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HUMAN PREPUBERTAL AROMATASE DEFICIENCY: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL LESSONS LEARNED FROM THIS EXPERIMENT OF NATURE

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RESUM

En els humans, el cP450arom és el producte d'un gen únic (CYP19), localitzat en el cromosoma 15q21.1. A la placenta, l'aromatització activa dels andrògens protegeix el fetus femella i la mare de les accions virilitzants dels andrògens fetals. De fet, els nadons 46XX amb deficiència completa de l'aromatasa neixen amb una ambigüitat dels genitals externs. Els estudis en pacients afectats per la deficiència completa del cP450arom han contribuït considerablement a l'anàlisi de la importància de l'activitat de la cP450arom en la diferenciació sexual, en el patró de secreció de les gonadotrofines, en la capacitat reproductora, en el metabolisme lipídic i la sensibilitat a la insulina, i en el creixement i la maduració esquelètica, en ambdós sexes. La seqüència codificant de la proteïna està continguda en nou exons (exons 2-10), que s'estenen aproximadament al llarg de 35 kb de DNA. Els codons d'iniciació i terminació de la traducció són als exons 2 i 10, respectivament. Fins ara, s'han documentat onze casos de deficiència completa de l'aromatasa secundaris en mutacions del gen CYP19. El patró dels nivells sèrics hormonals indica una baixa concentració d'estròGens i alta d'andròGens, FSH, i, ocasionalment, també d'LH, sempre, però, depenent de l'edat i del sexe. Durant la infantesa, s'observa un dimorfisme sexual en el paper dels estròGens en la regulació de la secreció de les gonadotrofines. Aquests pacients presenten un risc elevat de desenvolupar quists d'ovari, fins i tot abans de la pubertat. Finalment, l'estudi d'aquests pacients també ha estat útil per il·lustrar el paper dels estròGens en el desenvolupament esquelètic, en la maduració de les epífisis i en el brot de creixement puberal, en ambdós sexes.

Paraules clau: aromatasa, ambigüitat sexual, creixement puberal, malaties genètiques.

SUMMARY

In humans, cP450arom appears to be the product of a single gene (CYP19), located on chromosome 15q21.1. In the placenta, the active aromatization of androgens protects the female fetus and the mother from the virilizing actions of fetal androgens. Indeed, 46, XX neonates with complete aromatase deficiency are born with ambiguous external genitalia. Studies carried out on patients with complete cP450arom deficiency have also contributed considerably to the analysis of the importance that cP450arom activity has on sexual differentiation, the pattern of gonadotropin secretion, reproductive capacity, lipid metabolism and insulin sensitivity, as well as growth and skeletal maturation in both sexes. The protein coding sequence is contained within nine exons (exons 2-10), which span approximately 35 kb of DNA. The translation, initiation and termination codons are present in exons 2 and 10, respectively. Eleven well-documented cases of complete aromatase deficiency, secondary to mutations of the CYP19 gene, have been reported. Very low estrogens and high androgens, FSH, and sometimes LH, depending on age and sex, characterize the pattern of serum hormones. During infancy, a sexual dimorphism has been observed in the role that estrogens play in the regulation of gonadotropins. These patients are at risk of developing ovarian cysts, even before puberty. Finally, the study of these patients has also been useful to illustrate the essential role of estrogens in skeletal development, epiphysial maturation, and in the pubertal growth spurt in both sexes.

Keywords: Aromatase, sexual ambiguity, puberal growing, genetic illnesses.

