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Aromatase Deficiency in a Female Who Is Compound Heterozygote for Two New Point Mutations in the $P450_{arom}$ Gene: Impact of Estrogens on Hypergonadotropic Hypogonadism, Multicystic Ovaries, and Bone Densitometry in Childhood*

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ABSTRACT

We report on a female who is compound heterozygote for two new point mutations in the CYP19 gene. The allele inherited from her mother presented a base pair deletion (C) occurring at P408 (CCC, exon 9), causing a frameshift that results in a nonsense codon 111 bp (37 aa) further down in the CYP19 gene. The allele inherited from her father showed a point mutation from G->A at the splicing point (canonical GT to mutational AT) between exon and intron 3. This mutation ignores the splice site and a stop codon 3 bp downstream occurs.

Aromatase deficiency was already suspected because of the marked virilization occurring prepartum in the mother, and the diagnosis was confirmed shortly after birth. Extremely low levels of serum estrogens were found in contrast to high levels of androgens. Ultrasonographic follow-up studies revealed persistently enlarged ovaries (19.5–22 mL) during early childhood (2 to 4 yr) which contained numerous large cysts up to 4.8 × 3.7 cm and normal-appearing large tertiary follicles already at the age of 2 yr. In addition, both basal and GnRH-induced FSH levels remained consistently strikingly elevated. Low-dose estradiol (E_2) (0.4 mg/day) given for 50 days at the age of 3 9/12 yr resulted in normalization of serum gonadotropin levels, regression of ovarian size, and increase of whole body and lumbar spine (L1-L4) bone mineral density. The FSH concentration and ovarian size returned to pretreatment levels shortly (150 days) after cessation of E_2 therapy. Therefore, we recommend that affected females be treated with low-dose E_2 in amounts sufficient to result in physiological prepubertal E_2 concentrations using an ultrasensitive estrogen assay. However, E_2 replacement needs to be adjusted throughout childhood and puberty to ensure normal skeletal maturation and adequate adolescent growth spurt, normal accretion of bone mineral density, and, at the appropriate age, female secondary sex maturation. (*J Clin Endocrinol Metab* **82:**1739–1745, 1997)

AROMATASE ($P450_{arom}$) catalyses the conversion of androgens to estrogens, which is a key step in estrogen biosynthesis. $P450_{arom}$ is encoded by the CYP19 gene localized on chromosome 15p21.1 and belongs to the cytochrome P-450 superfamily (1). The enzyme is mainly located in the endoplasmatic reticulum of estrogen-reproducing cells such as the ovary and placenta (2, 3), and additionally, is widely expressed in other tissues including Leydig cells of the testis (4), brain (5), adipose tissue (6), liver (7), muscle (8), and hair follicles (9).

Aromatization of fetal adrenal androgens is essential for production of estrogens during pregnancy by the human placenta (10). Therefore, a placental defect in aromatization results in low estrogen production during pregnancy. During gestation, placental $P450_{arom}$ converts androstenedione (Δ^4A) and testosterone (T) derived from fetal and maternal adrenal dehydroepiandrosterone sulfate (DHEA-S) to estrone (E_1) and estradiol (E_2), whereas 16-α OH-androstenedione (16-OH-Δ^4A) derived from fetal 16-α OH-dehydroepiandrosterone sulfate (16-OH-DHEA-S) is converted to estriol (E_3) (10). Thus, the principal products of placental aromatase activity are E_3, E_1, and E_2 (10).

Aromatase deficiencies are rare, and five well-documented cases have been described so far (11–17). The first reported female was homozygous for a consensus 5′-splice donor sequence mutation from GT-GC, resulting in the use of a cryptic splice donor site further downstream in intron 6 and, therefore, in a protein with an insert of 87 bp encoding 29 amino acids (12). By transient expression in COS-7 cells, the aromatase complementary DNA of this patient was found to produce a protein with traces of activity (<0.3%) (12). Ito *et al.* (13, 15) reported a compound heterozygote female patient who had two missense mutations, one at position 1303 bp (C->T; R435C) and the other at 1310 bp (G->A; C437Y) in exon 10. Assays of the expressed mutated proteins showed that R435C mutant had 1.1% the activity of the wild-type $P450_{arom}$ enzyme, whereas the C437Y mutant demonstrated no activity (15). The two siblings reported by Morishima *et al.* (16) demonstrated a single base change at bp

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1123 (C->T), a highly conserved region, that resulted in a cysteine instead of an arginine at position 375 (R375C) in exon 9. The expression of the mutant cDNA in COS-1 cells revealed an activity of 0.2% when compared with the wild type (16). Most recently, Portrat-Doyan *et al.* (17) reported a female with a homozygous mutation R457X, resulting in a stop codon in exon 10.

