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Impact of Estrogen Replacement Therapy in a Male with Congenital Aromatase Deficiency Caused by a Novel Mutation in the CYP19 Gene

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Recent reports of the impact of estrogen receptor α and aromatase deficiency have shed new light on the importance of estrogen for bone formation in man. We describe a novel mutation of the CYP19 gene in a 27-yr-old homozygous male of consanguinous parents. A C to A substitution in intron V, at position −3 of the splicing acceptor site before exon VI of the CYP19 gene, is the likely cause of loss of aromatase activity. The mRNA of the patient leads to a frameshift and a premature stop codon 8 nucleotides downstream the end of exon V. Both parents were shown to be heterozygous for the same mutation. Apart from genua valga, kyphoscoliosis, and pectus carniatus, the physical examination was normal including secondary male characteristics with normal testicular size. To substitute for the deficiency, the patient was treated with 50 μg transdermal estradiol twice weekly for 3 months, followed by 25 μg twice weekly. After 6 months estrogen levels (<20 at baseline and 45 pg/ml at 6 months; normal range, 10–50) and estrone levels (17 and 34 ng/ml; normal range, 30–85) had normalized. Bone maturation progressed and the initially unfused carpal and phalangeal epiphyses began to close within 3 months and were almost completely closed after 6 months. The bone age, assessed by roentgenographic standards for bone development by Gruelich and Pyle, was 16.5 at baseline and 18–18.5 yr after 6 months of treatment. Bone density of the distal radius (left), assessed by quantitative computed tomography, increased from 52 to 83 mg/cm^3 (normal range, 120–160) and bone mineral density of the lumbar spine, assessed by dual-energy x-ray-absorptiometry, increased from 0.971 to 1.043 g/cm^2 (normal range, >1.150). Osteocalcin as a bone formation parameter increased from 13 to 52 μg/l (normal range, 24–70) and aminoterminal collagen type I telopeptide as a bone resorption parameter increased from 62.9 to 92.4 nmol/mmol creatinine (normal range, 5–54). Semen analysis revealed oligoazoospermia (17.4 million/ml; normal >20) at baseline. After 3 months of treatment, the sperm count increased (23.1 million/ml) and decreased rapidly (1.1 million/ml) during the following 3 months. The sperm motility was reduced at baseline and decreased further during treatment. Area under the curve of insulin, C-peptide, and blood glucose levels during oral glucose tolerance test decreased after 6 months (insulin: 277 *vs.* 139 μU/ml·h; C-peptide 52 *vs.* 15 ng/m·h; area under the curve glucose: 17316 *vs.* 12780 mg/d·min). Triglycerides (268 *vs.* 261 mmol/liter) and total cholesterol levels (176 *vs.* 198 mmol/liter) did not change significantly, but the low-density lipoprotein/high-density lipoprotein ratio decreased from 5.37 to 3.56 and lipoprotein (a) increased from 19.9 to 60.0 mg/dl (normal range, <30). In this rare incidence of estrogen deficiency, estrogen replacement demonstrated its importance for bone mineralization and maturation and glucose metabolism in a male carrying a novel mutation in the CYP19 gene. (*J Clin Endocrinol Metab* 87: 5476–5484, 2002)

IN A WIDE VARIETY of tissues, including testis, ovary, placenta, and adipose tissue, the aromatase cytochrome P450, as the product of the CYP19 gene, catalyzes the conversion of androgens to estrogens (1, 2). Reports of osteopenia and osteoporosis in animals and humans with gene defects in the estrogen receptor (3–5) and both in females and males with aromatase deficiency (6–9) have called attention to the importance of estrogen for skeletal maturation (10). The precise role of estrogen in human male physiology remains largely unknown, especially which effects on bone mineralization and metabolism in the male are mediated by estrogens derived from the aromatization of androgens. Many studies have demonstrated that gonadal failure in males is associated with a decrease in bone mass, but less is known about the role of genetic disorders associated with estrogen resistance or deficiency (11). First descriptions of young men affected by congenital estrogen deficiency have shed new light on the importance of estrogen for bone formation in man (7, 8, 11, 12). These findings suggest that epiphyseal closure does not develop without the action of estrogen even in males and that androgen alone is not sufficient to promote normal skeletal mineralization. Recently two mutations in the CYP19 gene in males have demonstrated the role of estrogen on bone mineralization and their effect on glucose and lipid metabolism (6–9, 13, 14).

We now present the third case of a 27-yr-old man with open epiphysis caused by a new mutation in the CYP19 gene (aromatase deficiency), the effect of estrogen replacement on bone mineralization/maturation and glucose and lipid metabolism.

Abbreviations: AP, Alkaline phosphatase; AUC, area under the curve; DEXA, dual-energy x-ray absorptiometry; DHEAS, dehydroepiandrostendione; dNTP, deoxynucleotide triphosphate; HDL, high-density lipoprotein; HOMA IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; QCT, quantitative computed tomography.

Subjects and Methods

Case report

The propositus was the only child of consanguineous parents (second cousins, Fig. 1). His mother described clinical signs of virilization during

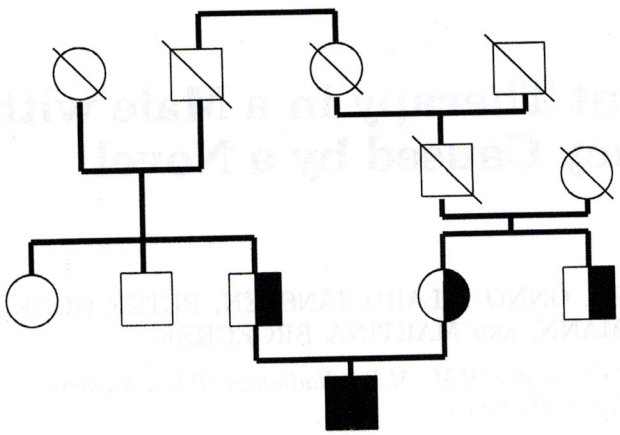

FIG. 1. Pedigree of the affected patient indicating the consanguineous marriage of his parents.