INTRODUCTION

Aromatase is the enzyme complex that catalyzes the synthesis of estrogens from androgens. Therefore, the activity arising from this enzyme complex affects both androgen metabolism and estrogen synthesis. The biological importance of aromatase complex activity is related not only to its role in the synthesis of estrogens, but also to its potential influence in the balance of the androgen-estrogen ratio in several tissues. This enzyme complex, which is expressed in the endoplasmic reticulum of cells, consists of two components: a cytochrome P450 (cP450arom) and coupled to it, a ubiquitous flavoprotein, NADPH-cytochrome P450 reductase. The catalytic process requires the sequential transfer of three pairs of electrons, and it consumes three moles of reduced NADPH to catalyze a series of three hydroxylations in the synthesis of one mole of estrogen. Even though androstenedione and testosterone are the most common and the most physiologically important substrates, 16OH-dehydroepiandrosterone sulfate (16OH-DHEAS), arising from the fetal liver hydroxylation of fetal adrenal DHEAS, is also an important substrate for placental estriol synthesis during pregnancy in both humans and high primates. Moreover, the fact that the affinity of androstenedione for cP450arom is in the nanomolar range (at least one order of magnitude higher than that of most other microsomal steroid hydroxylases) represents an important mechanism to facilitate estrogen synthesis in peripheral, non-classical steroidogenic tissues.

In humans cP450arom appears to be the product of a single gene (CYP19 gene) that is localized on chromosome 15q21.1. The protein coding sequence is contained within nine exons (exons 2-10), which span approximately 35 kb of DNA (Means *et al.*, 1989). The translation, initiation and termination codons are present in exons 2 and 10, respectively. However, there are multiple first exons that are involved in tissue-specific expression. These

exons are not translated, and they generate alternative splicing in such a fashion that the coding region and, hence, the protein sequence, is conserved in every tissue. These promoters are found within a 90 kb region, upstream of the coding region (Means *et al.*, 1989). The most highly conserved region (the core region), defined by a three-dimensional model of the cP450arom, consists essentially of a four-helix bundle: two beta sheets and the heme-binding region. cP450arom is expressed in a number of tissues, including the syncytiotrophoblast layer of the placenta, the gonads, adipose tissue, bone, brain (including the hypothalamus, hippocampus and amygdala), coronary arteries and various fetal tissues, including the liver, skin, the intestine, testes and ovaries (Graham-Lorence *et al.*, 1995).

CYP19 gene expression is subjected to multi-factorial regulations by a diverse group of hormones and factors that differ markedly in different tissues. These regulators, which act via specific receptors that bind responsive cis-acting elements at the 5′ flanking region, include: gonadotropins, cyclic AMP analogs, phorbol esters, growth factors, such as epidermal growth factor and transforming growth factor beta, and steroids. Thus, a strict control over tissue-specific expression is necessary for an exquisite regulation of estrogen synthesis, as early as during fetal development, as well as during the post-natal life span. In humans, several molecular CYP19 gene alterations, associated with complete cP450arom deficiency, have been described. They have contributed considerably to the analysis of the importance of cP450arom activity on different body tissues, on sexual differentiation, the pattern of gonadotropin secretion by the hypothalamo-pituitary axis and reproductive capacity, lipid metabolism and insulin sensitivity, and growth and skeletal maturation in both sexes.

THE ROLE OF AROMATASE IN THE PLACENTA

The placenta is derived from two major cell lineages: the embryonic trophoblasts that give rise to the epithelial parts and the extra-embryonic mesoderm that gives rise to the stromal cells and blood vessels of the placenta. The precursor trophoblastic lineage differentiates either into extra-villous cytotrophoblasts (inherently invasive) that are associated with maternal blood vessels in the outer layer of the placenta or multi-nucleated syncytiotrophoblasts, located in the chorionic villae in the innermost layer.

It has been known for many years that there is a large production of steroids after the second month of pregnancy. In most physiological instances, including ovarian secretion, estrogen synthesis is the consequence of the cooperative effect of two cells, one with the capability of synthesizing androgens and the other, of expressing aromatase. Pregnancy is no exception to this rule. Indeed, there is an interaction between the fetal adrenal gland, site of the synthesis and secretion of androgen precursors, and the placenta, rich in aromatase and other enzymes necessary to convert androgens into estrogens (Siiteri *et al.*, 1963). The aromatase gene is specifically expressed in syncytiotrophoblasts, and it is under the regulation of a placental-specific enhancer.