The present case describes the clinical entity of female pseudohermaphroditism at birth and hypergonadotropic hypogonadism secondary to total aromatase deficiency. Molecular biological studies revealed compound heterozygosity for a point mutation/deletion resulting in two stop codons. One is a one base (C) deletion occurring at P408 (CCC; exon 9), which corresponds to the aromatic consensus region of the aromatase enzyme and results in a frameshift and nonsense codon 111 bp (37 aa) downstream in the aromatase transcripts. The other is a point mutation G->A at the splicing site (canonical GT-> mutational AT) between exon 3 and intron 3 yielding a stop codon 3 bp downstream.

Materials and Methods

Plasma LH and FSH were measured by a fluorometric enzyme immunoassay using Stratus (DADE International, Miami, FL). Serum E_1, 17-β E_2, E_3, 17-αOH progesterone, free T and DHEA and DHEA-S, were determined by commercially available RIA kits (Diagnostic Systems Labs., Diagnostic Products Corp., Los Angeles, CA). The conversion factors are as follows: E_3, serum: pmol/L = 3.67 $\times$ pg/mL; E_2, serum: pmol/L = 3.67 $\times$ pg/mL; DHEA-S, serum: μmol/L = 0.0027 $\times$ ng/mL; DHEA, serum: nmol/L = 3.467 $\times$ μg/L; free T, serum: pmol/L = 3.467 $\times$ pg/mL; Δ^4A, serum: pmol/L = 0.028 $\times$ ng/dL; cortisol, serum: nmol/L = 27.6 $\times$ μg/dL; LH, plasma: IU/L: 1.0 $\times$ mIU/mL; and FSH, plasma: IU/L = 1.0 $\times$ mIU/mL.

Dual energy x-ray absorptiometry (DEXA)

Whole body and lumbar spine bone mineral density (BMD) was measured three times using DEXA (Hologic QDR 4500, Hologic, Waltham, MA) as previously described (18). To take into account the influence of vertebral growth between each measurement, the lumbar spine BMD (g/cm^2) was corrected using a simple mathematical method (19). Briefly, volume density (d) of the lumbar spine, approximately a cylinder of height (h), and diameter (A; A and h are obtained from DEXA analysis), was expressed in g/cm^3.

The equation we used was: $d = 4/\pi \times h \times BMD/A$ (19). The volume related BMD (d) was a constant value of 0.255 $\pm$ 0.015 g/cm^3 (19).

DNA isolation

Genomic DNA was isolated from peripheral leukocytes of the affected subjects and relatives, as previously described (20). The concentration of each sample was determined by measuring the optical density of the purified DNA at 260 and 280 nm.

Amplification, subcloning, and sequencing of genomic DNA

Each exon of the CYP19 gene, including the 5′-flanking regions, were amplified using the primers as shown in Table 1. Placenta-specific exon 1, exons 2–9, and their boundary intron and promoter regions were amplified by PCR of genomic DNA using a pair of Pro-1F and In-1R, In-1F and In-2R, In-2F and In-3R, In-3F and In-4R, In-4F and In-5R, In-5F and In-6R, In-6F and In-7R, In-7F and In-8R, and In-8F and In-9R, respectively. Exon 10 and the intron 9-exon 10 junctions were divided into three portions, and they were amplified by PCR using a pair of In-9F and Ex-10R1, Ex-10F1 and Ex-10R2, or Ex-10R3. The PCR was performed in a total volume of 50 μL containing 2 ng genomic DNA, 0.2 micromolar deoxynucleotide triphosphates, 50 pmol each of PCR primers, 2 U recombinant Taq DNA polymerase XL (Perkin-Elmer, Cetus, Norwalk, CT) for 38 cycles as follows: 94 C for 20 sec, 58 C for 30 sec, and 72 C for 30 sec. After an extra 10-min extension period at 72 C in the final cycle, PCR products were purified with QIAquick spin column (QIAGEN Inc., Santa Clarita, CA) and subcloned into Bluescript (Stratagene, La Jolla, CA). DNA sequences of at least 20 independent clones for each PCR product were determined by dideoxy-chain terminator method on a DNA sequencer 373A (Applied Biosystems, Foster City, CA). Possible mutation sites in exon 3-intron 3 junction and exon 9 were further confirmed by repeated DNA sequencing of at least 30 independent clones from genomes DNAs of the patient and her family members.

