Novel Insights from Clinical Practice

HORMONE RESEARCH IN PÆDIATRICS

Horm Res Paediatr
DOI: 10.1159/000492128

Received: January 8, 2018
Accepted: July 13, 2018
Published online: August 31, 2018

Accelerated Pubertal Tempo in a 46,XY Aromatase-Deficient Patient

Mariana Costanzo[a] José Garcia-Feyling[c] Nora Saraco[a, b] Roxana Marino[a] Natalia Pérez Garrido[a] Maria Sol Touzon[a] Gisela Viterbo[a] Juan Manuel Lazzati[a] Hellem Carolina Patiño[a] Celeste Mattone[a] Mercedes Maceiras[a] Alicia Belgorosky[a, b] Gabriela Guercio[a, b]

[a]Servicio de Endocrinología, Hospital de Pediatría Garrahan, Buenos Aires, Argentina; [b]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; [c]Hospital Regional de Concepción, Tucumán, Argentina

Established Facts

- Aromatase deficiency is a rare condition.
- 46,XY-affected patients usually remain undiagnosed until adulthood or late puberty, when signs and symptoms become noticeable (continuous linear growth, delayed bone age, osteoporosis, and increased basal serum FSH with normal to increased testosterone and inhibin B levels).

Novel Insights

- Here we report accelerated pubertal progression in a boy with aromatase deficiency.
- Patients have apparently normal pituitary gonadal function during prepuberty as well as early and advanced puberty.
- Rapid pubertal progression may be an additional feature in the phenotype of male aromatase deficiency that could be useful to improve the identification of affected boys who are currently underdiagnosed.

Keywords
Aromatase deficiency · Hypothalamic-pituitary-gonadal axis · Puberty · Bone mineral density

Abstract
Background: Aromatase deficiency is a rare autosomal recessive disorder. 46,XY-affected patients often remain undiagnosed until late puberty. Only 2 pediatric cases have been reported. Data on pubertal development in affected males are scarce. ***Aim:*** To report the clinical phenotype and hormonal studies of an aromatase-deficient boy during the prepubertal and early pubertal period. ***Results:*** The patient was the older brother of a 46,XX girl with aromatase deficiency. Molecular analysis revealed a previously reported homozygous mutation (Arg192Cys) in the *CYP19A1* gene. Pubertal onset was at 9.8 years. At 11.3 years of age, signs of rapidly progressive puberty were seen. Laboratory tests revealed

E-Mail karger@karger.com
www.karger.com/hrp

Alicia Belgorosky, MD, PhD
Servicio de Endocrinología, Hospital de Pediatría Garrahan
Combate de los Pozos 1881
Buenos Aires C1245AAM (Argentina)
E-Mail abelgo12345@gmail.com

normal pubertal basal and GnRH-stimulated gonadotropin levels, normal Sertoli cell markers, and increased testosterone. The prepubertal lumbar spine bone mineral density (BMD) was normal but pubertal bone mineral accrual was incomplete, leading to osteopenia. ***Conclusion:*** Estrogen restraint on gonadotropin secretion has been demonstrated in animal and human models. Interestingly, our patient presented with accelerated puberty and apparently normal pituitary gonadal function. These findings suggest that aromatase activity may be required to define pubertal progression in boys. Estrogen deficiency due to aromatase deficiency is responsible for insufficient bone mineral accrual during puberty.

Introduction

The human cytochrome P450 aromatase (cP450 arom) enzyme is the product of a single gene located on chromosome 15q2, named *CYP19A1* [1]. The protein-coding sequence is contained within 9 exons (E2 to E10) spanning approximately 35 kb [2, 3]. Multiple untranslated first exons (E1) involved in tissue-specific expression have been reported; however, as E1 is not translated the protein sequence is always preserved [4].

cP450 arom is located on the endoplasmic reticulum membrane of a variety of tissues, such as gonads, brain, placental syncytiotrophoblast, breast, and adipose tissue [1]. The enzyme is essential for the biosynthesis of estrogens from androgen precursors. The biological relevance of cP450 arom is related not only to its role in estrogen biosynthesis but also to its potential influence on the androgen-estrogen ratio in the different tissues [1].

cP450 arom deficiency is a rare autosomal recessive condition caused by *CYP19A1* mutations. Disorders of sexual development have been reported in 46,XX-affected patients. In these girls, moderate-to-high increases in serum FSH levels and, occasionally, mild increases in serum LH have been reported [5, 6].

