

Brief Report

EFFECT OF TESTOSTERONE AND ESTRADIOL IN A MAN WITH AROMATASE DEFICIENCY

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RECENT reports of disruptive mutations of the genes for the estrogen receptor or for cytochrome P-450 aromatase¹⁻⁶ have shed new light on the role of estrogen. In females the lack of estrogen due to aromatase deficiency leads to pseudohermaphroditism and progressive virilization at puberty, whereas in males pubertal development is normal. In members of both sexes epiphyseal closure is delayed, resulting in a eunuchoid habitus, and osteopenia is present.⁶ These findings suggest a crucial role of estrogen in skeletal maturation.¹⁻⁶ We describe the responses to androgen and estrogen in a man with a novel, homozygous inactivating mutation of the P-450 aromatase gene.

CASE REPORT

The proband, the second of 10 siblings, was born after an uncomplicated pregnancy. His parents were first cousins (Fig. 1). The patient's early growth and pubertal development were normal, although his testicular volume remained subnormal. At 18 years of age he was 170 cm tall (25th percentile), and he continued to grow thereafter. At the age of 28 years, x-ray films of the right arm obtained after an injury revealed unfused epiphyses and osteopenia. At the age of 29 years, he married a woman who did not conceive despite regular unprotected intercourse. Semen analysis one year later⁷ revealed a sperm count of 1 million per milliliter (normal, >20 million) with 100 percent immotile spermatozoa. The patient was treated with 150 IU of human menopausal gonadotropin and 1000 IU of human chorionic gonadotropin intramuscularly three times weekly for four months, with no change in the sperm count.

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In 1988, at the age of 31, the patient was evaluated because of a four-year history of persistent linear growth, infertility, and moderate skeletal pain, especially in the knee, that limited his ability to walk. He weighed 96.5 kg and was 187 cm tall (97th percentile). His arm span was 204 cm, and the ratio of the upper segment to the lower segment was 0.85. Physical examination revealed bilateral genu valgum. The patient's blood pressure was normal. He had normal optic fundi and no gynecomastia, acromegaly, goiter, or acanthosis nigricans. The volume of each testis was 8 ml. His penis size and pattern of pubic hair were normal. His sexual identity and psychosexual orientation as assessed by questionnaire⁸ were heterosexual, and his libido was normal. He had spontaneous erections sufficient for intercourse.

The patient had normal concentrations of serum testosterone, undetectable concentrations of estradiol, slightly elevated concentrations of follicle-stimulating hormone, and concentrations of luteinizing hormone at the upper limit of the normal range (Table 1). After he received an intravenous bolus dose of 100 μ g of gonadotropin-releasing hormone (GnRH), his serum concentration of luteinizing hormone rose from 6 to 18 IU per liter after 60 minutes (when the peak response occurs), and the concentration of serum follicle-stimulating hormone rose from 14 to 19 IU per liter. The serum concentrations of dehydroepiandrosterone sulfate, 17-hydroxyprogesterone, androstenedione, parathyroid hormone, free thyroxine, and thyrotropin were normal. The serum concentration of growth hormone rose from 0.8 to 6.2 ng per milliliter after the administration of levodopa. The serum concentration of insulin-like growth factor I was 332 ng per milliliter (normal range at the age of 25 to 35 years, 193 to 575). The serum concentrations of total cholesterol and triglycerides were high, and the serum concentration of high-density lipoprotein (HDL) cholesterol was low (Table 1).

X-ray films of the left wrist and hand revealed open metacarpal and phalangeal epiphyses; the bone age was 14.8 years (Fig. 2). X-ray films of the tibias, knees, and pelvis showed diffuse bone demineralization and lack of epiphyseal fusion. A bone biopsy of the iliac crest after labeling with tetracycline revealed several slightly widened areas of osteoid seams lined by active osteoblasts.

A semen analysis⁷ revealed a sperm count of less than 1 million per milliliter, with 100 percent immotile spermatozoa. A testicular biopsy showed hypospermatogenesis and germ-cell arrest, mainly at the level of primary spermatocytes. The karyotype was 46,XY.

In an attempt to arrest his persistent linear growth and stimulate epiphyseal closure, the patient, after giving informed consent, was treated with 250 mg of testosterone enanthate intramuscularly every 10 days for 6 months. There were no clinical, behavioral, hormonal, or metabolic changes, except for a small decrease in the serum concentration of HDL cholesterol (Table 1). His bone age did not change, and moderate bone pain persisted. He interrupted the treatment spontaneously in 1989 because of its ineffectiveness and because he believed it was rendering him irretrievably infertile.

