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Intrauterine growth retardation associated with precocious puberty and Sertoli cell hyperplasia

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Abstract

The original description of patients with Russell-Silver syndrome included precocious puberty, the mechanism of which was unclear. We describe a child with a Russell-Silver syndrome-like phenotype who presented with precocious puberty that was associated with hyperplasia of the Sertoli cells. The patient was found to have an immature cryptorchid testicle; hyperplastic Sertoli cells were also aneuploid carrying trisomy 8. This chromosomal abnormality was present in Sertoli cells *only* and could not be detected in peripheral lymphocytes, tunica vaginalis, or other, normal, testicular tissue. Sertoli cells in culture showed excess aromatization providing an explanation for the rapid advancement of the patient's bone age. We conclude that in a patient with a Russell-Silver syndrome-like phenotype, Sertoli cell hyperplasia was associated with somatic trisomy 8, increased aromatization and gonadotropin-independent precocious puberty.

INTRODUCTION

Precocity and elevated urinary gonadotropins were features of the first described patients with Russell-Silver syndrome (RSS), a genetic condition with a variable phenotype and, to date an unknown molecular cause [1–3]. Patients with RSS have short stature and their defective growth starts *in utero*, a feature that is important in the diagnosis of this condition and emphasized by Dr. Russell [4]. RSS also has skeletal manifestations and is associated

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with asymmetry and several other dysmorphic features. Patients with RSS are prone to fasting hypoglycemia mostly in infancy, excess sweating in later childhood, and a tendency to develop a variety of tumors [5]. Precocious puberty and/or adrenal hyperplasia are inconsistent but recurrent findings. Although uniparental disomy (UPD) for chromosome 7 was suggested as a molecular mechanism for this disorder as early as in 1995, it is not present in the majority of patients tested to date [6–8]. DNA hypomethylation at the telomeric imprinting control region on chromosome 11p15 has been identified in approximately 30% of patients with RSS [9].

On the other hand, a variety of chromosomal disorders have an RSS-like phenotype, including patients with triploidy and diploid/triploid mixoploidy syndrome with mosaicism for trisomy 8 and a single patient with a interstitial deletion of proximal 8q [10–12]. In this report, we investigated precocious puberty in a child with an RSS-like phenotype and medical history. Bone age advancement was significant and beyond what one would expect from his other signs of puberty. Cryptorchid testicular tissue was found to harbor immature Leydig cells and other structures, but also Sertoli cell hyperplasia (SCH) which expressed aromatase (the P450aromatase enzyme), as in other patients with Sertoli cell lesions [13,14]. More importantly, the hyperplastic Sertoli cells, cultured *in vitro*, showed excess aromatization and when studied cytogenetically, trisomy 8. The latter was not present in other tissues of the patient that were investigated, pointing to a somatic defect. The findings in this report have implications for both the care of patients with RSS-like syndromes, but also for the molecular investigation of this condition and of Sertoli cell tumors, in general.

MATERIALS AND METHODS: LAB PROCEDURES

During the patient's initial evaluation, routine analytical tests were performed at the Children's Hospital of Buffalo. All subsequent routine analytical tests and immunohistochemistry were performed at the NIH Clinical Center laboratory in Bethesda, MD. Chromosome analysis of the testicular tissue was performed by Quest Diagnostics Nichols Institute, Chantilly, VA; a cell line was prepared from primary tissue fragments as previously published [15]. In brief, the patient's Sertoli cell testicular cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) media containing 10 % fetal calf serum. Twenty G-banded metaphases were analyzed from both this cell line as well as the patient's peripheral blood. Fluorescence in situ hybridization (FISH) was performed on interphase nuclei to search for multiple copies of chromosome 8, as previously published [16]. FISH was performed using a probe for the centromere of chromosome 8 (CEP8) (Abbott Molecular Inc, Des Plaines, IL) on fixed interphase nuclei from the testicular cell line, as well as on touch preps from the tunica vaginalis and normal testicular tissue. Two hundred interphases were scored for each tissue type.