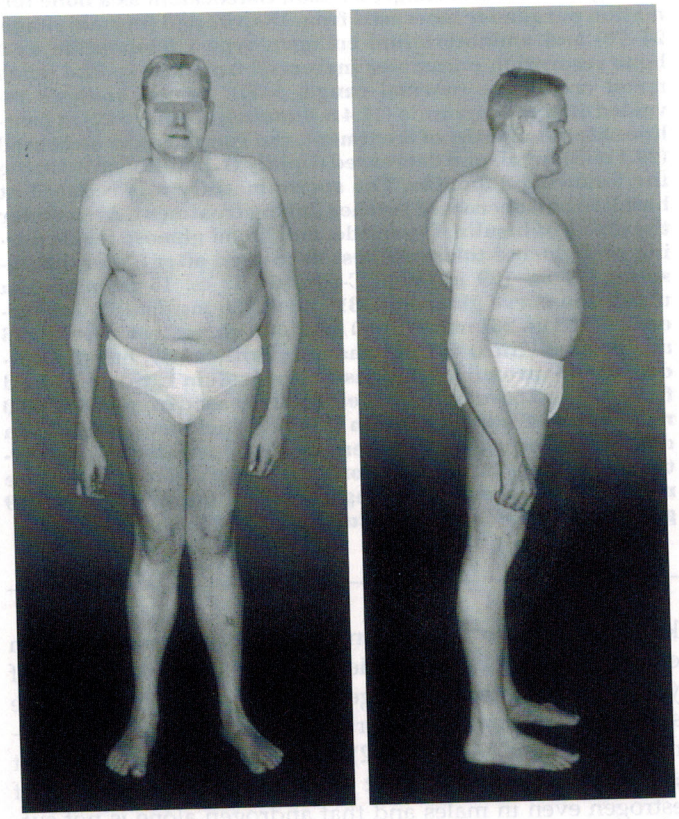

FIG. 2. The 27-yr-old male with aromatase deficiency (with permission of the patient to reproduce these pictures).

the second trimenon of pregnancy (hirsutism grade 29 of the scoring scale of Ferriman and Gallway, acne, frontal balding, and coarsening of the voice) that regressed postpartum. She was not able to produce milk post partum. The patient's childhood and pubertal development were unremarkable. At 14 yr of age he was 170 cm tall (97th percentile), and he continued to grow until the age of 24 yr. At initial presentation at our outpatient clinics, the 27-yr-old patient weighed 120 kg with eunuchoid proportions (Fig. 2), was 197 cm tall, and had an arm span of 204 cm and shoe size of 15. Physical examination was unremarkable except for bilateral genu valgum, kyphoscoliosis, abdominal striae, and pectus carniatus. Pubic and axillary hair were normal as well as the size (13 and 14 ml) and consistency of the testis. His sexual identity and psychosexual orientation as assessed by questionnaire were heterosexual, and his libido was normal. He had spontaneous erections sufficient for intercourse. He lived with his parents and his behavior was very friendly. The patient was single, had no offspring, and was employed as a civil servant. His intellectual and physical capacities were normal. His blood pressure was normal (125/80 mm Hg). His waist circumference was 124 cm and waist to hip ratio was 1.02. X-Ray of the hand revealed open epiphyses. Androstenedione (3.5 μg/liter; normal range, 1.25–3.1) and serum testosterone (9.0 ng/ml; normal range, 2.7–8.7) were elevated and estradiol (<20 pg/ml; normal range, 20–50) and estrone levels (17 ng/ml; normal range, 30–85) were diminished (Table 1). The karyotype was 46, XY. A semen analysis revealed a sperm count of less than 1 million per milliliter, with 100% immotile spermatozoa. Bone density of the distal radius (left) (52 mg/cm^3; normal range, 120–160), assessed by quantitative computed tomography (QCT), and bone mineral density of the lumbar spine (0.971 g/cm^2; normal range, >1.150) measured by dual-energy x-ray absorptiometry (DEXA) were both diminished (Table 2). Osteocalcin as a bone formation parameter was low at 13 μg/l (normal range, 24–70) and aminoterminal collagen type I telopeptide as a bone resorption parameter was elevated at 62.9 nmol/mmol creatinine (5–54). 25-Hydroxyvitamin D was low at (7 ng/ml; normal range, 12–120). Plasma glucose levels during an oral glucose tolerance test were normal but insulin resistance [homeostasis model assessment of insulin resistance (HOMA IR)] was elevated (Table 3). Triglycerides and total cholesterol were normal, but high-density lipoprotein (HDL) cholesterol was low. The patient gave his informed consent to treatment with estrogen. All examinations conformed with the declaration of Helsinki.