For steroidogenesis, the feto-placental unit synthesizes cholesterol in the fetal liver, which is transported to the fetal adrenal as LDL cholesterol. The fetal zone of the fetal adrenal is quite enlarged, and it has the biosynthetic machinery to form DHEAS from cholesterol in large quantities. Cholesterol is converted into Δ_5-pregnenolone by the enzyme cytochrome P450scc. A deficiency in 3β-hydroxysteroid dehydrogenase (3β-HSD), present in the fetal zone, is a pre-requisite for the synthesis of this and other Δ_5-steroids. An active 17α-hydroxysteroid dehydrogenase/17,

20 desmolase is also necessary. Finally, Δ_5-steroid sulfoconjugation is also very active in the fetal zone of the fetal adrenal. The fetal liver is also rich in 16α-hydroxylase, which contributes to the synthesis of 16α-DHEAS. After secretion, DHEAS and other sulfoconjugates are transported, via the umbilical artery, to the placenta.

The placenta is an efficient extractor and processor of Δ_5-sulfoconjugates. The placenta is rich in sulfatases, 3β-HSD and aromatase, all necessary steps in the synthesis of estrogens from the DHEAS and 16α-DHEAS, provided by the fetus. DHEA is first converted into Δ_4-androstenedione by 3β-HSD, followed by the generation of the biological active androgen testosterone through the action of 17β-HSD. Finally, the very active placental aromatase allows for the synthesis of estrone, estradiol and estriol. The maternal blood concentration of these three estrogens increases gradually during pregnancy to reach values, at term, 50-fold higher than those in non-pregnant women's blood.

The active aromatization of androgens protects the fetus and the mother from the virilizing actions of androgens. In the case of the female fetus, this is particularly important, in order to avoid an androgenic effect, not only on the differentiation of external genitalia, but also on the central nervous system, which could program the brain during fetal and neonatal life for non-cyclic hypothalamic GnRH function, male sex self-identity, and male sexual behavior.

Aside from being essential to the neutralization of androgens, it has been postulated that during pregnancy estrogens are important for pregnancy maintenance and the maturation of maternal and fetal organ systems (Pepe *et al.*, 1995). Estrogens could regulate the functional differentiation of placental trophoblasts and steroid biosynthetic pathways within the feto-placental unit, as well as utero-placental blood flow. Along with progesterone, they inhibit uterine contractility and the immune system to allow for fetal antigen tolerance. Mammary gland development, fetal lung maturation and the regulation of placental 11β-hydroxysteroid dehydrogenase for cortisol-cortisone metabolism are also among the important actions attributed to estrogens during pregnancy.

MOLECULAR ANALYSIS IN cP450arom DEFICIENT PATIENTS

The first case in the literature was reported by Harada *et al.* (1992) on a 24 year-old primigravida. She showed progressive virilization during her third trimester of pregnancy, and her husband had consanguinity in his pedigree. The newborn girl was homozygous for a consensus 5′-splice donor sequence mutation from GT-GC, which resulted in the use of a cryptic donor site further downstream in intron 6, and which, therefore, generated a transcript with an insert of 87 base pairs, resulting in the translation of an abnormal protein molecule with 29 extra amino acids. After transient expression in COS-7 cells, the aromatase cDNA of the patient was found to produce a molecule with a trace of activity (less than 0.3%).

The next report was described by Conte *et al.* (1994) on a prepubertal girl. The patient was a compound heterozygote for two missense mutations in exon 10, in the putative heme-binding region of the enzyme. These missense mutations resulted in a cysteine, instead of the highly-conserved arginine, at amino acid 435 (R435C), and a tyrosine, instead of the conserved cysteine, at amino acid 437 (C437Y). An assay on the expressed mutated proteins showed that the R435C mutant had less than 1.1 % of the enzyme activity of the wild-type protein molecule and that the C437Y mutant had no aromatase activity.

The following case was described by Morishima *et al.* (1995) on two adult siblings, male and female, of a consanguineous pedigree.

The molecular analysis indicated a homozygous point mutation at position 375 in exon 9 (C-T) in a highly conserved region, resulting in a cysteine instead of an arginine (R375C). Expression of the mutant cDNA showed that the R375C mutation had 0.2% of the aromatase activity of the wild-type enzyme.