IMMUNOHISTOCHEMISTRY

Five-micron slides from formalin-fixed paraffin embedded tissue samples were used for immunohistochemistry. Slides were deparaffinized in 3 changes of xylene for 5 min each followed by rehydration in graded alcohols. Antigen retrieval was achieved by heating the slides in Tris-EDTA buffer pH 8.0 in a microwave oven at 95°C for 20 min. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide in methanol for 10 min. Sections were then incubated at room temperature with the following primary antibodies: rabbit anti-luteinizing hormone receptor (LHr) (1:2000 Sigma, Saint Louis, MO), rabbit anti-Aromatase (1:1500, Sigma, Saint Louis, MO), and rabbit anti-17-Alpha hydroxylase (1:1000; Abcam, Cambridge, MA). Rabbit immunoglobulins were used at 1 ug/mL instead of primary antibodies as negative controls. Horseradish peroxidase-dextran polymer conjugate goat anti-rabbit (Envision™ PO System; Dako, Carpinteria, CA) was

used as secondary antibody and reported as previously described, with 3,3-diaminobenzidine as chromogen [17]. The sections were then briefly counterstained with Mayer's haematoxylin, dehydrated in graded alcohols, cleared in xylene and permanently mounted.

AROMATASE ASSAY

Aromatase activity testing was done at the University of Maryland, Baltimore, MD as previously published [18]. Radioactive ligand for the aromatase assay, 1β^{3}H androstenedione (23.5 Ci/mmole) was purchased from Perkin Elmer (Boston, MA). Aromatase activity in cells was measured using radiometric ^3H_2O release assay as described earlier by measuring ^3H_2O formed on conversion of 1-β^{3}H androstenedione (substrate) to estrone. About 150,000 cells were plated in phenol red free medium and allowed to attach for 24 hours. Next day, cells were incubated with 1β^{3}H androstenedione for 24 hours. At the end of the incubation period, the medium was removed and residual steroids were extracted with chloroform followed by treatment with 2.5% charcoal. The radioactivity in the aqueous layer was measured using a scintillation counter.

INFORMED CONSENT

Our patient was treated under Institutional Review Board (IRB) approved protocols 97CH0076, 00CH0180 and 00CH160 of the National Institute of Child Health and Human Development (NICHD) and informed consent was obtained.

PATIENT REPORT

The patient was initially referred to the pediatric endocrinology clinic at the age of 19 months for evaluation of intrauterine growth retardation (IUGR) and failure to thrive. He had been previously diagnosed with an RSS-like condition. The patient was the product of a 37-week gestation complicated by severe IUGR, and was delivered via cesarean section secondary to fetal distress and oligohydramnios. Birth weight was 940 grams (Z score −3) and birth length was 33 cm (Z score −6). The patient had multiple dysmorphic features including micropenis, chordee and hypospadias, as well as a non-palpable right testis. Hand deformities included bilateral clinodactyly of the fifth digit, webbing defects, and a bifid left thumb. The karyotype was 46XY. Family history is notable for a sibling with trisomy 21. The patient was hospitalized in the neonatal intensive care unit for the first five months of life, his course complicated by respiratory distress, hypoglycemia, and feeding difficulties requiring G Tube placement.

At the patient's initial evaluation at the pediatric endocrinology clinic when he was 19 months of age, he was noted to have developmental delay and pronounced growth retardation with height and weight both remaining well below the third percentile for age (Figure 1). On examination, he had a narrow, triangular face with thin upper lip consistent with RSS. A small prepubertal testis of 0.6 cm was palpable on the left while no testis was palpable on the right, phallic length was 2.3 cm.

At 2 years of age, an MRI of the brain revealed a small pituitary gland and a shallow sella turcica. Growth hormone treatment was initiated; the patient responded well to GH with a doubling of his growth rate. At the age of 4 3/12 yrs the patient had developed Tanner III pubic hair, acne, body odor and phallic enlargement. Left testis was also larger (2 cm length), while the right testis continued to be non palpable. A Leuprolide test was done which revealed baseline FSH of 1.0 IU/L and peak response of 9.4 IU/L at 90 minutes and baseline LH of 0.1 IU/L with peak of 1.9 IU/L at 90 minutes. At this time, he was also found to have an elevated free testosterone of 1.8 pg/ml (prepubertal: 0.15–0.6), a total testosterone of 8.5ng/dL (prepubertal: 3–10 ng/dL), a mildly elevated 17 OH Progesterone of 161ng/dL

(1.0–120 ng/dL) and elevation of DHEAS at 149 ug/dl (2–37 ug/dL) (Table 1). An ACTH stimulation test was performed and was consistent with exaggerated adrenarche but not diagnostic for late onset congenital adrenal hyperplasia (Table 2). A CT of the abdomen revealed minimally prominent bilateral adrenal glands of unknown significance, and bilateral hypo-dense lesions on the kidneys, thought to be cysts. He was started on LHRH agonist (Leuprolide) 7.5 mg IM every 28 days at 4 5/12 years with the presumed diagnosis of early- stage or “transitional” central pubertal development due to the FSH response to Leuprolide stimulation and the slightly high free testosterone level.