Amplification and direct sequencing of genomic DNA

Genomic DNA was prepared from whole blood of the patient, his parents, the sister and brother of the father, the brother of the mother (Fig. 1) and 10 normal control persons using the QIAamp DNA blood minikit (QIAGEN, Hilden, Germany). All coding exons with their flanking intron sequences of the CYP19 gene and 5′ untranslated exons were amplified using the primers described previously (15). PCR was carried out in 50-μl reactions containing 100 ng genomic DNA, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 20 pmol of each primer, 1.5 mM $MgCl_2$, and 1.2 U Taq-DNA polymerase (Roche Diagnostics, Mannheim, Germany) for 35 cycles. PCR products were analyzed by agarose gel electrophoresis, and DNA fragments were cleaned using QIA spin columns (QIAGEN).

DNA sequences of both the sense and antisense strands of each PCR product were determined by cycle sequencing on a DNA sequencer 310 (ABI PE Applied Biosystems, Foster City, CA) and compared with published sequences of the human CYP19 gene (16, 17).

RNA isolation and RT-PCR

RNA was isolated from peripheral leukocytes of the patient, his parents, and three normal male and female control persons by the RNA blood minikit (QIAGEN). The protocol included a DNase digestion step to eliminate traces of contaminating genomic DNA. The reverse transcription reaction was performed in a total volume of 20 μl and contained 0.5 μg total RNA, 5 mM hexanucleotides (Roche Diagnostics), 1 mM of each dNTP, 10 mM dithiothreitol, and 200 U Superscript II reverse transcriptase (Life Technologies, Inc., Karlsruhe, Germany). The reverse transcription reaction was carried out at 42 C for 60 min, followed by 6 min at 94 C to inactivate the enzyme.

Five microliters of cDNA were amplified by PCR using primers located within exons IV and VII of the human CYP19 gene (Ex4/1, 5′-tcatatttaacaacaatccagag; Ex7/2, 5′-tgtggaaatcctgcgtctttt). PCR was carried out in 50-μl reactions containing 0.2 mM of each dNTP, 20 pmol of each primer, 1.5 mM $MgCl_2$, and 1.2 U Taq-DNA polymerase (Roche Diagnostics) for 35 cycles (94 C for 30 sec, 58 C for 30 sec, and 72 C for 45 sec). PCR products were analyzed by agarose gel electrophoresis. DNA bands were eluted from the gels, purified with QUIAGEN spin columns, and sequenced as described.

Measurement of the bone mineral density

Bone mineral density was assessed by DEXA (model DPX-L; Lunar Corp., Madison, WI). Analysis was performed using software version 1.31 (DPX-L, Hanheim, Germany). The T-score was defined as the de-

TABLE 1. Changes in plasma hormone levels during estradiol replacement therapy in the propositus

	Baseline	3 months	6 months	Normal
Estradiol (pg/ml)	<20	27	45	20–50
Estrone (ng/ml)	17	31	34	30–85
17-OH-progesterone (μg/liter)	1.9	0.27	0.15	0.3–2.0
Testosterone (ng/dl)	899	519	107	270–870
Androstenedione (μg/liter)	3.5	1.7	1.5	1.25–3.1
DHEAS (μg/liter)	1.9	0.27	0.15	0.3–2.0
SHBG (nmol/liter)	12	17	18	15–70
LH basal (mIU/ml)	6.0	2.1	1.9	2–10
LH 30 min after GnRH (mIU/ml)	39.0	20.1	12.8	(3-fold of the basal value)
FSH (mIU/ml)	11.0	1.8	1.2	1–7
FSH 30 min after GnRH (mIU/ml)	18.5	4.8	2.4	(2-fold of the basal value)
GH (μg/liter)	<0.5	<0.5	<0.5	<5
IGF-I (μg/liter)	156	160	144	122–400
Cortisol, serum 0800 h (μg/dl)	14.6	8.2	6.7	6.52–23.2

TABLE 2. Changes in bone metabolism during estradiol replacement therapy in the propositus

	Baseline	3 months	6 months	Normal
Bone density (QCT) of the distal radius (left) (mg/cm^3)	52	66	83	120–160
Lumbar spine (DEXA)				
BMD (g/cm^2)	0.971	0.996	1.043	>1.150
T-score	−2.24	−1.74	−1.64	<−1.0
Z-score	−2.98	−2.47	−2.37	<−1.0
Ward triangle (DEXA)				
BMD (g/cm^2)	n.d.	0.575	0.584	>1.020
T-score	n.d.	−2.96	−2.89	<−1.0
Z-score	n.d.	−3.56	−3.49	<−1.0
Calcium (mg/dl)	9.4	9.4	9.4	8.5–10.5
Phosphorous (mg/dl)	3.5	3.4	3.5	2.5–4.5
PTH intact (pg/ml)	32	48	46	12–72
25-Hydroxyvitamin D (ng/ml)	7	6.4	14.8	12–120
Osteocalcin (μg/liter)	13	n.d.	52	24–70
AP (U/liter)	217	285	195	60–180
Bone-specific AP (U/liter)	61	87	65	10–26
NT_X (nmol/nmol creatine)	62.9	82.9	92.4	5–54

BMD, Bone mineral density; NT_X, aminoterminal collagen type I telopeptide; n.d., not done.