Portrait *et al.* (1996) described a study on a girl with a homozygous point mutation at position 457 in exon 10 (R457X) in a highly conserved region, and the mutant protein was probably inactive.

Mullis *et al.* (1997) described the case of a female, diagnosed at 2 weeks of post-natal life. Molecular analysis showed a compound heterozygote for a point mutation /deletion, resulting in two stop codons. The point mutation was G-A at the splicing site between exon 3 and intron 3, yielding a stop codon 3 bp downstream. The other defect was a base pair deletion (C) in the Pro (P408, CCC, exon 9), which corresponded to the consensus aromatase region of the cP450 arom enzyme. A frameshift mutation occurred, resulting in a nonsense codon 111 bp (37 aa) downstream in the aromatase transcript. Carani *et al.* (1997) described the case of an adult man of consanguineous pedigree, and Ludwig *et al.* (1995) that of a girl also of consanguinous pedigree; both studies found two homozygous point mutations in exon 9, at positions 365 (G-A, R365Q) and 370 (G-A, V370M), respectively, in a highly conserved region of the cP450arom molecule. Expression studies on COS 1 cells, showed that the cP450arom activity of the R365Q mutant protein was 0.4% that of the wild-type.

The first case of an infant boy with cP450arom deficiency (consanguineous pedigree) was described by Deladoey *et al.* (1999). Molecular analysis of the CYP19 gene revealed homozygosity for a C-base pair deletion in exon 5, causing a frame shift mutation and a stop codon 21 codons downstream. The resulting protein was inactive, since functional regions of the protein were not present in the molecule.

Herrmann *et al.* (2002) described, in an adult male of consanguineous parents, a homozygous C-A (C-A transition) substitution in intron 5 at position –3 of the splicing acceptor site, before exon 6, causing a frameshift and a premature stop codon, 8 bp downstream at the end of exon 5. In the patient referred to above, the resulting peptide, if processed, would result in a non-functional cP450arom enzyme, since it lacked the functional, highly-conserved region of the protein, including the substrate-binding pocket, the electron-accepting site, and the heme-binding site.

Finally, we recently described another cP450arom deficiency case (Belgorosky *et al.*, 2003) on a neonatal girl, admitted at 7 days of age. The sequence analysis revealed a compound, heterozygous for a point mutation from G-A at the consensus 5′-splice donor sequence, a GAA- AAA mutation at c DNA position 655 in exon 5, probably resulting in a cryptic donor site, and an A base pair deletion, occurring in a Glu aa (Glu 412; GAA, exon 9), which caused a frameshift mutation and generated a stop codon 98 bp (33 aa) downstream. As in the patients above, this resulted in a prematurely terminated, non-functional protein.

AROMATASE DEFICIENCY AND SEXUAL DIFFERENTIATION

As explained above, the active aromatization of androgens protects the fetus against the virilizing action of fetal androgens. It has been known for many years that the external genitalia of the embryo and the fetus are extremely sensitive to androgens from any source. By a mechanism similar to that, which happens in congenital adrenal hyperplasia (CAH) (i.e., excessive adrenal androgens, virilizing tumors of the pregnant mother (tumor androgens), etc.) or during the administration of androgenic compounds to the mother,

the external genitalia of the female fetus can be masculinized to several degrees. In most of the described cases of female aromatase deficiency, a marked masculinization of the external genitalia has been reported: e.g., an enlarged clitoris, marked or even complete labioscrotal fusion, but non-palpable gonads. As expected, in all of these cases, the female internal genitalia differentiation was not affected.

In these newborns, the diagnosis of 46, XX ambiguous genitalia, secondary to congenital adrenal hyperplasia, which is much more frequent, should be discarded. A useful clinical fact is that, in the case of aromatase deficiency, though not in CAH, signs of virilization in the mother have been reported during pregnancy. CAH is confirmed by finding a high serum 17α-hydroxyprogesterone concentration and, in the salt losing form, a low serum sodium and high serum potassium. If CAH is discarded, other etiologies of 46, XX intersex should be considered, such as true hermaphroditism, gonadal dysgenesis, virilizing tumors of the mother, etc. Aromatase deficiency is confirmed when loss-of-function mutations of the CYP19 gene are found in the two alleles.