Four months after initiating GnRH agonist therapy, a Leuprolide test indicated adequate suppression of gonadotropins 20 minutes post Leuprolide injection. Despite remaining on Leuprolide, the patient continued to have rapid progression in skeletal maturation. At a chronological age of 6 11/12 years, his bone age was 9 years. Leuprolide was discontinued given the relentless bone age progression over the course of nearly two years of treatment. A repeat cosyntropin stimulation test was performed, which again showed stimulated androgen levels that were in a pubertal range but not consistent with congenital adrenal hyperplasia (Table 2). A right inguinal exploration performed by a pediatric urologist when the patient was 6 10/12 years failed to identify a right testis.

The patient was referred to the NIH for further evaluation at the age of 7 years 5 months; at that time his bone age was advanced at 10 years of age. He was noted to have normal testosterone levels 12 ng/dL (<7–20), elevated DHEA-S of 159 ug/dL corresponding to a Tanner III male (<15–312), LH <1 IU/L and FSH <1 IU/L, minimally elevated estrone of 17 pg/mL (0–16) and normal estradiol <10 pg/mL (Table 1). Ophthalmic evaluation revealed the presence of villiform protrusions on the irises (mamillations) (Figure 2A). The patient underwent a single dose peripheral hCG stimulation test to confirm the presence of responsive Leydig cells in which 100 IU/kg of hCG was administered intramuscularly [19]. The testosterone level went from a baseline of 29 to a peak of 323 ng/dL, consistent with functioning testicular tissue with greater than a 3-fold increase over the baseline level. A testicular ultrasound revealed left testis with microlithiasis 1.5 × 0.6 × 1 cm, and no visible right testis. A magnetic resonance scan of the abdomen revealed a 1.4 cm mass inferior to the right renal lower pole thought to be a candidate for an undescended gonad (Figures 2B and 2C). Tumor markers including Beta hCG, Alpha-Fetoprotein, CEA, and CA125 were all negative. The patient underwent testicular and adrenal venous sampling with hCG stimulation that showed a prominent peak in testosterone corresponding to the right testicular vein. Testosterone levels corresponded to the anatomic localization of the intraabdominal testicle on MRI scan, with a peak of 8,180 ng/dL in the right testicular vein versus only 315 ng/dL in the left testicular vein and 278 and 447 ng/dL in the left and right adrenal veins, respectively (Table 3). The right undescended testicle was felt to be the source of excess androgens and precocious virilization. As cryptorchidism is associated with an increased risk of testicular malignancy, the undescended testis was thought to be at risk of transforming into a gonadoblastoma [20].

The patient underwent an exploratory laparotomy; at operation, a cryptorchid right testis was removed. The testis correlated with the MRI scan findings and was consistent with testicular tissue on pathological examination. On gross examination, the tissue received in surgical pathology consisted of an immature right testis. On microscopic analysis, seminiferous tubules were populated with progenitor cells although some immature spermatids were also seen. Sertoli cell hyperplasia was noted, if one took into account the patient’s age and pubertal stage, as well as the overall otherwise immature testicular tissue. Calcifications were also present within the lumen of some seminiferous tubules (Figure 3 panels A and B). The presence of both relative hyperplasia and calcifications, along with the clinical evidence of aromatization suggested the beginning of a lesion that resembled an “early” large cell