TABLE 3. Changes in glucose metabolism and lipid parameter during estrogen therapy in the propositus

	Baseline	3 months	6 months	SI (normal range)
AUC glucose (mg/dl·min)	17316	13770	12780	
AUC insulin (μU/ml·h)	277	209	139	
AUC C-peptide (ng/ml·h)	52	17	15	
HOMA IR	3.6	3.5	3.5	(<2.4)
Triglycerides (mg/dl)	268	195	261	
Total cholesterol (mg/dl)	176	167	198	
LDL-cholesterol (mg/dl)	112	120	110	
HDL-cholesterol (mg/dl)	21	26	31	
LDL/HDL	5.37	4.66	3.56	
Lipoprotein a (mg/dl)	19.9	39.3	60	<30
Apolipoprotein A1 (mg/dl)	81.6	97.6	126.7	115–190
Apolipoprotein B (mg/dl)	101	102	112.3	60–160

viation from the mean bone density of healthy young adults of the same sex and ethnicity. The Z-score was defined from the mean bone density of adults of the same age and sex. Forearm measurement of the distal radius (left) was performed by QCT (model Stractec XCT-900, pQCT; Stratec Medizintechnik GmbH, Pforzheim, Germany).

Biochemical measurements

Blood samples were obtained by venipuncture after an overnight fast. Estradiol was determined by a competitive immunoassay (DPC Biermann GmbH, Bad Nauheim, Germany), estrone, and androstenedione by a RIA (Diagnostic Systems Laboratories GmbH, Sinsheim, Germany), 17-OH-progesterone by a RIA (Biosource Technologies, Inc. GmbH, Solingen, Germany), and dehydroepiandrostendione (DHEAS) by an electroluminescent method (DPC Biermann GmbH). Cortisol, PRL, testosterone, LH, and FSH were measured by a chemiluminescence method (Bayer Corp. Vital GmbH, Fernwald, Germany).

GH was determined by a chemiluminescence immunometric assay (Nichols Institute Diagnostics GmbH, Bad Nauheim, Germany) with an intra- and interassay coefficients of variation for a low point of the standard curve were 5.4% and 7.9%, respectively. IGF-I concentrations were measured by an immunoradiometric assay (Nichols Institute Diagnostics) with intra- and interassay coefficients of variation for low IGF-I concentrations of 2.4% and 5.2%, respectively.

PTH was determined by a chemiluminescent immunometric assay (DPC Biermann GmbH), 25-hydroxyvitamin D by a RIA (Byk-Sangtec Diagnostica GmbH, Dietzenbach, Germany), osteocalcin by a RIA (Brahms Diag-

nostica GmbH, Berlin, Germany), bone-specific alkaline phosphatase (AP) by an enzymatic luminescent method (Metra Biosystems GmbH, Osnabrueck, Germany), and the aminoterminal collagen type I telopeptide by an ELISA (Ortho-Clinical Diagnostics GmbH, Neckargemuend, Germany).

The HOMA IR [(fasting insulin microunits per milliliter × fasting plasma glucose millimoles per liter)/22.5] (18–20) as a well-accepted parameter to determine insulin resistance was performed before and after treatment of estrogen.

Results

Genetic analysis

All individual exons and flanking intron sequences of the human CYP19 gene of the patient and his parents were amplified, and both strands were sequenced. Comparison with published sequences of the human CYP19 gene (16, 17, 21) revealed a homozygous mutation of base −3 at the splicing acceptor site of exon VI (C to A transition) in the patient's genomic DNA (Fig. 3). Both parents were found to be heterozygous for this mutation (Fig. 3).

In addition, exon VI and flanking intron DNA fragments of three siblings of the patient's parents were sequenced. The brother of the mother was found to carry the same mutation on one allele, and both the sister and brother of the father had two normal alleles. As expected, all 10 controls also showed two normal alleles.

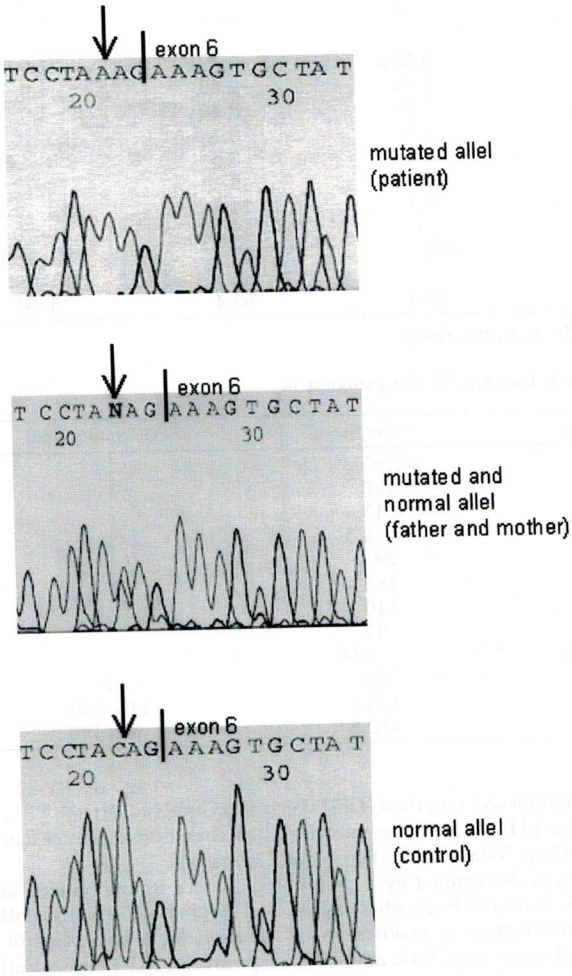

FIG. 3. Nucleotide sequence of the DNA of exon VI and the flanking regions of intron V. Genomic DNA was amplified by PCR, purified, and directly sequenced as described in *Subjects and Methods*. A homozygous mutation of base −3 at the splicing acceptor site of exon VI (C to A transition) was detected in the patient's genomic DNA (*top*). Both parents were found to be heterozygous for the mutation.