In 1995 the patient was 190 cm tall (above the 97th percentile), and his bone age and biochemical values had not changed appreciably (Table 1). The results of an oral glucose-tolerance test were normal. The similarity between his phenotype and that of a man with a mutated estrogen-receptor gene¹ prompted us to analyze the patient's DNA for a mutation in that gene or in the P-450 aromatase gene. As expected from the low serum estradiol levels, the estrogen-receptor gene was normal, but there was a single G \rightarrow A mutation at base pair (bp) 1094 in exon 9 of the P-450 aromatase gene, resulting in a glutamine instead of an arginine at position 365 (Fig. 3). This mutation abolishes a site cleaved by the restriction enzyme *Acc65I*; restriction analysis, used to determine the carrier status of other family members, showed that both parents were heterozygous for the mutation. Expression studies in COS-1 cells showed that the aromatase activity of the mutant protein was 0.4 percent of that of the wild-type protein in the presence of the same amount of total cellular protein, as measured by a Western blot assay corrected for the efficiency of transfection.

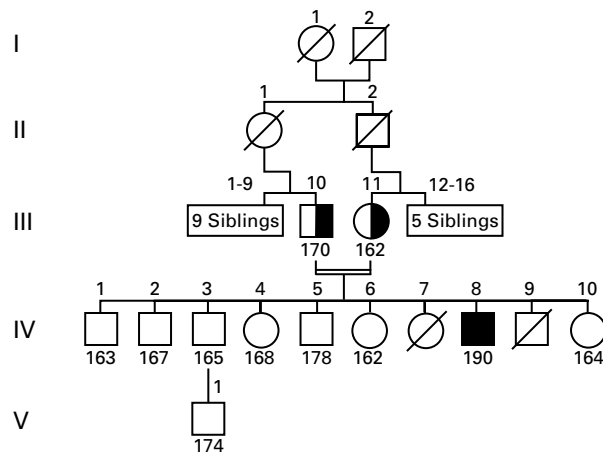


Figure 1. Pedigree of a Man with Aromatase Deficiency.

The proband (Subject IV-8) is indicated by the solid square. Sequence analysis of the P-450 aromatase gene was performed in the proband, his parents, two of his brothers (Subjects IV-3 and IV-5), and one of his nephews (Subject V-1). Squares denote male family members, circles female family members, slashes deceased family members, the double line consanguinity, and half-solid symbols family members heterozygous for the mutation in the P-450 aromatase gene. The values shown below the symbols are the heights (in centimeters) of the subjects in adulthood.

After giving informed consent and with the approval of the local university review board, the patient was treated with 50 μg of transdermal estradiol twice weekly. His bone pain improved after four months and resolved completely after six months. His serum concentrations of luteinizing hormone, follicle-stimulating hormone, and testosterone decreased, that of HDL cholesterol increased, and that of low-density lipoprotein (LDL) cholesterol decreased (Table 1). His fasting concentrations of serum insulin and blood glucose were normal. The serum concentrations of alkaline phosphatase and osteocalcin increased, as did the urinary excretion of pyridinoline, indicating active bone remodeling (Table 1). The bone mineral density of the lumbar spine was 0.93 g per square centimeter before treatment (normal range⁹ for adolescents in Tanner stage 5, 0.96 to 1.31) and was 1.05 and 1.17 g per square centimeter after four and seven months of treatment, respectively. Epiphyseal closure was documented after nine months of therapy, with a bone age greater than 16 years (Fig. 2). The treatment did not induce gynecomastia, hyperprolactinemia, or sexual dysfunction. Testicular volume and the results of semen analysis did not change. At this writing the patient is being treated with 25 μg of transdermal estradiol twice weekly.

METHODS

Biochemical Measurements

Blood samples were obtained by venipuncture after an overnight fast. Serum luteinizing hormone, follicle-stimulating hormone, and growth hormone were measured by an immunofluorimetric assay (Delfia kits, Pharmacia, Milan, Italy) according to the instructions of the manufacturer. All the other hormones were measured by commercially available radioimmunoassays.