TABLE 1. Oligonucleotide primers used for PCR

Pro-1F	5′-AGGAAGAAGAATCTGGACAG-3′	5′ Upstream of placenta specific exon 1
In-1R	5′-CTCCAACTCCAGTTCCAACAC-3′	Intron 1
In-1F	5′-TGTACAGCACCCTCTGAAGC-3′	Intron 1
In-2R	5′-ATGATGGACCAAAATCCCAAG-3′	Intron 2
In-2F	5′-GGAGTAACACAGAACAGTTGC-3′	Intron 2
In-3R	5′-ATTGCTCACTTCATTTCAGTGG-3′	Intron 3
In-3F	5′-CTAAGAGAGCTGCCTCCTAG-3′	Intron 3
In-4R	5′-ATAGAGTCAGAGCCTGTCTC-3′	Intron 4
In-4F	5′-TGCATGATTGTGGTGTGTGCC-3′	Intron 4
In-5R	5′-GGACAGATGGTCAAGATGTG-3′	Intron 5
In-5F	5′-GCTCAACTGCTCTTTCTTGTG-3′	Intron 5
In-6R	5′-AAGTTACCTGAGAGGCCAAG-3′	Intron 6
In-6F	5′-TCGCTAGATGTCTAAACTGAG-3′	Intron 6
In-7R	5′-TTTACACACCTCTACACAGTC-3′	Intron 7
In-7F	5′-GTTGATCCTTGAGTGTCACC-3′	Intron 7
In-8R	5′-GATGCTTAGGACATAAGAAATGG-3′	Intron 8
In-8F	5′-GCTTTAATACCAATCACAGATGG-3′	Intron 8
In-9R	5′-TTCAAGAGTGGCACAATCAG-3′	Intron 9
In-9F	5′-AGAGACTGAGTGACTCTAGA-3′	Intron 9
Ex-10R1	5′-ATGCTCCAGAGTGGGTACTG-3′	Intron 10
Ex-10F1	5′-CAGACAGGTGTCTGGAACAC-3′	Intron 10
Ex-10R2	5′-ACACTAGCAGGTGGGTTTGG-3′	Intron 10
Ex-10F2	5′-CAGGCCAATGTCAGGGTACC-3′	Intron 10
Ex-10R3	5′-CCACACTAATTGAGCTAAGC-3′	Intron 10

Case report

The history of this Swiss family was unremarkable, in particular there is no evidence of consanguinity. The patient is the second child of a 28-yr-old mother. The first child, a female as well, was born after a normal full-term pregnancy and has grown uneventfully. The affected female sibling was a 3.5-kg product of a full-term pregnancy. No medications were taken during the pregnancy. At about 12 weeks, the mother began to suffer from progressive virilization, including severe acne on her face, generalized pigmentation, lowering and breaking of her voice, hypertrophy of the clitoris, and masculinizing changes in her face. At about 20 weeks gestation, she was referred to a dermatologist for treatment of her severe acne. These virilizing signs worsened progressively until delivery.

Biophysical findings excluded fetal distress, and ultrasonographic examinations demonstrated that the size and the rate of growth of the fetus as well as placenta were appropriate for gestational age. No fetal abnormalities were diagnosed. At 39 weeks and 1 day, spontaneous onset of labor occurred 1 h after the rupture of the membranes, and the baby was vaginally delivered 2 h later with an Apgar score of 8, 8, and 9 at 10 min after birth. The infant had masculine-appearing external genitalia: a greatly enlarged phallus (2.9 cm long and 0.9 cm wide; Prader V), complete fusion of posterior scrotolabial folds, rugation of the scrotolabial folds as in a scrotum, and a single meatus on the top of a phallus. Unfortunately, the parents were told by the obstetrician and midwife that the baby was a boy. Following the examination by the family's pediatrician, female pseudohermaphroditism was suspected, and a comprehensive workup was initiated.

