functional activity despite comparable protein expression [17]. Interestingly, mRNA of an aromatase splice variant similarly lacking exon 5, along with normal full-length transcripts, was also found in steroidogenic tissues of normal individuals without the c.628G>A mutation, suggesting that exon 5 skipping in the aromatase gene might be a common phenomenon [17]. Finally, another female patient with aromatase deficiency was previously reported to have a large deletion mutation of 1600 base pairs encompassing exon 5, consequently resulting in a protein that similarly lacked exon 5 [33].

Based on the above-mentioned reports of mutation c.628G>A, three possible outcomes should be considered: one being a normally spliced gene containing an amino acid replacement E210K, another being an in-frame deletion protein lacking exon 5, and the final being a truncated protein due to intronic retention [11,17]. The latter two proteins would be expected to be inactive, while E210K has normal *in vitro* activity [11]. We propose that, in the background of mutation c.628G>A, differential regulation of splicing events may yield both active as well as inactive proteins, which could potentially result in a more mild form of estrogen deficiency, as proposed by Pepe et al. [17].

The first male patient with this mutation [11] was diagnosed at an adult age, similar to our patient. In contrast to our case, his phenotype, both clinically and biochemically, was more severe with noted eunuchoidal features, small inguinal testes, genu valgum, elevated FSH and LH levels as well as an increased BMI and insulin resistance. His testosterone levels, though initially in the low normal range, dropped below normal after start of therapy with low doses of estradiol [11]. This is most likely the consequence of coexisting primary hypogonadism secondary to cryptorchidism that was initially masked by impaired aromatization (and thus higher levels) of testosterone. In this context, it is remarkable that undescended testes or cryptorchidism have been reported in three [8,11,21] out of ten male patients (30%) with aromatase deficiency [8–13,18–21], a prevalence much higher than in the general population (1–4%, [34,35]). One could argue that prenatal estrogen deficiency may affect the physiologic inguinoscrotal descent of the male gonads in humans. Of note, a phenotype of abdominally retracted testes (possibly related to larger cremaster muscles) has been observed in estrogen receptor alpha knockout but not in aromatase knockout mice. Fertility was impaired in both young adult estrogen receptor alpha knockout and older aromatase knockout mice [5,15,36,37].

Similar to our case, two female patients with the homozygous mutation c.628G>A had a mild phenotype signified by mild clinical signs of virilization, only slightly elevated FSH and normal insulin levels [38]. On the other hand, another female in this study, with a compound heterozygous mutation c.628G>A and c.1235 delA (nucleotide deletion in exon 9 leading to a truncated protein), had a much more apparent phenotype. She presented with large polycystic ovaries, elevated FSH levels and diabetes mellitus with insulin resistance [38,39]. It is possible that the phenotype variation seen in the latter case is related to the second mutation present. The female patient with the intronic deletion mutation leading to an aromatase protein lacking exon 5 also showed a rather mild phenotype at first but later failed to undergo puberty [33]. Her ovaries were not cystic but extremely small. Thus, it was hypothesized that her phenotype may have been affected by an additional, as yet unrecognized pathology [33].

We conclude that the specific mutation c.628G>A could translate into expression of differentially functioning proteins (deletion exon 5, truncated protein = inactive, E210K = active protein) which in turn might explain variable, overall milder phenotypes of aromatase deficiency as the case reported here.

In summary, aromatase deficiency, an extremely rare disease, presents with relatively subtle features of osteopenia and unfused growth plates in males and thus is usually only diagnosed in early adulthood. With regard to our patient, the specific nature of the mutation c.628G>A, potentially resulting in an inactive enzyme lacking exon 5, an inactivated truncated protein, but also an amino acid substitution resulting in an active enzyme, may lend an explanation to the variable, overall milder phenotype associated with this particular genotype.

Disclosure

The authors state that they have no conflicts of interest.

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