Most 46,XY-affected patients present with normal external genitalia and the condition often remains undiagnosed until late puberty when the classic features, such as continuous linear growth, tall stature, unfused epiphyses, and eunuchoid body proportions, become more evident [7, 8]. In adult affected males, slight increments in basal serum FSH (and in some cases also basal serum LH) despite normal or even high serum testosterone (in one case also normal inhibin B) levels have been reported [9]. Adult men with aromatase deficiency are found to have various degrees of impaired carbohydrate metabolism and a decreased bone mineral density (BMD) [7, 8].

Data on the clinical course and biochemical findings in affected boys during prepuberty and puberty are scarce. To date, only 2 patients younger than 4 years of age and without long-term follow-up have been reported [10, 11].

Here we report the clinical phenotype and hormonal studies of a boy with aromatase deficiency during the prepubertal and early pubertal period.

Subjects and Methods

Patient

The patient was the older brother of a 46,XX girl with aromatase deficiency who was previously reported by our group [12]. The first evaluation at our institution was at the age of 8 years. Clinical features and hormonal data are presented in Figure 1 and Table 1 and in supplementary Figure 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000492128). Antropometric measures were assessed using Argentinian references. This study was approved by the Institutional Review Board of the Hospital de Pediatria Garrahan (Buenos Aires, Argentina). Written informed consent was given by the parents for the evaluation, including molecular biology analysis.