The hormonal changes in the mother before and after delivery are reported in Table 2. Importantly, 6 months after delivery, the maternal manifestations of virilization disappeared except for her broken voice. The karyotype of the patient was 46,XX. To rule out the possibility of

TABLE 2. Hormonal changes in mother before and after delivery

Serum hormones	39 Weeks 1 day of pregnancy		2 weeks after delivery	
	Mother	Control data	Mother	Control data
E_3 (pmol/L)	**204**	55,010.0 ± 20,150	<17	590 ± 328 (28)
E_2 (pmol/L)	**1053**	61,070 ± 8,722	**85**	520 ± 70 (28)
DHEA-S (mmol/L)	**3.41**	0.9 ± 0.2 (29)	**2.6**	0.2 ± 0.1 (28)
DHEA (nmol/L)	18.6	59.5 ± 7.0 (30)	15.3	14.4 ± 5 (28/31)
Free T (pmol/L)	**31.3**	2.5 ± 0.5	**3.6**	1.2 ± 0.3
[Δ^4A] (nmol/L)	**195.6**	20.2 ± 2.3 (30)	**12.6**	4.8 ± 1.3 (11)

Control values (mean ± SD) were taken from references on right of each value; values without references are normal ranges in our laboratory. Highly abnormal values are in **bold type**. Conversion factors (SI) are given in *Materials and Methods*.

46,XX true hermaphroditism, the infant was given 1500 U human CG, im daily for 3 days. No rise in serum free T was detected. Further, she was SRY negative, and the plasma concentration of the anti-Müllerian hormone was less than the sensitivity of the assay. These observations are consistent with the absence of testicular tissue. A blood screening test for 21-hydroxylase deficiency indicated normal concentration of 17-α-OH progesterone; a urine steroid profile was normal, and no evidence for a virilizing form of congenital adrenal hyperplasia was found. Accordingly, the diagnosis of a nonadrenal form of female pseudohermaphroditism was made.

Ultrasonographic examination (21) of the patient at the age of 6 months revealed slightly enlarged ovaries (2.1 × 1.4 × 1.8 cm; 2.6 mL) and a vagina that entered a persistent urogenital sinus. The hormonal changes in the baby are shown in Table 3. The patient was reevaluated at the age of 12 months. Both basal and GnRH-induced FSH levels were strikingly elevated (Table 4). A laparoscopy at the age of 2 yr identified enlarged (3.8 × 2.7 × 3.1 cm; 15.9 mL) ovaries with several cysts, the largest of which measured 4.8 × 3.7 cm; the fallopian tubes and uterus were normal in appearance. Biopsies of both ovaries were performed; histopathological examinations demonstrated many normal-appearing large tertiary follicles with an oocyte within a cumulus oophorus projecting into the antrum (Fig. 1). The bone age rated according to the method described by Tanner *et al.* (22) (TW2 RUS) was 1$^{6}/_{12}$ yr (chronological age: 2 yr) (22).

At the age of 3½ yr she was referred to the clinic again for reevaluation of her hormonal status and assessment of bone densitometry (Tables 4–6). The bone age was delayed at 2 $^{3}/_{12}$ yrs (22). FSH remained elevated at 24.5 IU/L, and GnRH testing showed a striking rise in FSH and LH (Table 4). A repeat pelvic ultrasound examination again revealed enlarged ovaries (4.1 × 2.6 × 3.2 cm; 17 mL). Because low levels of serum E_2 are detectable (23) in prepubertal females, we chose to administer estrogens (E_2: 0.4 mg/day for 2 months; 50 days) to study the effect of low levels of serum E_2 on the hypothalamo-pituitary feedback mechanisms. Previously, informed consent for estrogen treatment was obtained from both parents. The low-dose E_2 substitution resulted in a normalization of serum gonadotropin levels (Tables 4 and 5) and regression of the enlarged ovaries on pelvic ultrasound examination (2.3 × 1.2 × 1.6 cm; 2.2 mL). BMD increased drastically, whereas bone age did not advance accordingly (2$^{6}/_{12}$ yr; chronological age 3$^{8}/_{12}$ yr) (Table 6, Fig. 2). Four months (150 days) after cessation of the low-dose estrogen therapy, the child was reassessed. Once again a rise of unstimulated and stimulated gonadotropin levels was noted, and the ovaries had increased in size (3.1 × 1.8 × 2.2 cm; 6.1 mL) (Tables 4 and 5). In contrast, at the chronological age of 4 yr bone age remained unchanged and retarded at 2$^{6}/_{12}$ yrs (22).