TABLE 1. BIOCHEMICAL VALUES BEFORE AND AFTER SIX MONTHS OF TREATMENT WITH TESTOSTERONE ENANTHATE OR TRANSDERMAL ESTRADIOL IN A MAN WITH AROMATASE DEFICIENCY.*

VARIABLE	NORMAL RANGE	TESTOSTERONE		ESTRADIOL	
		BASE LINE (1988)	6 MO LATER	BASE LINE (1995)	6 MO LATER
In serum					
Estradiol (pg/ml)	20–90	<10	<10	<10	97
Testosterone (ng/dl)	360–900	390	1186	523	21
Follicle-stimulating hormone (IU/liter)	1.7–6.9	13.6	11.0	17.1	1.0
Luteinizing hormone (IU/liter)	1.4–8.9	8.9	7.3	5.6	0.8
Total cholesterol (mg/dl)	140–200	279	248	306	241
HDL cholesterol (mg/dl)	>45	42	29	43	53
LDL cholesterol (mg/dl)	<150	183	170	209	166
Triglycerides (mg/dl)	<175	257	287	305	200
Glucose (mg/dl)	60–110	84	93	89	82
Insulin ($\mu\text{U}/\text{ml}$)	5–25	ND	ND	22	14
Alkaline phosphatase (U/liter)	98–280	164	185	227	329
Osteocalcin (ng/ml)	5–18	ND	ND	9.7	22
In urine					
Pyridinoline (nmol/mmol of creatinine)	6–20	ND	ND	23	49

*To convert values for estradiol to picomoles per liter, multiply by 3.671; to convert values for testosterone to nanomoles per liter, multiply by 0.035; to convert values for total cholesterol, HDL cholesterol, and LDL cholesterol to millimoles per liter, multiply by 0.025; to convert values for triglycerides to millimoles per liter, multiply by 0.011; and to convert values for glucose to millimoles per liter, multiply by 0.055. ND denotes not determined.

Molecular Analysis of the Genes for the Estrogen Receptor and P-450 Aromatase

Genomic DNA was prepared from blood samples obtained from the patient, his parents, two of his brothers, one of his nephews, and a normal unrelated man.⁴ Single-strand conformation analysis of the estrogen-receptor gene was performed as previously reported.¹ To determine the complete sequence of the exons and the intron-exon junctions, each exon of the P-450 aromatase gene, including the 5' untranslated exons and their respective 5' flanking regions, was amplified as previously described.¹⁰ Both strands were sequenced to exclude artifacts. The complete sequence of each exon, including the 5' and 3' splice junctions, was compared with the published sequence.¹¹

Exon 9 of the genomic DNA from the normal subject, the proband, and the family members was amplified and digested with *Acc651* (Promega, Madison, Wis.), according to the specifications of the manufacturer, and subjected to electrophoresis in a 2 percent agarose gel. The digested fragments were visualized by staining with ethidium bromide.

P-450 aromatase complementary DNA (cDNA) was prepared from wild-type pCMV5arom.¹¹ The wild-type, mutant (R365Q), and vector-only constructs were transfected into COS-1 cells by lipofectamine (BRL, Grand Island, N.Y.). Aromatase activity was determined by the production of tritiated water from [1β -³H]androstenedione.¹² Incubations were conducted in triplicate 48 hours after transfection. Western blot analysis was performed as previously described.¹⁰

DISCUSSION

We studied the effects of estrogen therapy in a man with a loss-of-function mutation of the aromatase gene. Our first conclusion is that estrogen therapy had a large effect on the patient's skeletal growth and bone maturation, whereas androgen therapy did not. The dichotomy between the histologic picture of active bone formation and normal biochemical measures of bone metabolism suggests that testosterone exerted an active effect on osteoblasts, albeit an inefficient one. With estrogen treatment spinal bone mineral density increased, and complete epiphyseal closure was achieved after nine months. The increases in bone mineral density, serum levels of alkaline phosphatase and osteocalcin, and urinary excretion of pyridinoline were similar to those that occur during normal skeletal maturation during puberty.^{13,14} By contrast, testosterone had no effect on skeletal maturation. Therefore, the eunuchoid skeleton may result mainly from a deficiency of estrogen, rather than a deficiency of androgen. The lack of eunuchoid skeletal development in patients with complete androgen insensitivity supports this view.¹⁵ Conversely, patients of either sex who have a complete deficiency of 17α -hydroxylase or a combined



A



B

Figure 2. X-Ray Films of the Left Hand of the Proband.

When the patient was first admitted, at the age of 31 and immediately before his treatment with testosterone, the bone age was 14.8 years (Panel A). Seven years later, after nine months of treatment with transdermal estradiol (50 μ g twice weekly for six months, followed by 25 μ g twice weekly), the bone age was greater than 16 years (Panel B). A rapid increase in bone maturation, with closure of the metacarpal and phalangeal epiphyses, is evident.