THE HYPOTHALAMIC PITUITARY GONADAL AXIS

As described above, three girls and one boy (Harada *et al.*, 1992; Conte *et al.*, 1994; Deladoey *et al.*, 1999; Belgorosky *et al.*, 2003) were studied during the first month of post-natal life. However, the serum pattern of basal gonadotropins during this period was reported in only one cP450arom deficient girl (Belgorosky *et al.*, 2003). In this index case, serum basal LH and FSH levels were extremely high. They increased from 8 to 26 days of age, resulting in high androgen serum levels. In the other index cases, in both sexes, serum androgen levels, in particular androstenedione, were high as well. This abnormal serum gonadotropin pattern could reflect a central change in the activity of the GnRH pulse generator, and/or an effect at the pituitary level, presumably induced by the increment of androgens and the decrement of estrogens during fetal and neonatal life. However, in the former index girl at infancy, from 2 to 6 months of age, serum basal LH levels dropped dramatically, whereas serum basal FSH levels remained clearly high; on the other hand, in the same age period, basal levels of serum androgens remained high. Even though it has been proposed that in females the low estrogen levels of infancy are active components in the restraint of FSH and LH secretion, it can be proposed that androgens could also play a negative feedback role on gonadotropin secretion at this age. In keeping with this concept, it has been reported that androgens, acting directly through the androgen receptor-mediated pathway, repress GnRH gene expression in hypothalamic GnRH-secreting neurons. At the pituitary level, a suppression of LHβ gene transcription through a direct interaction of the androgen receptor, reducing the Sp1 site in the distal GnRH responsive promoter region, has also been described. Moreover, the decrement in serum gonadotropins and the lower values measured during the second semester of life are in contrast with the increase in basal serum LH and FSH, reported in Turner's syndrome during the first year of life. In another girl, reported by Mullis *et al.* (1997), basal serum FSH levels and the FSH response to GnRH were elevated at 2 months of age, though normal basal and post GnRH serum LH levels were found. Therefore, the persistence of high basal serum FSH levels, reported so far in the two affected infant girls, suggests that a minimal amount of estrogen is required for the feedback mechanism of FSH secretion in females during infancy. However, a large inter-individual variation of serum FSH levels has recently been described in normal infant girls.

In contrast to the affected girls, basal serum FSH levels and the FSH response to GnRH were normal in the affected boys during infancy, suggesting that estrogens were not involved in the regulation of FSH secretion in boys. Therefore, these data indicate a sexual dimorphism in the regulation of FSH secretion, at least during infancy. During the first two years of life, serum FSH is relatively higher in normal girls than in normal boys. The levels of FSH and inhibins are important biomarkers of gonadal function. However, since serum inhibin B levels are considerably lower in infant girls than in infant boys, it has been proposed that inhibin B is a major contributor to the regulation of serum FSH secretion in boys. The fact that in the affected boys serum-free testosterone and androstenedione levels were very high at 2 weeks of post-natal life and that they decreased to the normal range during the first month of life, reveals a role played by cP450arom in fetal and newborn testicular function. Indeed, it has been described that cP450arom is highly expressed in fetal and neonatal human testes, compared to the testes at older prepubertal ages. Moreover, we found that during the newborn period, there was a vigorous increment in the testicular cell populations (Berensztein *et al.*, 2002). This cell mass growth seemed to be mainly mediated by decreased apoptosis. We postulated that these changes, taking place during the neonatal period, could be important in defining the testicular function in adults. The factors that could modulate this growth are not known. Since it has been described that estrogens are strong inhibitors of cell apoptosis in several tissues, we can postulate that local testicular estrogen could be one of the candidates, among others, for modulating testicular growth during the newborn period. Even though small adult testes have been described in male cP450arom deficient patients, no information is available regarding the testicular growth pattern during the newborn and infant periods, childhood, or puberty.