Results

Genetic analysis

Genomic DNA was extracted, and the individual exons and flanking regions of the CYP gene were amplified by PCR. The DNA fragments were individually subcloned into Bluescript and separately sequenced. To exclude artefacts both strands were sequenced. Comparison with the published sequence of the human CYP19 gene (24–26) revealed compound heterozygosity for a point mutation and deletion within the CYP19 gene. The allele inherited from her mother (allele 2) presented a base pair deletion (C) occurring in the Pro (P408; CCC, exon 9), which corresponds to the consensus aromatic region of the aromatase enzyme. Thereafter, a frameshift occurs resulting in a nonsense codon 111 bp (37 aa) further down in the aromatase gene (Fig. 3). The allele inherited from her father (allele 1) showed a point mutation from G->A at the splicing point (canonical GT to mutational AT) between exon and intron 3 (24–26). This mutation ignores the splice site, and a stop codon 3 bp downstream occurs. Namely, ATCAGCAA/(splicing) GT–[Ile Ser Lys/splicing–] in normal is altered to ATCAGCAA/ATGA–[Ile Ser Lys/stop] in this allele. As expected there was no active transcript found.

Hormonal studies

The total lack of aromatase activity in the patient caused severe virilization of the mother as early as the 12th week of gestation. The female patient had striking masculinization of the external genitalia at birth. Aromatase deficiency was suspected in view of the prepartum hormone levels in the mother (Table 2), and the diagnosis was confirmed after birth

TABLE 3. Hormonal changes in baby after birth

Serum hormones	Mixed cord blood		2 Weeks after birth		4 Weeks after birth	
	Patient	Control data	Patient	Control data	Patient	Control data
E_3 (pmol/L)	**160**	246,717 ± 38,517 (32)				
E_2 (pmol/L)	**630**	29,600 ± 14,700 (32)	**5**	15–275	**<5**	20–175
DHEA-S (μmol/L)	**6.5**	2.0 ± 0.3 (30)	0.7		0.5	
DHEA (nmol/L)	21.5	36.5 ± 6.1 (30)	8.5	11.9 ± 3.2 (31)	2.0	
Free T (pmol/L)	**361**	30.2 ± 1.4	<0.9	2.9 ± 1.2	<0.9	2.9 ± 1.2
[Δ^4A] (nmol/L)	**751.5**	3.3 ± 1.3 (33)			**4.6**	0.7 ± 0.2 (33)
Cortisol (nmol/L)	460	630 ± 120	159			

Control values (mean ± SD) were taken from references on right of each value; values without references are normal ranges in our laboratory. Highly abnormal values are in **bold type**. Conversion factors (SI) are given in *Materials and Methods*.

TABLE 4. Basal and stimulated (100 μg GnRH iv) plasma concentrations of LH and FSH in baby and controls

	Controls		Baby		
	Mean (SD; range)	2 months	3 6/12 yr (start E_2)	3 6/12 yr (end E_2)	4 yr (after E_2)
FSH (IU/L)					
Basal levels	4.7 (3.7; 0–12.5) (35)[a] 1.2 (0.3–2.5) (34)[b]	**52.3**	**24.5**	1.0	**21.5**
Peak levels	33.3 (20, 13.5–58.5) (35)[a] 8.3 (2.5–14) (36)[b]	**214.5**	**96.8**	**18.2**	**93.2**
LH (IU/L)					
Basal levels	5.8 (4.5; 0–13) (35)[a] 0.9 (0.3–2.0) (36)[b]	10.3	<1.0	<1.0	**2.7**
Peak levels	18.2 (11.3; 8.5–40) (35)[a] 2.7 (1.4–4.1) (36)[b]	28.7	**24.7**	**6.3**	**29.7**

Control values (mean, SD; range) were taken from references on right of each value. Highly abnormal values are in **bold type**. E_2 therapy: 0.4 mg E_2 po was given for 2 months (50 days).

[a] Normal values for infants.

[b] Normal values for females aged 2–4 yr. Conversion factors (SI) are given in *Materials and Methods*.