Analysis of CYP19 mRNA

For further analysis of the mutation at the 3′-splicing acceptor site between intron V and exon VI of the CYP19 gene, RT-PCR of peripheral blood leukocyte mRNA of the patient, his parents and three male and female control persons was performed. All samples contained small amounts of CYP19 mRNA that were sufficient for RT-PCR amplification. Gel electrophoretic analysis and sequencing of a 410-bp fragment between exon IV and VII revealed the normal cDNA sequence in all control persons. In contrast, PCR of the patient's cDNA yielded a single PCR fragment of only 295 bp (Fig. 4). Sequence analysis showed that it consisted of exons IV, V, and VII of the CYP19 gene, with exon VI completely excised (Fig. 5). The mRNA of the patient leads to a frameshift and a premature stop codon 8 nucleotides downstream the end of exon V (Fig. 5). The heterozygous parents of the patient both displayed two RT-PCR bands corresponding to the normal and the mutant allele (Figs. 4 and 5).

Pedigree analysis

Pedigree analysis of the consanguinous family with a history of consanguinity is consistent with an autosomal recessive mode of inheritance, as previously reported (8, 22–24). The paternal grandfather, maternal grandfather, and maternal great-grandmother of the propositus appear to have been heterozygous carriers of the mutation (Fig. 1).

Treatment

At initial presentation, the patient had elevated testosterone and androstenedione levels, undetectable estradiol levels, and open epiphyses. His bone age at the hand and wrist was 16.5 yr. The patient was treated with 50 μg transdermal estradiol (Estraderm TTS, Novartis Pharma GmbH, Nürn-

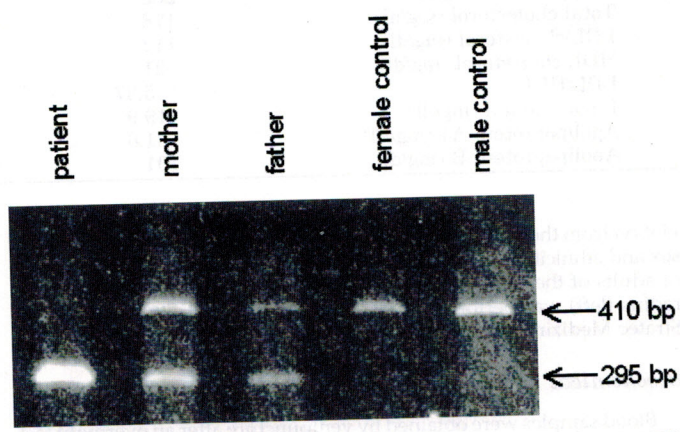

FIG. 4. Allele-specific PCR of CYP19 cDNA fragments (exons IV–VII) from peripheral blood leukocytes of the patient, his parents, and two control persons. The patient's cDNA yielded a single PCR fragment of 295 bp, and control cDNA displayed a PCR fragment of 410 bp. The heterozygous parents' cDNA yielded both fragments.