During childhood, in the five studied girls affected with aromatase deficiency, both basal serum and GnRH-induced FSH levels were clearly elevated, suggesting again that minimal circulating levels of estrogens (or androgens as substrates) are required, not only during infancy, but also in childhood, to restrain pituitary FSH in normal prepubertal females. Basal serum LH levels were normal in all of the reported cases. Serum LH response to GnRH was elevated during childhood. However, in the only longitudinal study of serum LH response to GnRH and serum androstenedione and testosterone levels during prepuberty (Belgorosky *et al.*, 2003), carried out on an affected girl, GnRH testing was performed at three different ages, once in early prepuberty and twice during late prepuberty, after 5.5 years of age, when a gradual increase in serum androgens was detected. This finding was in agreement with previous reports, indicating that the ovary was not hormonally quiescent during prepuberty. It was interesting to find that in early puberty, along with normal serum androgen levels, peak serum LH response to an acute GnRH test was 10 times lower than in late prepuberty. It has been shown that during prepuberty, the sex hormones of extra-gonadal origin (adrenal) or those administrated exogenously, produced not only the development of secondary sexual characteristics, but also an advance in the induction of the onset of GnRH-dependent puberty, both in boys and in girls. Therefore, as we previously proposed (Belgorosky *et al.*, 1998), it is possible to speculate that the pubertal serum LH response to acute GnRH stimulation, which was detected in this aromatase deficient girl, could have been secondary to the prolonged effect of androgens on the central nervous system; this could include, among other effects, the maturation of the GH/IGF-1axis, which in turn, could re-

sult in an irreversible maturation of the GnRH pulse generator.

Puberty was followed in two affected females: a 14.2 year-old girl (Conte *et al.*, 1994) and a 12.6 year-old girl (Morishima *et al.*, 1995). Clinical signs of virilization were found in the two patients. In the younger girl, Tanner stage 2 pubic hair, facial comedones and acne were noted, while in the older one, Tanner stage 4 pubic hair, abundant axillary hair, an increment in clitoris size, an undeveloped labio minora and an unstimulated vagina were found. There was no breast development or menarche in either case. Basal and post GnRH test serum LH and FSH levels were elevated. The serum androgen levels (testosterone and androstenedione) were clearly high, compared to normal values for the age and state of sexual development, whereas the serum estradiol levels were extremely low. Therefore, this hormonal pattern supported the concept of a main role for estrogens in the feedback mechanism of gonadotropin secretion within the hypothalamo-pituitary-gonadal axis in females. Although detailed psychosexual studies were not reported, female psychosexual orientation was observed in the two patients.

In affected males, pubertal development was reported to take place at the normal age. Three young adult patients were studied. In one of the patients (Morishima *et al.*, 1995), an enlarged testicular volume was observed, and an increment in basal serum androstenedione, testosterone, LH and FSH was found. However, in the other two patients, testis volume was slightly decreased in one subject (Hermann *et al.*, 2002) and was very small in the other (Carani *et al.*, 1997). Serum androgens and basal gonadotropins were normal or slightly increased (mainly FSH). However, a markedly elevated LH and FSH response to GnRH was found. Thus, estrogens appeared to be one of the components for normalizing the gonadal feedback of FSH and LH in adult men, similar to the report on women. The fact that the serum gonadotropin was not as high as in castrated subjects indicated that other factors, such as testosterone and inhibin B, were also involved in the regulation of the hypothalamo-pituitary-testicular axis. Unfortunately, no information is available on serum inhibin B in cP450arom deficient adult male patients. The differences reported in testes size were striking. A spermogram was evaluated on the two patients with reduced testicular volume; a decrease in motility and in the number of spermatozoa was found in both subjects. Similar data has been reported in the ArKO and EαrKO mouse models (Robertson *et al.*, 1999; Eddy *et al.*, 1996), indicating that estrogens are necessary for fertility in this species, as well as in humans. Male gender identity and psychosexual orientation were observed in all of the affected male patients described so far.