calcifying Sertoli cell tumor or LCCSCT. Inhibin A, a specific marker that serves to differentiate testicular sex cord-stromal tumors from germ cell tumors and is enerally high in LCCSTs, was highly expressed in the cytoplasm of Sertoli cells (Figure 3 panels B and C). Aromatase was expressed in the cytoplasm of Sertoli cells (Figure 3 panels E and F), as it is known to be expressed in LCCSCTs but not in normal Sertoli cells. The LH receptor (LHR) was also strongly expressed by Leydig cells in the cell membrane and cytoplasm, but staining was also present to a lesser degree in the Sertoli cells of the seminiferous tubules (Figure 3 panels G and H). The patient's cultured Sertoli cells exhibited increased aromatase activity (Figure 5), which was further exacerbated by dexamethasone in the culture medium. Chromosome analysis performed on cultured Sertoli cells revealed trisomy 8 in all metaphases examined; FISH analysis showed 3 copies of chromosome 8 in 98% of interphases from this cell line, also consistent with trisomy 8 (Figure 4). By contrast, normal G-band or FISH results were obtained on the patient's peripheral lymphocytes, tunica vaginalis, and testicular tissue. The cytogenetic studies are suggestive of trisomy 8 limited to the hyperplastic Sertoli cells since the patient's peripheral lymphocytes as well as the tunica vaginalis tissue and testicular tissue showed no evidence of trisomy 8.

Four days post-operatively, when the patient underwent a repeat hCG stimulation test, the serum testosterone at baseline was 12 ng/dL and rose only to a peak of 17 ng/dL. In the months following surgery, however, the patient developed bilateral gynecomastia and persistent elevation of estrone. In addition, testosterone and gonadotropin gradually rose to pubertal levels. He was subsequently treated with letrazole (2.5 mg po daily) to block aromatization and with monthly Leuprolide injections (11.25 mg IM) for central precocious puberty. After several months, Leuprolide was discontinued after insertion of a histrelin acetate subcutaneous implant (Supprelin LA, 50 mg). On follow up, he has good suppression of testosterone and estrogen while on an aromatase inhibitor and GnRH agonist therapy. His most recent bone age was stable at 12 years.

DISCUSSION

The patient described presented with RSS-like phenotype in conjunction with precocious puberty. The source of his precocious puberty was due to the hormonally active non-descended right testicle in which there was coincident SCH. Results of testing performed on the patient's cultured testicular cells showed that his Sertoli cells behaved like those in other LCCSCTs and were capable of aromatizing estrogen. In addition, immunohistochemistry performed on the patient's Sertoli cells was positive for aromatase expression that was further exacerbated by glucocorticoids. LCCSTs are known to be associated with Peutz-Jeghers syndrome (PJS) and Carney complex (CNC); increased cytochrome P-450 aromatase expression in gonadal tissue has been described in patients with PJS and sex-cord tumors [13,21]. Aromatase expression in our patient, similar to LCCSTs, is the likely explanation for his elevated estrone levels and subsequent bone age advancement, as well as for the mild gynecomastia that developed following right orchiectomy. The pathophysiology of increased testosterone secretion by the cryptorchid right testis, however, has not been clarified.

Aromatase inhibitors have been used to decrease estrogen production and slow skeletal maturation in patients with PJS [22]. In our patient, aromatase inhibition in conjunction with GnRH agonist therapy has served thus far to stall bone age advancement and decrease estradiol levels.

The finding of trisomy 8 in the patient's testicular cells is particularly interesting. Multiple chromosome copies are frequently observed in tumor development as acquired changes. Karyotype analysis of testicular and ovarian germ cell tumors has previously revealed

trisomy 8 in these lesions [23–25]. The variable genetic findings in patients with RSS, from UPD of chromosome 7 to mixed polyploidy and chromosome 8 abnormalities [6,7,10], along with the family's history of a patient who has a brother with trisomy-21, are in support of a more general defect in RSS that may affect chromosomal integrity and/or segregation, as recently suggested by Dias et al. [26].

Constitutional mosaic trisomy 8 (CT8M), also known as *Warkany syndrome*, has a variable phenotype that may include developmental delay, renal anomalies, dysmorphic facial features, and cardiac defects that sometimes mimic RSS [27]. It is also associated with an increased risk of neoplasia, particularly myeloid hematologic malignancies, although solid tumors have also been reported in association with this karyotype [28–30]. Ophthalmic defects including optic disc coloboma and chorioretinal defects have also been associated with CT8M, and may be related to our patient's unusual ophthalmologic findings [31].