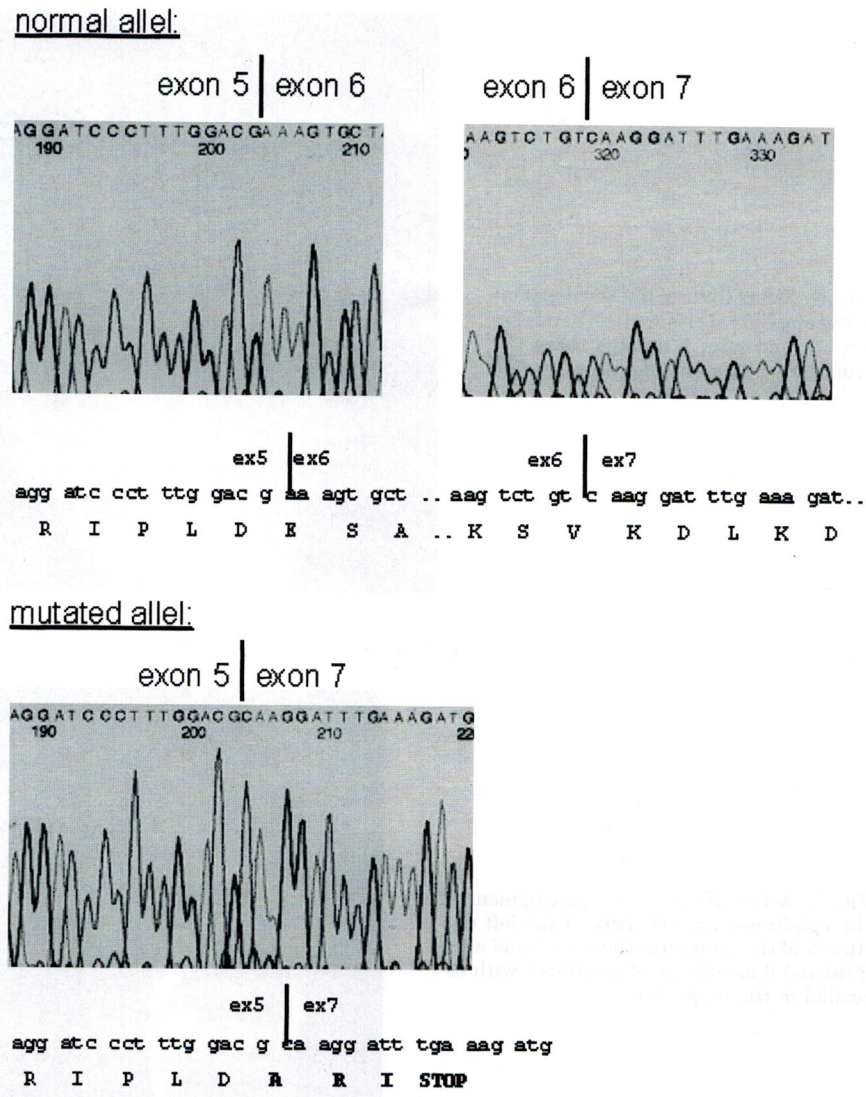

FIG. 5. Partial nucleotide sequences of CYP19 cDNA fragments (exons IV–VII) and corresponding amino acid sequences. The 410-bp fragment corresponds to the normal allele (*top*), and the 295-bp fragment corresponds to a shorter mRNA with excision of exon VI. The mutation leads to a frameshift and a premature stop codon after 8 nucleotides (*bottom*).

berg, Germany) twice weekly (0.83 μg/kg·wk) for 3 months. In concordance with the recommendations of Rochira *et al.* (9), the dosage was then reduced to 25 μg estradiol twice weekly (0.42 μg/kg·wk). Estradiol (undetectable before treatment) and estrone levels increased, and testosterone, androstenedione, and DHEAS decreased after treatment (Table 1). The markedly elevated LH response to a GnRH bolus (100 μg iv) decreased after 3 and 6 months (Table 1). The elevated FSH levels, both basal and GnRH stimulated, also decreased after 3 and 6 months of estrogen treatment.

Bone mineral density of the lumbar spine and femoral neck (ward triangle) and the T-score increased with estradiol treatment (Table 2). The initially unfused epiphyses began to close after 3 months and were almost complete closed after 6 months (Figs. 6 and 7). The bone age, assessed by roentgenographic standards for bone development by Gruelich and Pyle, was 16.5 yr at baseline and 18–18.5 yr after 6 months of treatment.

Biochemical markers of bone turnover (osteocalcin, AP, bone-specific AP, and the aminoterminal collagen type I telopeptide) rose after treatment with normalization of AP and bone-specific AP after 6 months (Table 2). Serum calcium and phosphorous did not change significantly during treatment.

Plasma glucose levels during oral glucose tolerance test before treatment with estradiol were normal, but the area under the curve (AUC) of glucose, insulin, and C-peptide levels decreased after 3 and 6 months of treatment (Table 3 and Fig. 5). The HOMA IR was elevated before (3.6) and after (3.5) treatment with estrogen indicating modest insulin resistance (normal value, <2.4). Triglycerides, total cholesterol, and low-density lipoprotein (LDL) cholesterol did not change significantly, but HDL cholesterol increased after 6 months of treatment. Consequently, the ratio of LDL/HDL decreased after treatment (5.37 and 3.56, respectively; Table 3). Lipoprotein (a) increased after 6 months from 19.9 to 60.0 mg/dl (normal range, <30) and apolipoprotein A1 increased from 81.6 to 126.7 mg/dl (normal range, 115–190). Body mass index (30.9 kg/m^2), body length, and waist to hip ratio did not change during treatment.

Treatment with estradiol did not induce gynecomastia, hyperprolactinemia (PRL levels 0, 3, and 6 months: 6.2, 6.5,

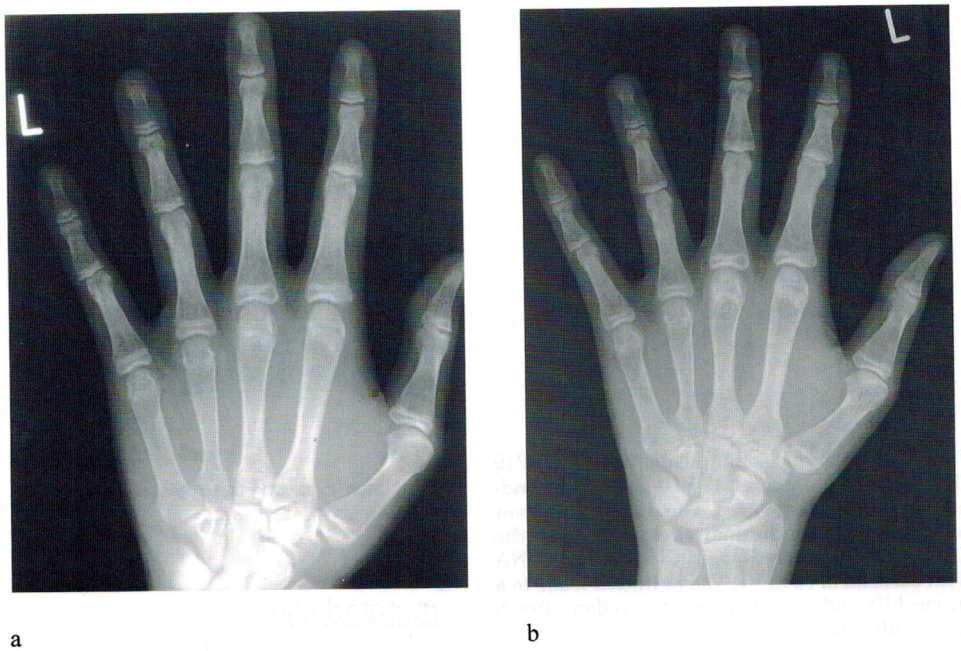

FIG. 6. X-Ray films of the development of the epiphyseal closure (left hand) before (a) and after 6 months (b) of the propositus of treatment with estradiol.