OVARIAN CYST FORMATION

Large hemorrhagic cystic ovarian follicles have been described in human aromatase deficient girls, as well as in ArKO mice, not only in childhood, but also during infancy (Britt *et al.*, 2000). It has been proposed that chronic exposure to abnormally high LH levels could stimulate ovarian cyst development. However, female mice, homozygous for a targeted disruption of the FSH beta subunit gene, exhibited an approximate 5-fold increase in serum LH, but did not develop enlarged cystic follicles in the ovary, suggesting that FSH played a role in this process, as well. Weil *et al.* (1999) showed that testosterone increased follicular FSH receptors, and it has been suggested that androgens could promote follicular growth by amplifying the FSH effect. It is possible that this could partially explain the enhanced responsiveness to gonadotropin stimulation noted in women with polycystic ovary syndrome. According to these data, we can speculate that, in aromatase deficient

prepubertal patients, an amplification of FSH signaling could occur in the presence of high intra-ovarian androgen production and that this mechanism could be involved in the development of ovarian follicular cysts.

In one pubertal girl, a large bilateral cyst mass was observed in a pelvic ultrasonography. After abdominal exploration, several multi-lobulated cystic masses were found in the two ovaries, confirming that large follicular cysts can be observed from infancy to adulthood in cP450arom deficient patients. It is remarkable that the histopathology of the cystic masses of this patient was compatible with the diagnosis of polycystic ovary syndrome.

GROWTH, SKELETAL MATURATION AND BONE MASS

Longitudinal bone growth results from the expansion of the growth plate cartilage by regional chondrocyte proliferation, hypertrophy, and the secretion of the extra-cellular matrix. The extra-cellular matrix produced by chondrocytes calcifies and is replaced by bone during the process of endochondral ossification. The epiphyseal fusion of long bones is the result of a progressive decrease in cartilage expansion. Thus, when the vascular invasion exceeds cartilage expansion, there is a progressive thinning of the growth plate. This mechanism is mediated in large part by the action of estrogen during puberty. In this regard, aromatase transcripts and the cP450arom protein have been detected in the human growth plate. In addition, it is also known that estrogen receptors are expressed in chondrocytes. These data support the concept that local estrogens play a prominent role in the control of long bone growth and growth plate maturation (Turner *et al.*, 1994).

In both sexes, bone mass clearly increases during puberty through a combination of increased bone length, bone diameter, cortical bone width, and cancellous bone mass. It has been proposed that these effects are modulated by sex steroids and other hormonal changes, which take place during puberty. Cortical bone mass could continue to increase after final height is achieved (Turner *et al.*, 1994).

In one estrogen receptor α affected adult male and in three cP450arom deficient adult males, tall stature, unfused epiphyses, osteopenia, eunuchoid skeletal proportions, and genu valgum were observed. Pubertal height velocity was apparently constant, and tall stature was the consequence of a lack of epiphyseal fusion. These cases illustrated the essential role of estrogens in skeletal development and in the growth spurt in males. Furthermore, in 46, XY phenotypic females with complete androgen insensitivity, the age of peak height velocity, the pattern of skeletal maturation, and the timing of epiphyseal fusion were comparable to that of normal females. On the other hand, while aromatase inhibitors decrease rapid growth and skeletal maturation, anti-androgens do not affect skeletal maturation, strongly supporting the essential role of estrogens on the pubertal growth spurt, skeletal maturation, and body proportions. During childhood, even though normal growth was observed in the three affected girls, a delayed bone age was also found, indicating the critical role of estrogen on skeletal maturation during prepubertal years. However, information on the role of estrogen on bone mineral acquisition is scarce. In the Mullis *et al.* case (1997), low bone mineral density (BMD) was found, whereas in a case recently reported by us, on a 7-year-old girl, BMD was normal. Further information is required to clarify this important issue during prepuberty in children of both sexes.