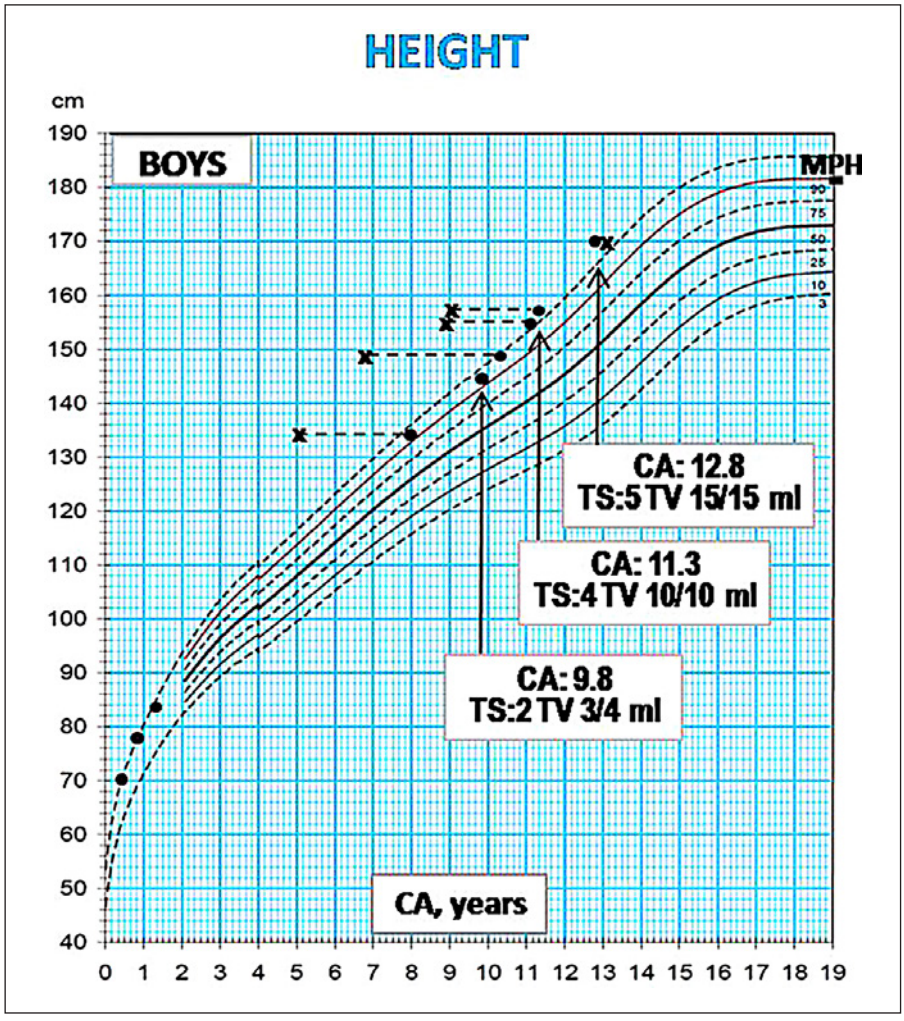
Laboratory Assays

Serum hormone levels were measured as follows: LH, FSH, and insulin by automated chemiluminescent microparticle immunoassay (ARCHITECT i4000; Abbott); testosterone, estradiol, DHEAS, and cortisol by chemiluminescent assay (IMMULITE2000; Siemens); delta 4 androstenedione by RIA (Dia Source); and inhibin B and AMH by 2-site ELISA (Beckman Coulter and Beckman Coulter Gen II, respectively). The assays were performed according to the standard operating procedures recommended by the manufacturer. The detection limits and interassay coefficients of variation (CV%) were as follows: LH, 0.06 mIU/mL and 4.4; FSH, 0.06 mIU/mL and 2.9; testosterone, 0.05 nmol/L and 12.6; estradiol, 55.1 pmol/L and 5.8; DHEAS, 81.4 nmol/L and 8.6; cortisol, 5.5 nmol/L and 8.9; delta 4 androstenedione, 0.35 pmol/L and 9.5; inhibin B, 2.6 pg/mL and 6.8; and AMH, 0.6 pM and 7.7, respectively. The normal reference values for age, sex, and Tanner stage are listed in Table 1 [13–16].

The hCG stimulation test was performed as follows: 2 IM doses of 2,500 IU hCG on days 0 and 3; the response was evaluated on day 7. The GnRH test was performed by measuring LH and FSH levels at 0, 20, and 60 min after a GnRH (100 μg) IV bolus. Glucose tolerance was determined according to WHO criteria using an oral glucose tolerance test [17].

Serum fasting total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and triglyceride levels were measured using commercially available kits.

BMD was determined via a dual-energy absorptiometry scan (DXA) of the whole body and the lumbar spine employing a Lunar Prodigy advance bone densitometer (GE Medical Systems, Madison, WI, USA) using published reference values [18, 19].



Color version available online

Fig. 1. Growth chart, Argentinian references. Bone age is depicted by small X; horizontal slashed lines connect each bone age with the corresponding height at chronological age (CA). Pubertal milestones are highlighted in boxes; TS, Tanner stage; TV, testicular volume. The small bar at the right edge represents the midparental height (MPH) calculated as follows: (paternal height [cm] + maternal height [cm] + 12.5)/2.

Mutation Analysis

Molecular studies were performed as previously reported [12]. Briefly, genomic DNA was isolated from peripheral leukocytes of the affected subject and his parents using standard techniques. Each coding exon (E2 to E10) and the flanking intronic regions of the *CYP19A1* were PCR amplified and automatedly sequenced. The nucleotide sequences obtained were compared with the National Center for Biotechnology Information entry of *CYP19A1* (NG_007982.1). The sequence homology-based tool SIFT (Sorting Intolerant From Tolerant; http://sift.jcvi.org/) version 2.0.6 and the structure-based tool PolyPhen (Polymorphism Phenotyping; http://www.genetics.bwh.harvard.edu/pph) were used.

Results

Clinical Case Report

The patient was studied at our institution because his younger sister had been found to have aromatase deficiency confirmed by mutational analysis of the *CYP19A1* gene [12]. Briefly, the girl (patient 4 in our previous report) was born with ambiguous genitalia (Prader IV). Congenital adrenal hyperplasia and disorder of gonadal development were ruled out. At 16 months of age the girl was referred to our hospital for further evaluation. The parents are of Caucasian descent, denied consanguinity, and did not have additional children. The family history was negative for obesity but positive for adult-onset type 2 diabetes in the maternal grandfather and uncles and the paternal grandmother. Maternal virilization (progressive hirsutism and acne) was present during both pregnancies and disappeared immediately after delivery. When the diagnosis of aromatase deficiency was confirmed, her brother was called for evaluation.

The clinical parameters and biochemical assessments of the boy are shown in Figure 1 and Table 1, respective-

Table 1. Biochemical studies

	Chronological age, years						
	8		9.8 basal	11.3		12.8	
	basal	GnRH test		basal	GnRH test	basal	GnRH test
LH, MIU/mL	<0.10	<0.10/0.83/1.14		3.8	3.8 /17.2 /18.9	5.28	4.36/19.9/19.6
FSH, MIU/mL	0.47	0.82/2.14/4.08		2.8	2.8/3.9/4.7	2.88	3.7/4.39/5.1
Testosterone, nmol/L	<0.2	<0.2	**1.98**	**20.8**		**30.9**	
Androstenedione, nmol/L	<0.35	0.63		4.19		4.33	
Estradiol, pmol/L	<55	<55		<55		<55	
DHEAS, nmol/L	896			2193		3,121	
AMH, pmol/L	829			104.9		256.4	
Inhibin B, pg/mL	90.7			369.7		343.5	