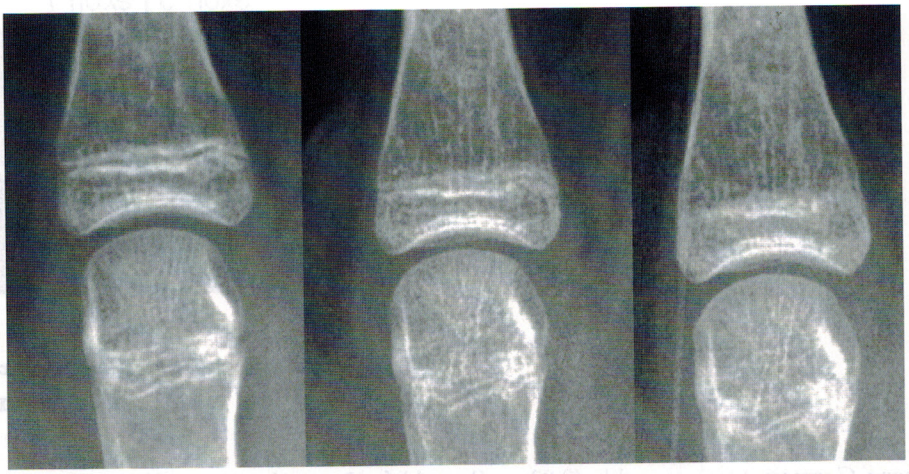

FIG. 7. X-Ray films of the development of the epiphyseal closure (base of the left digitus IV of the propositus) before (a) and after 3 (b) and 6 months (c) of treatment with estradiol in the propositus.

and 6.5 ng/ml; normal range, <20), or sexual dysfunction. Testicular volume decreased (right, 14 ml before *vs.* 10 ml after 6 months of estradiol treatment; left, 13 ml *vs.* 9 ml). Semen analysis revealed oligoazoospermia with a sperm count of 17.4 million/ml (normal, >20 million/ml) and a sperm vitality of 55% (normal, >50%) at baseline (Table 4). After 3 months of treatment, the sperm count increased (23.1 million/ml) and decreased rapidly (1.1 million/ml) during the following 3 months of treatment with a reduced dosage of estradiol (25 μg twice weekly). All sperm analyses were performed after at least 4 d of sexual abstinence. The sperm motility was reduced at baseline and decreased thereafter.

Discussion

New tools to examine the role of estrogen in human male physiology became available when the first two descriptions of young men affected with congenital estrogen deficiency were reported (7, 8).

The propositus in our study was affected by an aromatase deficiency caused by a novel homozygous splicing junction defect, *i.e.* a mutation of base −3 at the splicing acceptor consensus site of exon VI of the CYP19 gene (C to A transition). Both parents, who were consanguineous as in the two previously described cases (7, 8), were shown to carry the mutation heterozygously. Because the propositus mutation was not part of the 3′ acceptor splice site consensus sequence $(Y)_{11}$NYAGA of eucaryotic genes, RT-PCR of his CYP19 mRNA was performed. The mutation was shown to disrupt the splicing acceptor site of exon VI, resulting in its excision from the mature mRNA. As a consequence, a frameshift results in a premature stop codon 8 bp downstream the end of exon V. The resulting peptide most likely will not be

TABLE 4. Results of semen analysis during estrogen therapy in the propositus

	Baseline	3 months	6 months	SI (normal range)
Volume (ml)	4	3	1.5	ml
Sperm count (million/ml)	17.4	23.1	1.11	>20
Sperm vitality (%)	55	45	70	>50
Sperm motility (WHO A+B after 1 h) (%)	10	5	0	>25
Sperm morphology (normal) (%)	46	48	10	>30

processed, but even if it were, it would not result in a functional aromatase because it lacks the substrate-binding pocket (I-helix), the electron-accepting site, and the heme-binding site (1).

Although most of the mutations of the CYP19 gene described so far are missense mutation in highly conserved regions that cause single amino acid substitutions in regions of the protein critical for its activity (11). Some of these mutations were studied in expression systems. The new mutation described here disrupts an intron-exon splice junction and leads to a premature stop codon. Two other mutations affecting mRNA splicing of the CYP19 gene have been described so far: Harada and Yamada (17) and Harada (21) described a homozygous point mutation at the donor site of exon VI in a female that leads to an in-frame incorporation of an 87-bp insert into the coding region and an aromatase enzyme with additional 29 amino acids. Another female patient was found to be compound heterozygous for a base pair deletion in exon IX and a point mutation at the splicing donor consensus site of exon III (15). However, in both studies, only DNA analyses were performed, and mRNA analysis to directly demonstrate their effect on transcription were not performed.

The absence of active aromatase leads to the transfer of large amounts of androstenedione and testosterone to the fetal and maternal circulation because these androgenic steroids are not converted to estrone, estradiol, and estriol. The virilization of the mother during pregnancy is a result of elevated androgen levels. This contrasts to defects of the estrogen receptor with similar defects in male children but no virilization of the mother (11). Elevated serum levels of FSH and the marked GnRH stimulation at baseline despite the strikingly high circulating testosterone levels suggest that estradiol levels have an important feedback role on the gonadotropins in the male (25, 26). These observations are consistent with previous studies in which estradiol has been shown to inhibit LH secretion by decreasing LH pulse amplitude and LH responsiveness to GnRH (27–29). Moreover, normalization of estradiol levels can reduce basal gonadotropin levels despite low or reduced testosterone levels (30). In addition, administration of a selective aromatase inhibitor, anastrozole, or antiestrogens to healthy men result in an increase in LH pulse frequency and responsiveness to GnRH (31–33). In summary, estradiol has dual sites of negative feedback, acting at the hypothalamus to decrease GnRH pulse frequency and at the pituitary to decrease responsiveness to GnRH (33, 34).