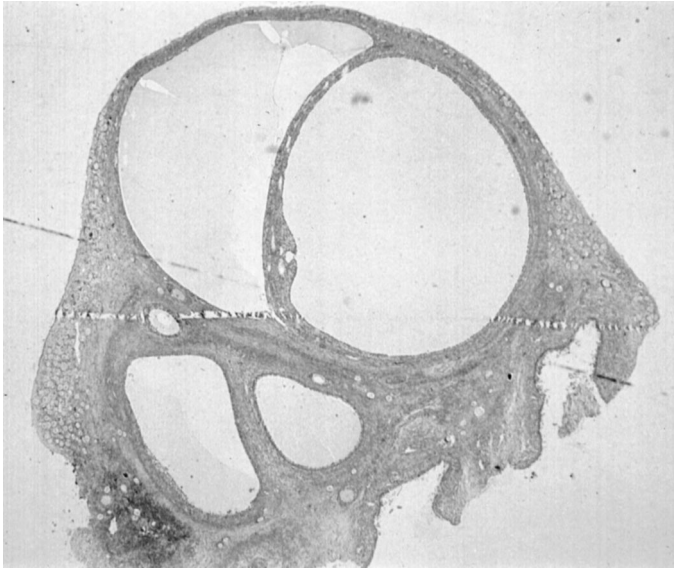

FIG. 1. Photomicrograph of biopsy of right ovary at age 2 yr. Many large primary and secondary follicles are seen. Note normal-appearing large tertiary follicles that contained an oocyte within a cumulus oophorus that projected into the antrum.

TABLE 5. Hormonal changes in patient before, on, and after 0.4 mg E_2/day

Serum hormones	3 6/12 Yr before E_2	3 8/12 Yr on E_2	4 Yr after E_2	Control data[a] (3–4 yr)
E_3 (pmol/L)	<0.02	<0.02	<0.02	
E_2 (pmol/L)	<5	19	<5	37 (<25–43)
E_1 (pmol/L)	<40	114	<40	52 (25–63)
DHEA-S (μmol/L)	<0.81	<0.8	<0.8	1.1 ± 0.4
DHEA (nmol/L)	1.6	2.4	2.3	5.98 ± 2.36
Free T (pmol/L)	<2	<2	<2	0.7–2.1
[Δ^4A] (nmol/L)	0.5	0.7	0.9	1.08 ± 0.26
Cortisol (nmol/L)	558	664	632	200–630

[a] All serum control data were taken from Ducharme and Forest (34). Conversion factors (SI) are given in *Materials and Methods*.

in the patient following hormonal analysis (Tables 2 and 3). At that time, the very low levels of estrogens were in remarkable contrast to the high levels of androgens. Results of further clinical and hormonal investigations suggested a

TABLE 6. Whole body and lumbar spine BMD

	Controls	Patient[a]		
	3.5–4 yr (mean (SD)	Day 0	Day 50	Day 200
Whole body BMD (g/cm^2)	0.682 (0.05)	0.665	0.728	0.747
Lumbar spine BMD (L1-L4; g/cm^2)	0.594 (0.029)	0.553	0.585	0.586
Lumbar spine BMD (volume) (L1-L4; g/cm^3)	0.255 (0.015)	0.212	0.245	0.242

[a] Day 0 indicates beginning of E_2 (0.4 mg/day), day 50 end of E_2 substitution, day 200 4 months later without E_2. Control data (mean, SD) are from Cochat *et al.* (19) and are identical with our own unpublished control data.

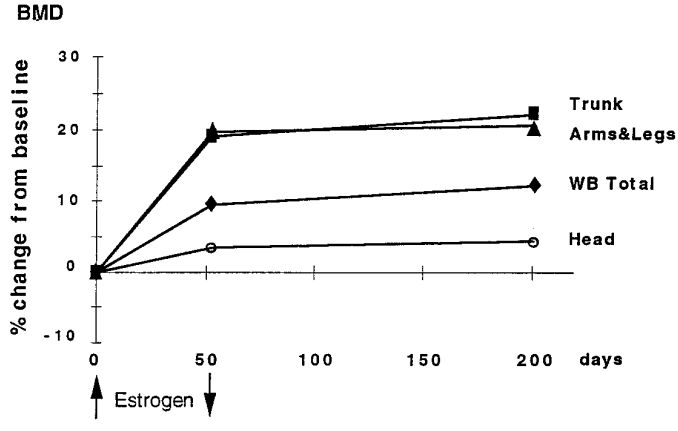

FIG. 2. Estrogen substitution caused an increase of BMD that remained unaltered after E_2 therapy was discontinued.

total absence of aromatase activity and, therefore, a generalized defect. Furthermore, ultrasonographic follow-up studies revealed persistently enlarged ovaries (19.5–22 mL) in early childhood (age: 2–4 yr), which contained normal-appearing large tertiary follicles and numerous large cysts up to 4.8 × 3.7 cm by the age of 2 yr. Both basal and GnRH-induced FSH levels were consistently strikingly elevated throughout the childhood. Low-dose E_2 (0.4 mg/day) given for 50 days resulted in normalization of serum gonadotropin levels and regression of the enlarged ovaries and ovarian cysts and in an increase of whole body