	Basal	OGTT		Basal	OGTT	
		basal	120		basal	120
Glucose, mg/dL	76	76	94	76	76	87
Insulin, IU/mL	4.3	4.3	55.1	2.7	2.7	15.9
Total cholesterol, mg/dL	146			164		
HDL	53.2			53.9		
LDL	80			96		
Triglycerides	64			80		

Bold numbers represent values outside of the reference range. OGTT, oral glucose tolerance test. Normal reference ranges [13–16]. Tanner stage 1: LH 0.05 ± 0.05 MIU/mL, FSH 0.97 ± 0.59 MIU/mL, testosterone <1.38 nmol/L, androstenedione 0.17–1.95 nmol/L, estradiol <55 pmol/L, DHEAS 460 ± 370 nmol/L, and AMH 236–1,831 pmol/L. Tanner stage 4: LH 2.65 ± 1.81 MIU/mL, FSH 4.47 ± 1.88 MIU/mL, testosterone 3.64–18.9 nmol/L, androstenedione 0.69–3.59 nmol/L, estradiol 55–190 pmol/L, DHEAS 2,980 ± 1,480 nmol/L, and AMH 33–164 pmol/L. Tanner stage 5: LH 2.76 ± 1.12 MIU/mL, FSH 7.64 ± 2.50 MIU/mL, testosterone 7.6–28 nmol/L, androstenedione 1.95–8.73 nmol/L, estradiol 73–230 pmol/L, DHEAS 2,980 ± 1,480 nmol/L, and AMH 38–195 pmol/L. Inhibin B: age 8–9 years, 30–250 pg/mL; age 11–12 years, 50–370 pg/mL; and age 12–13 years, 70–410 pg/mL. GnRH test: Tanner stage 1: LH maximum response 1.8 ± 1.3 MIU/mL and FSH maximum response 4.7 ± 2.2 MIU/mL; Tanner stage 4–5: LH maximum response 42 ± 23 MIU/mL and FSH maximum response 11 ± 5.6 MIU/mL.

ly. The first evaluation at our hospital was at the age of 8 years. On the physical exam, the height was 134.2 cm (1.63 SD). Based on his parents' heights, his midparental height was at 1.25 SD. His weight and BMI were within the normal range (weight: 28.3 kg, BMI: 15.7; –0.03 SD). The boy presented with normal male prepubertal external genitalia. Bone age was 3 years delayed. Laboratory findings showed normal prepubertal basal serum LH and FSH (including an adequate response to the GnRH stimulation test), inhibin B, AMH, testosterone, and androstenedione levels (Table 1). The hCG stimulated testosterone level was 10.2 nmol/L (normal response ≥3.47 nmol/L). Oral glucose tolerance test results, basal insulin, and glucose levels as well as the basal lipid profile were normal (Table 1). During follow-up, clinical and biochemical evidence of central pubertal onset was observed at 9.8 years based on a genital exam (the Tanner stage was G1 and PH1, and the testicular volume was: right, 4 cm^3; left, 3 cm^3) and basal serum testosterone levels (1.98 nmol/L, prepubertal reference male value <1.36 nmol/L). At 11.3 years of age, the physical exam revealed a rapid progression of secondary sexual characteristics, reaching Tanner stage 4 in 1.5 years (G4, PH4). The testicular volume was 10 mL each and the bone age was 2 years delayed. At the last evaluation, the patient was 12.8 years old, his height was above the reference range (i.e., 170 cm, 2.46 SDS), his weight was appropriate for his height (i.e., 52.5 kg, BMI 18.2, 0.07 SDS), his bone age was according to his chronological age, the Tanner stage was G5 and PH4, and the testicular volume reached 15 mL each (Fig. 1; online suppl. Fig. 1). During puberty, laboratory tests showed serum basal and GnRH-stimulated gonado-

Table 2. Bone mineral density

	Chronological age, years	
	8	12.8
L2 to L4		
g/cm^2	0.64	0.842
Z score	0.53	–0.2
Height-adjusted *Z* score	–0.24	**–2.79**
Whole body		
g/cm^2	–	0.941
Z score		0.7
Height-adjusted *Z* score		–1.65

BMD measured by DXA, Lunar Prodigy Advance. The bold number represents a value outside of the reference range. Height-adjusted *Z* scores are according to Zemel et al. [19].

tropin levels within the normal pubertal range while serum testosterone levels were increased for the age and Tanner stage. Estradiol levels remained undetectable. Serum inhibin B levels were in the upper normal range (Table 1). Oral glucose tolerance test results, basal insulin, glucose levels, and the lipid profile remained within the normal range (Table 1). An abdominal ultrasound showed a normal liver structure.