Spermatogenesis has been known for many years to be regulated by FSH and androgens. In recent years evidence from animal models, including transgenic mice, suggests that estrogens may play an important role for spermatogenesis (35–40). The present case could provide essential information about spermatogenesis and fertility because only one of the known adult males with aromatase deficiency has had a semen analysis (7). However, the azoospermia of this patient (7) may be due to a second genetic disorder, because he had a brother with normal CYP19 gene who also was infertile and had azoospermia. Unlike the estrogen-resistant male with normal sperm count but a reduced vitality of 18% (3), our patient had an oligoazoospermia with a normal vitality of 55% (normal, >50%). Furthermore, the sperm motility was reduced but the sperm morphology was normal (Table 4). Sperm analysis after 3 months of treatment with estradiol (normal testosterone and estradiol serum levels) revealed an increase of sperm count but a further decrease of sperm motility. After 6 months of treatment with estradiol (low testosterone and normal estradiol serum levels), the sperm count as well as the number of normal sperm morphology and motility decreased, probably because of low testosterone levels. Whether normalization of estradiol levels with maintenance of normal testosterone levels in males may induce spermatogenesis and prevent further spermatogenic damage needs future investigation.

The tall stature of the patient was the consequence of the prolonged growth and delayed skeletal maturation. This also explains the discordance between linear growth and skeletal maturation because of estrogen deficiency despite elevated circulating androgens. The observation of the improvement in bone mass in our patient after estradiol replacement therapy is remarkable, considering the fact that administration of estrogen reduced the androgen levels. Thus, distinct levels of estrogens may also be important to achieve normal peak bone mass in males. The high bone turnover, verified by absorption and resorption parameters, showed that estrogen controls skeletal maturation in females as well as in males. The decrease of androgens to subnormal levels prompted us to reduce the transdermal estradiol to 12.5 μg twice weekly after 6 months in concordance with the recommendations of Rochira (9). This dose reduction induced a normalization of the testosterone level and maintained normal estradiol, LH, and FSH levels. Unfortunately, the patient denied further semen analyses.

The lipid profile of our patient is similar to patients with a metabolic syndrome and showed elevated triglyceride levels with a low HDL level (41). After treatment with estradiol, the HDL level increased, the ratio of LDL/HDL decreased, and the insulin secretion decreased after an oral glucose load indicating an apparent insulin resistance state of patients with aromatase deficiency, similar to the observations of a man with aromatase deficiency (8). In contrast to the lipid pattern of the aromatase-deficient men, the estrogen receptor α-resistant man had low serum levels of total cholesterol, LDL, and HDL cholesterol, but serum levels of triglycerides were normal (42). The very low concentration of HDL cho-

lesterol could be due to the unopposed action of androgen, which is considered the most important determinant of differences between females and males in serum HDL cholesterol (43). The effects of high-dose exogenous testosterone administration on lipid profile in the previous patient with aromatase deficiency (8) resulted in a decrease of HDL cholesterol, thereby confirming the role of elevated testosterone levels in decreasing HDL cholesterol, as shown in our aromatase-deficient patient. In contrast, transdermal estrogen treatment caused an increase of HDL cholesterol levels and a decrease of the LDL/HDL ratio, but triglyceride and total cholesterol levels did not change. Even though these changes may at least in part be the result of reduced concentrations of androgens, it is now evident that abnormal lipid pattern can be modified by estrogen treatment in aromatase-deficient men.

Lipoprotein a, as an independent risk factor of cardiovascular diseases, increased after 6 months of estradiol replacement therapy, indicating the influence of androgens and estrogens. This observation is consistent with the results of Zmuda *et al.* (44, 45), who have shown that testosterone or testosterone plus the aromatase inhibitor testolactone reduce lipoprotein a levels in males.

The causal relationship between estrogen deficiency and carbohydrate metabolism remains unclear because the mechanism by which the lack of estrogen may induce insulin resistance is still unknown. In the estrogen receptor α-deficient man, an impaired glucose tolerance and insulin resistance were found in association with clinically evident bilateral axillary acanthosis nigricans 3 but in only one of two known aromatase-deficient men an increase in the concentration of fasting insulin associated with normal blood glucose was found. To determine insulin resistance, we used HOMA IR (19, 20). During treatment with estradiol, HOMA IR, which was elevated, did not change, although AUC insulin decreased, indicating a beginning improvement of the postprandial insulin secretion. Further determinations of HOMA IR and AUC insulin after a longer period of estradiol treatment should address this issue.

Interestingly, body mass index did not change after treatment with estradiol in agreement to the observations of Mauras *et al.* (46, 47) who have shown that in contrast, body composition did not change after 10 wk of treatment with an aromatase inhibitor of healthy volunteers. Consequently, the reduction of insulin levels in our patient may not be due to change of body fat. Whether the reduction of insulin levels is the consequence of changes of lipids or may be due to direct estrogen action (8, 48, 49) must be evaluated in further investigations.

The present case illustrates the essential role of estrogens in skeletal development in males, verified by a novel mutation of the gene encoding the aromatase cytochrome P450. From this case we conclude that epiphyseal closure does not develop without the action of estrogen in males and that androgen alone is not sufficient to promote normal skeletal mineralization. Moreover, lipid abnormalities and an impairment of glucose tolerance are present in male patients with estrogen deficiency.

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