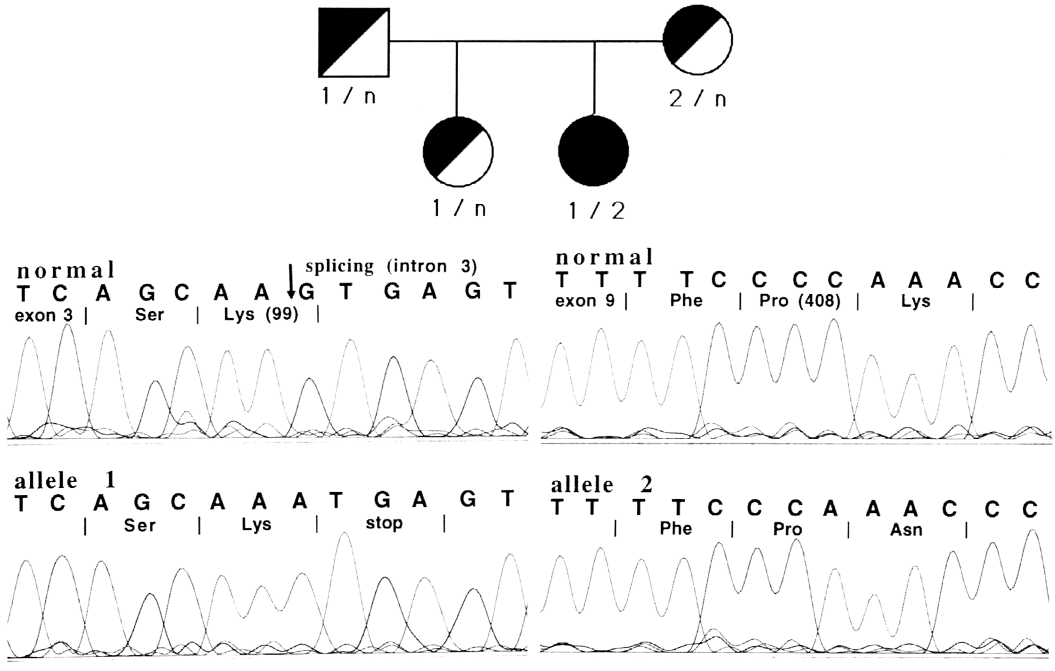

FIG. 3. Pedigree and nucleotide sequences of patient and a normal control are shown. Allele inherited from her father (allele 1) showed a point mutation from G->A at the splicing point (canonical GT to mutational AT) between exon and intron 3. This mutation ignores the splice site and a stop codon 3 bp downstream occurs. Namely, ATCAGCAA/(splicing) GT–[Ile Ser Lys/splicing–] in normal is altered to ATCAGCAA/ATGA–[Ile Ser Lys/stop] in this allele. Allele inherited from her mother (allele 2) presented a base pair deletion (C) occurring in Pro (P408; CCC, exon 9), which caused a frameshift that resulted in a nonsense codon 111 bp (37 aa) downstream in the aromatase gene.

and lumbar spine BMD. The plasma FSH returned to pretreatment levels, and ovarian size increased shortly after cessation (150 days).

BMD

Absolute values of whole body and lumbar spine BMD are given in Table 6. Figure 2 depicts the percentage change from baseline of values determined immediately before the beginning of estrogen (E_2) substitution therapy. Although the pretreatment values for whole body BMD were within the normal range for age-matched females (Table 6) (19), the lumbar spine BMD (L1-L4), when corrected for the volume (g/cm^3) to integrate the vertebral growth, was lower than in controls. Therefore, there was densitometric evidence of osteoporosis already at the age of 3 9/12 yr, which normalized with low-dose E_2 treatment (Table 6).

Discussion

The affected female is a compound heterozygote for two newly described point mutations in the CYP19 (aromatase) gene. In one allele inherited from the mother, there is 1 bp (C) at position Pro 408 deleted causing a frameshift and a stop codon 111 bp further down in the transcript. The mutation inherited from the father is a single point mutation (GT->AT) in the splice donor sequence of exon-intron 3 yielding a stop codon 3 bp downstream. Therefore, no active transcript of the aromatase gene could be found, which resulted in a female without any apparent active aromatase activity. This fact may be of importance in determining the severity of the clinical manifestation of an altered CYP19 gene in the fetus.