Prepubertal assessment of BMD at the age of 8 years was within the normal range even when adjusted for height (Table 2) [19]. At the last evaluation, at the age of 12.8 years, a significant decrease in BMD was observed, with a lumbar spine BMD of 0.842 g/cm^2 (*Z* score, –0.2; height-adjusted *Z* score, –2.79) and a whole body BMD of 0.941 g/cm^2 (*Z* score, 0.7; height adjusted *Z* score, –1.65) (Table 2).

CYP19A1 *Gene Analysis*

As previously reported, the patient and his younger sister were found to be homozygous for a C-to-T transition at cDNA position 574 bp in exon 5 (c.574C>T, the A of the ATG of the initiator Met codon is denoted as nucleotide 1) [12]. Briefly, the variation resulted in an arginine-to-cysteine substitution at codon 192 of the mature protein, i.e., Arg192Cys. Both parents were found to be heterozygous for this *CYP19A1* gene mutation. This variant was not found in the 1000 Genomes Project or exome sequencing projects (http://www.ensembl.org). To determine whether this alteration is present in the general population, 60 control subjects (120 alleles) were screened for this mutation using DNA sequencing. No allele carrying this mutation was detected, suggesting that it would not be a common polymorphism. Sequence alignment of cP450arom proteins from different species showed that the amino acid substitution affects a highly conserved amino acid of the aromatase protein from other species. We also applied an evolutionary perspective to screen the c.574C>T substitution using the sequence homology-based SIFT tool, which predicted this variant to affect protein function, with a highly deleterious tolerance index score of 0.00. In addition, the effect of the c.574C>T mutation was predicted to be probably damaging, with a score of 1.000 (sensitivity, 0.00; specificity, 1.00) using the PolyPhen-2 structure-based approach.

Discussion

We report a boy with aromatase deficiency and accelerated progression of secondary sexual characteristics during puberty. To our knowledge, this rapid pubertal progression has not been previously reported in male aromatase-deficient patients. The description of pubertal milestones in the reported cases is particularly scarce. This case represents an interesting natural model to analyze the role of estrogens in regulation of the pubertal tempo in male humans.

Different studies have evaluated the role of estradiol in the control of gonadotropin secretion in males. Indirect evidence suggests that androgen aromatization is required for the sex steroid inhibitory effect on gonadotropin secretion in adult men [20]. A modulatory and inhibitory effect of estradiol on pituitary release and/or synthesis of LH and FSH has been demonstrated [21–23] and in adult men with aromatase deficiency slight increments in basal serum FSH (and in some cases also basal serum LH) despite normal or even high serum testosterone and normal inhibin B levels have been reported [8, 9].

The results concerning sex steroid-mediated regulation of gonadotropin secretion in adult men may not be applicable to infancy and prepuberty. Hormonal studies conducted in aromatase-deficient boys and the case reported here showed normal basal and GnRH-stimulated gonadotropin levels and normal serum inhibin B and AMH during infancy and prepuberty [10, 11]. These findings suggest that in normal boys estrogens do not seem to play an important role in the regulation of gonadotropin secretion during these periods. In agreement with these observations, no changes in basal gonadotropin levels were found with use of the potent aromatase inhibitor letrozole in prepubertal boys with idiopathic short stature [23].

On the other hand, data from early- and midpubertal boys receiving estrogens or aromatase inhibitors suggest that, once puberty starts, estrogens become involved in the negative feedback control of gonadotropin secretion [24, 25]. The use of aromatase inhibitors to improve adult height in pubertal boys with idiopathic short stature has been associated with an increase in serum gonadotropin levels and a subsequent supraphysiological rise in testosterone concentrations, raising concern regarding pubertal progression [23]. Even though normal basal and stimulated gonadotropin levels were found, an increase in LH pulsatility and/or pulse amplitude could not be ruled out, since both effects have been previously demonstrated after estrogen suppression [26].