ESTROGEN TREATMENT

In pubertal females with cP450arom defi-

ciency (Conte *et al.*, 1994; Morishima *et al.*, 1995), daily estrogen replacement therapy, associated with a progesterone compound, not only resulted in breast development, menarche, growth spurt and fused epiphyses, but it also resulted in decreases in the plasma concentrations of gonadotropins and androgens and in the regression of ovarian cysts. Thus, treatment confirmed the critical role of estrogens on the hypothalamo-pituitary-ovarian axis and on bone skeletal maturation. Even though there is no information regarding the bone mineral mass of affected females on estrogen replacement therapy, it is logical to assume that estrogen treatment could be important to improve bone mineral accretion.

The indication for estrogen treatment during the infancy and childhood of affected female patients is less clear. As mentioned above, estrogen replacement therapy at puberty arrests the development of ovarian cysts and could also contribute to an increased BMD. Mullis *et al.* (1997), reported that low doses of estradiol (0.4 mg/day), given to a 3 year-old affected girl for 50 days, resulted in the normalization of serum gonadotropins, the regression of her enlarged ovaries and an improvement in BMD. However, plasma FSH returned to pre-treatment levels, and ovarian size increased shortly after the cessation of estrogens. Although the ovary is not quiescent during the prepubertal period and a mini-puberty has recently been described during the first three months of age in normal girls, there has not been much experience with regards to the side effects of a chronic, low dose administration of estradiol during early prepuberty. Therefore, in order to prevent the risk of torsion of the enlarged ovaries and ovarian cysts during infancy and early childhood, long-term treatment with a GnRH analogue could be considered as a therapeutic option. However, the consequences of a lack of estrogens during infancy and early childhood on BMD are not clearly established.

In affected adult males, the administration of low doses of estrogens inhibited gonadotropin secretion, as well as serum testosterone levels, supporting the idea that estrogens play a role in the regulation of the hypothalamo-pituitary-testicular axis. However, sperm quality and count remained abnormal. This lack of response could have been, in part, secondary to a low intratesticular concentration of testosterone, in turn, secondary to the inhibition of serum LH. On the other hand, lower doses of estrogens (transdermal estradiol 12.5 mg, twice weekly) were indicated for 6 months and normalized serum testosterone, as well as the serum LH and FSH in one case (Hermann *et al.*, 2002). However, sperm studies were not performed in that case. Even though the role of estrogens on testicular development and function is not clearly understood, an irreversible alteration of testicular function cannot be ruled out, due to a lack of intra-testicular estrogens at a critical period of testicular development, such as during fetal or neonatal life.

INSULIN SENSITIVITY AND LIPID METABOLISM

Insulin resistance (Morishima *et al.*, 1995; Hermann *et al.*, 2002) and lipid profile (Carani *et al.*, 1997), as well as some clinical features, similar to the metabolic syndrome, have been described in adult male cP450arom deficient patients. However, studies on insulin sensitivity and lipid profiles have not been performed on adult affected females or on children of either sex.

To date, a causal relationship between estrogen deficiency and carbohydrate metabolism has yet to be demonstrated. Although the abnormal lipid pattern could be related in part to a direct effect of androgens, it has been reported that this abnormal lipid pattern could be improved through estrogen treatment (Hermann *et al.*, 2002).

CONCLUSIONS

Patients with aromatase deficiency represent another "experiment of nature" that has proven to be a fertile source of knowledge for estrogen physiology and pathology. In particular, studies on these patients have demonstrated the role of estrogens on bone maturation, gonadotropin modulation in both sexes, ovarian cyst formation, and testicular function, and these data have been reinforced by the generation of aromatase and estrogen receptor knock-out mice.

From the point of view of the clinical diagnosis, it is important to consider this deficiency in the differential diagnosis of 46, XX patients with ambiguous genitalia and in the association of clinical signs of virilization and a lack of breast development at puberty. Moreover, the finding of delayed bone age, associated with high serum androgen levels, is highly suggestive of some degree of aromatase deficiency during childhood and the early pubertal years. This diagnosis should also be considered in men with excessive postpubertal growth and infertility.

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