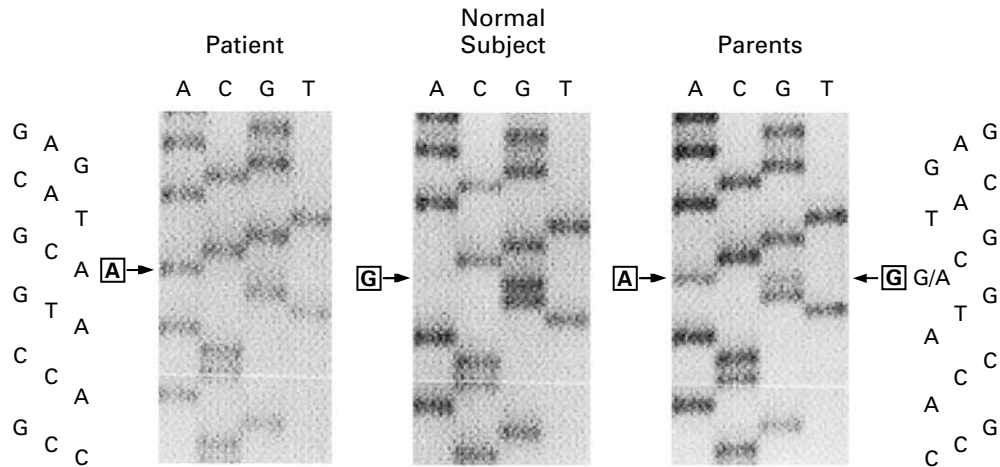


Figure 3. Nucleotide Sequence of a Region of Exon 9 of the P-450 Aromatase Gene in the Patient, a Normal Subject, and the Patient's Parents.

Exon 9 of the P-450 aromatase gene was amplified by PCR and sequenced directly, as described in the Methods section. A single-base change at bp 1094 (G→A) was detected. The parents were heterozygous for this mutation.

deficiency of 17 α -hydroxylase and 17,20-lyase have tall stature, retardation of bone age, osteoporosis, and a eunuchoid skeleton¹⁶ — a phenotype classically related to the poor production of sex steroids, which can now be explained by a deficiency of estrogen. As is consistent with these findings, estrogen seems required for epiphyseal fusion, an event that takes longer in patients with hypogonadism, who produce insufficient androgens for aromatization. Such fusion never takes place in men with estrogen deficiency or estrogen resistance.

Estrogen treatment induced substantial decreases in the ratio of serum LDL cholesterol to serum HDL cholesterol and in serum triglycerides in our patient (Table 1). Although this effect may depend at least in part on reduced concentrations of serum testosterone, it is clear that the abnormal lipid profile in an aromatase-deficient subject can be modified with estrogen treatment.¹⁷

Our patient did not have insulin resistance, unlike previously described patients with aromatase deficiency or estrogen insensitivity.^{1,4} This finding raises the possibility that insulin resistance is an unrelated phenomenon. His serum concentrations of luteinizing hormone and follicle-stimulating hormone were normal or slightly elevated and responded normally to GnRH stimulation. However, estrogen treatment caused complete suppression of serum gonadotropins whereas androgen treatment did not. In contrast, serum gonadotropins are hyperresponsive to GnRH in female patients with aromatase deficiency,³ because there is a complete absence of steroid feedback. These results indicate that the mechanism of sex-steroid–gonadotropin feedback in male pa-

tients is mainly mediated by testosterone, but that some testosterone must be converted to estrogen.¹⁷⁻²² This conclusion is supported by a report that the concomitant administration of testosterone and an aromatase inhibitor prevents testosterone-induced suppression of gonadotropin,²⁰ whereas dihydrotestosterone has no effect.²¹

Unlike the other two men with estrogen deficiency or resistance described to date, our patient had small testicles and severe oligozoospermia. Azoospermia and infertility were also reported in one of his brothers (Subject IV-5), who had a normal P-450 aromatase gene. Therefore, spermatogenic damage may also be a primary event in the proband, independent of estrogen deficiency. Mouse germ cells express aromatase,²³ and mice in which the estrogen-receptor gene is knocked out have reduced testicular volume and are infertile, indicating that estrogen is necessary for fertility in that species.²⁴ In adult men, aromatase is located in Leydig cells, but its function is unknown.²⁵ The ineffectiveness of estrogen therapy in inducing spermatogenesis in our patient argues against estrogen-dependent spermatogenic damage.

In conclusion, we describe a therapeutic response to estrogen therapy, but not to androgen therapy, in a man with aromatase deficiency. When to initiate treatment, at what doses, and for how long all remain uncertain.

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