Interestingly, our aromatase-deficient patient presented with accelerated progression of secondary sexual characteristics and apparently normal pituitary gonadal function. The time between pubertal onset and Tanner stage 4 (G4 and PH4) was 1.5 years. The progression of genital virilization in early puberty was consistent with the significant increase in basal serum testosterone levels, which were higher than expected for his age and for the Tanner stage. In adult male aromatase-deficient patients the reported final testicular volume is variable, ranging from macroorchidism [27] to normal [28–30] or even microorchidism [31, 32]. Therefore, although testicular volume is a useful marker of pubertal progression in normal boys, we do not consider it to be reliable in the assessment of Tanner stage or pubertal tempo in cases of male aromatase deficiency. In our patient, the testicular volume reached 15 ml at Tanner stage 5, coexisting with normal serum FSH and inhibin B levels. However, further testicular volume progression may still be observed in the follow-up.

The presence of normal serum FSH levels during puberty was an unexpected finding. It is widely known that testosterone aromatization is required to suppress FSH secretion [20, 22]. As in our patient, in two previously reported aromatase-deficient men, normal FSH levels were found together with increased serum testosterone [28, 30]. Hence, we may speculate that in the presence of high testosterone levels, minimal residual aromatase activity could already be enough for gonadotropin suppression. Even though functional studies of the Arg192Cys variant found in this case are lacking, a previous report on another substitution involving the same amino-acid residue revealed 19% wild-type aromatase catalytic activity [11].

Data regarding inhibin B levels in the affected adult patients are very scarce (only available in 2 cases) [9]. It has been proposed that in normal adult men inhibin B secreted by testicular Sertoli cells represents the main component involved in the negative feedback regulation of FSH secretion [33]. Therefore, we may speculate that the normal serum FSH levels found in our patient might be related to the presence of inhibin B levels in the upper normal range. Unfortunately, there is no information regarding serum inhibin B levels in affected adults with normal FSH.

Insulin resistance, an abnormal lipid profile, and other features of the metabolic syndrome have been described in aromatase-deficient adult men [8]. During childhood and puberty, insulin resistance and glucose intolerance have been reported in a few affected females [12, 34]; however, no information is available on affected boys. A normal lipid profile and glucose homeostasis were found in our patient despite significant familial antecedents of type 2 diabetes on both sides. This finding suggests that, at least during prepubertal and pubertal years, the lack of estrogens might not affect glucose homoeostasis or the lipid metabolism. In line with these observations, the use of aromatase inhibitors in pubertal boys has been associated with neutral [35] or even beneficial [36] effects on insulin sensitivity, while even short-term use in adult men has been associated with a decrease in insulin sensitivity [37]. In agreement with these observations, mouse aromatase deficiency models progressively developed the metabolic syndrome phenotype (obesity, glucose intolerance, and decreased insulin sensitivity) after the first 10–12 weeks of age (in adulthood) [38]. On the other hand, an important aspect to consider is that every aromatase-deficient man in whom an abnormal metabolic profile was reported had a BMI >25. The lack of estrogens associated with unopposed androgen action may represent a high-risk condition for the development of insulin resistance that needs the cooperative role of an increased fat mass to fully develop.

A delay in bone maturation has previously been reported in aromatase deficiency [6] and became clearly evident in late prepuberty. In the case of our patient, bone age remained delayed in prepubertal and early pubertal years.

A singular feature of aromatase deficiency in adults – both male and female – is the decrease in BMD. In adult affected men, estrogen treatment improved BMD in a dose-dependent manner even when the treatment was started in adulthood, supporting an important role of estrogens in the preservation of bone health [8, 28, 39–41]. Low BMD has also been observed in different animal models of estrogen deficiency [41]. Nevertheless, data on the role of estrogens in bone mineralization during child-

hood are scarce. In this case, the result of prepubertal assessment of BMD was within the normal range even when adjusted for height but it was decreased during puberty. This finding emphasizes the important role of estrogens in bone mass acquisition during male puberty.

Even though aromatase deficiency is a recessive disorder, more affected females (n = 26) than males (n = 12) have been reported. The underrepresentation of 46,XY patients might be explained by the lack of significant findings in the first decades of life that could delay or impair the diagnosis. Rapid pubertal progression may be an additional feature in the phenotype of male aromatase deficiency that could be useful to improve the identification of affected boys who are currently underdiagnosed.

In summary, aromatase deficiency is a useful human model to evaluate the role of estrogens in the regulation of gonadotropin secretion as was previously described. The accelerated pubertal progression observed in this 46,XY aromatase-deficient patient suggests that aromatase activity may be involved in the male pubertal tempo. Interestingly, the finding of rapid progression of secondary sexual characteristics in a boy may be an early sign that should alert the physician to consider aromatase deficiency, especially when delayed bone age is associated.

Disclosure Statement

The authors have nothing to disclose.

Funding Sources

This study was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Fondo para la Investigación Científica y Tecnológica (FonCyT) of Argentina.