In the absence of $P450_{arom}$, the androgenic steroids (Δ^4A, T, and 16-α OHΔ^4A) cannot be converted to E_3, E_1, or E_2 and, therefore, large quantities of Δ^4A and T are transferred to the maternal and fetal circulation, which results in a virilization of the mother during pregnancy, as well as masculinization of the urogenital sinus and external genitalia of the female fetus.

About 1% placental aromatase activity of wild-type $P450_{arom}$ enzyme seems to be enough to prevent virilization of the mother during pregnancy (Table 7). In all affected females reported so far, the exposure to an excess of androgens resulted in a variable virilization of the mother and masculinization of the external genitalia. Interestingly, the lower the aromatase activity (Table 7), the greater was the degree of masculinization of the external genitalia at birth. Our patient had an extreme degree of female pseudohermaphroditism, and virilization of the mother began as early as 12 weeks gestation, when fetal age differentiation of the fetal genital groove is determined (27). Additionally, in contrast to previously reported affected females, we found elevated FSH levels and striking GnRH-induced gonadotropin responses not only in infancy but in childhood as well. This suggests that a minimal amount of estrogen is needed not only in infancy but also during childhood to contribute to the inhibition of FSH secretion in prepubertal females.

Recently an ultrasensitive recombinant cell bioassay for determination of E_2 was developed that enabled the measurement of low concentrations of serum E_2 in prepubertal females (23). Because prepubertal females have an 8-fold higher estrogen level than boys without any correlation with age and body mass index, we suggest that low E_2 concentrations during childhood are important and may be respon-

TABLE 7. Review of clinical features and $P450_{arom}$ activities

	Gene defect	Pregnancy (mother)	Childhood	Aromatase activity (%)
Kanazawa (11, 14)	Homozygous splice junction defect exon VI	Virilization (at 30 weeks)	Female Greatly enlarged phallus Complete fusion of scrotolabial fold Single meatus at base of phallus	0.3
San Francisco (13, 15)	Compound heterozygote for two missense mutations	No virilization	Female Phallic like structure	
	C437Y (exon X) R435C (exon X)		Rugated labia majora Posterior labial fusion	0 1.1
	R435C (exon X)		Rugated labia majora Posterior labial fusion	0 1.1
New York (16)	Homozygous for point mutation (R375C, Exon IX)	Virilization (at 5th month)	Female Phallus (2.3 cm × 0.8 cm) Complete labioscrotal fusion Single meatus at base of phallus	0.2
Lyon (17)	Homozygous point mutation (R457X, exon X)	Virilization (unknown)	Female Ambiguous genitalia, Prader IV	
Bern	Compound heterozygote exon III (splice site) and exon IX (P408)	Virilization (at 12 weeks)	Female Greatly enlarged phallus (2.9 cm × 0.9 cm) Complete fusion of scrotolabial fold Single meatus at top of phallus	0

sible for the faster rate of skeletal maturation and earlier pubertal onset in females (23). Furthermore, the absence of estrogen synthesis because of complete deficiency of aromatase activity resulted in high concentrations of basal FSH, in a brisk GnRH-induced gonadotropin response combined with highly FSH-stimulated and enlarged multicystic ovaries in childhood, and in a lumbar spine BMD lower than in age-matched controls (19). In our patient, during a period of 50 days a dose of 0.4 mg E_2/day lead to the normalization of plasma gonadotropin levels and regression of ovarian size to normal on pelvic ultrasound. After cessation of E_2 therapy, a relapse occurred, and gonadotropin and ovarian size returned to pretreatment levels. These observations suggest that low levels of circulating E_2 are essential in childhood and prepuberty as a component of the restraint of FSH and LH secretion during early childhood (27). Further, BMD increased remarkably on E_2 replacement, whereas in the subsequent 150 days without E_2 the BMD remained unchanged.

In conclusion, we suggest that in females with a complete lack of aromatase activity, a minimal dose of exogenous E_2 should be given in childhood. The dose of E_2 needs to be adjusted by the use of an ultrasensitive estrogen assay, determination of plasma gonadotropins, and pelvic ultrasonography. The aim will be to administer an adequate dose of E_2 throughout childhood and puberty to ensure a physiological skeletal maturation, a normal adolescent growth spurt, normal accretion of bone mineral, and, at appropriate age, female secondary sex maturation.

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