References

1 Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev. 1994 Jun;15(3):342–55.
2 Means GD, Mahendroo MS, Corbin CJ, Mathis JM, Powell FE, Mendelson CR et al. Structural analysis of the gene encoding human aromatase cytochrome P-450, the enzyme responsible for estrogen biosynthesis. J Biol Chem. 1989 Nov;264(32):19385–91.
3 Harada N, Yamada K, Saito K, Kibe N, Dohmae S, Takagi Y. Structural characterization of the human estrogen synthetase (aromatase) gene. Biochem Biophys Res Commun. 1990 Jan;166(1):365–72.
4 Means GD, Kilgore MW, Mahendroo MS, Mendelson CR, Simpson ER. Tissue-specific promoters regulate aromatase cytochrome P450 gene expression in human ovary and fetal tissues. Mol Endocrinol. 1991 Dec;5(12):2005–13.
5 Belgorosky A, Guercio G, Pepe C, Saraco N, Rivarola MA. Genetic and clinical spectrum of aromatase deficiency in infancy, childhood and adolescence. Horm Res. 2009;72(6):321–30.
6 Belgorosky A, Pepe C, Marino R, Guercio G, Saraco N, Vaiani E 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 Nov;88(11):5127–31.
7 Bulun SE. Aromatase and estrogen receptor α deficiency. Fertil Steril. 2014 Feb;101(2):323–9.
8 Rochira V, Carani C. Aromatase deficiency in men: a clinical perspective. Nat Rev Endocrinol. 2009 Oct;5(10):559–68.
9 Rochira V, Zirilli L, Genazzani AD, Balestrieri A, Aranda C, Fabre B et al. Hypothalamic-pituitary-gonadal axis in two men with aromatase deficiency: evidence that circulating estrogens are required at the hypothalamic level for the integrity of gonadotropin negative feedback. Eur J Endocrinol. 2006 Oct;155(4):513–22.
10 Deladoëy J, Flück C, Bex M, Yoshimura N, Harada N, Mullis PE. 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 Nov;84(11):4050–4.
11 Bouchoucha N, Samara-Boustani D, Pandey AV, Bony-Trifunovic H, Hofer G, Aigrain Y et al. Characterization of a novel CYP19A1 (aromatase) R192H mutation causing virilization of a 46,XX newborn, undervirilization of the 46,XY brother, but no virilization of the mother during pregnancies. Mol Cell Endocrinol. 2014 Jun;390(1-2):8–17.
12 Marino R, Perez Garrido N, Costanzo M, Guercio G, Juanes M, Rocco C 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 Feb;100(2):E301–7.
13 Guercio G, Rivarola MA, Chaler E, Maceiras M, Belgorosky A. Relationship between the GH/IGF-I axis, insulin sensitivity, and adrenal androgens in normal prepubertal and pubertal boys. J Clin Endocrinol Metab. 2002 Mar;87(3):1162–9.
14 Neely EK, Hintz RL, Wilson DM, Lee PA, Gautier T, Argente J et al. Normal ranges for immunochemiluminometric gonadotropin assays. J Pediatr. 1995 Jul;127(1):40–6.
15 Grinspon RP, Bedecarrás P, Ballerini MG, Iñiguez G, Rocha A, Mantovani Rodrigues Resende EA et al.; LAREP Group. Early onset of primary hypogonadism revealed by serum anti-Müllerian hormone determination during infancy and childhood in trisomy 21. Int J Androl. 2011 Oct;34(5 Pt 2):e487–98.
16 Kelsey TW, Miles A, Mitchell RT, Anderson RA, Wallace WH. A normative model of serum inhibin B in young males. PLoS One. 2016 Apr;11(4):e0153843.
17 Puavilai G, Chanprasertyotin S, Sriphrapradaeng A; World Health Organization. Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria. Diabetes Res Clin Pract. 1999 Apr;44(1):21–6.
18 Crabtree NJ, Shaw NJ, Bishop NJ, Adams JE, Mughal MZ, Arundel P et al.; ALPHABET Study Team. Amalgamated reference data for size-adjusted bone densitometry measurements in 3598 children and young adults—the ALPHABET Study. J Bone Miner Res. 2017 Jan;32(1):172–80.
19 Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S et al. Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. J Clin Endocrinol Metab. 2010 Mar;95(3):1265–73.

20 Bagatell CJ, Dahl KD, Bremner WJ. The direct pituitary effect of testosterone to inhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. J Androl. 1994 Jan-Feb;15(1):15–21.
21 Finkelstein JS, O'Dea LS, Whitcomb RW, Crowley WF Jr; Effects of Estradiol Administration in Normal and Gonadotropin-Releasing Hormone-Deficient Men. Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab. 1991 Sep;73(3):621–8.
22 Pitteloud N, Dwyer AA, DeCruz S, Lee H, Boepple PA, Crowley WF Jr et al. The relative role of gonadal sex steroids and gonadotropin-releasing hormone pulse frequency in the regulation of follicle-stimulating hormone secretion in men. J Clin Endocrinol Metab. 2008 Jul;93(7):2686–92.
23 Hero M, Norjavaara E, Dunkel L. Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. J Clin Endocrinol Metab. 2005 Dec;90(12):6396–402.
24 Kletter GB, Padmanabhan V, Beitins IZ, Marshall JC, Kelch RP, Foster CM. Acute effects of estradiol infusion and naloxone on luteinizing hormone secretion in pubertal boys. J Clin Endocrinol Metab. 1997 Dec;82(12):4010–4.
25 Wickman S, Sipilä I, Ankarberg-Lindgren C, Norjavaara E, Dunkel L. A specific aromatase inhibitor and potential increase in adult height in boys with delayed puberty: a randomised controlled trial. Lancet. 2001 Jun;357(9270):1743–8.
26 Hayes FJ, Seminara SB, Decruz S, Boepple PA, Crowley WF Jr. Aromatase inhibition in the human male reveals a hypothalamic site of estrogen feedback. J Clin Endocrinol Metab. 2000 Sep;85(9):3027–35.
27 Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab. 1995 Dec;80(12):3689–98.
28 Bouillon R, Bex M, Vanderschueren D, Boonen S. Estrogens are essential for male pubertal periosteal bone expansion. J Clin Endocrinol Metab. 2004 Dec;89(12):6025–9.
29 Lanfranco F, Zirilli L, Baldi M, Pignatti E, Corneli G, Ghigo E et al. A novel mutation in the human aromatase gene: insights on the relationship among serum estradiol, longitudinal growth and bone mineral density in an adult man under estrogen replacement treatment. Bone. 2008 Sep;43(3):628–35.
30 Miedlich SU, Karamooz N, Hammes SR. Aromatase deficiency in a male patient - case report and review of the literature. Bone. 2016 Dec;93:181–6.
31 Maffei L, Murata Y, Rochira V, Tubert G, Aranda C, Vazquez M 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 Jan;89(1):61–70.
32 Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J et al. Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med. 1997 Jul;337(2):91–5.
33 Hayes FJ, Pitteloud N, DeCruz S, Crowley WF Jr, Boepple PA. Importance of inhibin B in the regulation of FSH secretion in the human male. J Clin Endocrinol Metab. 2001 Nov;86(11):5541–6.
34 Guercio G, Di Palma MI, Pepe C, Saraco NI, Prieto M, Saure C et al. Metformin, estrogen replacement therapy and gonadotropin inhibition fail to improve insulin sensitivity in a girl with aromatase deficiency. Horm Res. 2009;72(6):370–6.
35 Mauras N, Gonzalez de Pijem L, Hsiang HY, Desrosiers P, Rapaport R, Schwartz ID et al. Anastrozole increases predicted adult height of short adolescent males treated with growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years. J Clin Endocrinol Metab. 2008 Mar;93(3):823–31.
36 Wickman S, Saukkonen T, Dunkel L. The role of sex steroids in the regulation of insulin sensitivity and serum lipid concentrations during male puberty: a prospective study with a P450-aromatase inhibitor. Eur J Endocrinol. 2002 Mar;146(3):339–46.
37 Gibb FW, Homer NZ, Faqehi AM, Upreti R, Livingstone DE, McInnes KJ et al. Aromatase inhibition reduces insulin sensitivity in healthy men. J Clin Endocrinol Metab. 2016 May;101(5):2040–6.
38 Takeda K, Toda K, Saibara T, Nakagawa M, Saika K, Onishi T et al. Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. J Endocrinol. 2003 Feb;176(2):237–46.
39 Herrmann BL, Janssen OE, Hahn S, Broecker-Preuss M, Mann K. Effects of estrogen replacement therapy on bone and glucose metabolism in a male with congenital aromatase deficiency. Horm Metab Res. 2005 Mar;37(3):178–83.
40 Bilezikian JP, Morishima A, Bell J, Grumbach MM. Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med. 1998 Aug;339(9):599–603.
41 Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev. 1999 Jun;20(3):358–417.