Whether trisomy 8 is an acquired change confined to the testicular tissue alone or is a constitutional abnormality present in the patient's other tissues remains unclear; trisomy 8 was not found in other cell populations in this patient, including tunica vaginalis and peripheral lymphocytes. The most likely explanations for these findings are that (1) the patient survived as an embryo due to placental rescue of an otherwise lethal aneuploidy that was present in all, or almost all, cells of the conceptus, as is the case in other patients with similar abnormalities [32,33]: chromosome mosaicism is detected in about 1–2% of chorionic villi samples, and may be due to a postzygotic nondisjunction event generating a trisomic cell line in an initially normal conceptus (pointing to a mitotic origin) or the postzygotic loss of one chromosome in an initially trisomic conceptus (indicating a meiotic origin and subsequent trisomy rescue) [32]; or (2) the trisomy 8 was present in Sertoli cells only and caused ICH, as a tumor-related, confined somatic event that occurred late in development or during the differentiation of these cells, as in other tumors that have chromosome 8 abnormalities [23–25]. What is against that latter possibility is the fact that this patient had bilateral and apparently multicentric disease (as evidenced by the microcalcifications in the remaining gonad), and that he also had several other phenotypic features consistent with mosaicism (albeit low grade) for chromosome 8 aneuploidy.

Finally, this patient raises interesting questions regarding the diagnosis and management of precocious puberty. It underscores the importance of surgical diagnosis in patients with presumed anorchia, as well as the usefulness of the hCG stimulation test to localize functioning testicular tissue. The resulting increase in the conversion of androgens to estrogens by the hyper-functioning Sertoli cells likely contributed to the accelerated bone age seen in this patient, and treatment with aromatase inhibitor and GnRH agonist may improve the patient's height outcome and control the precocious pubertal development.

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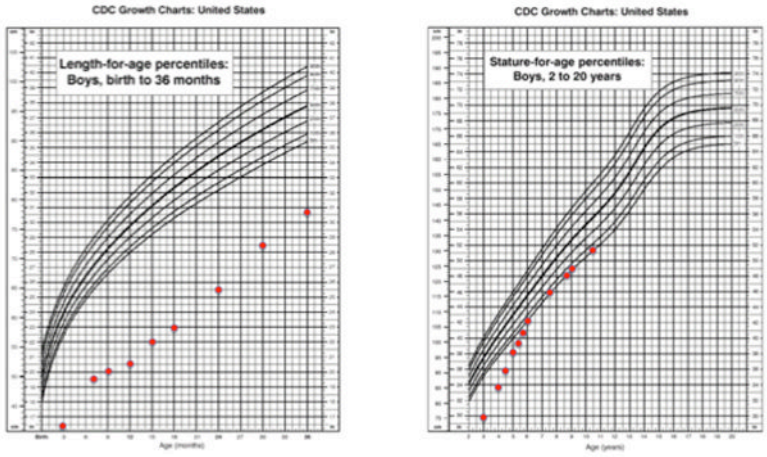

Figure 1.
Growth chart

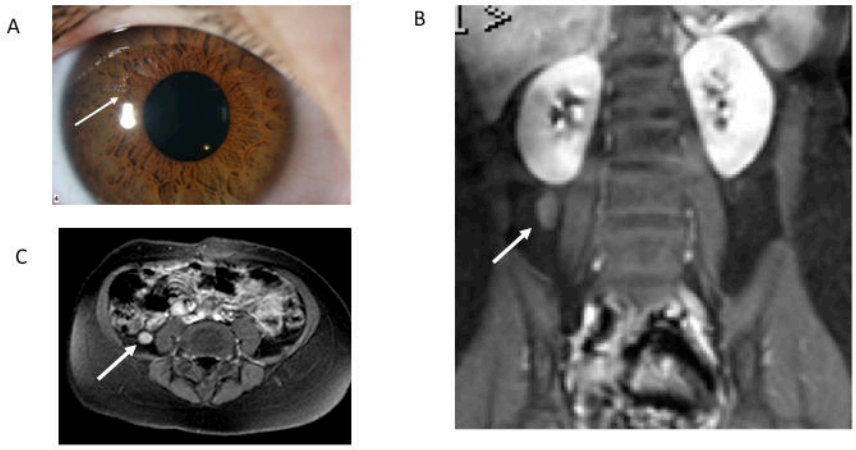

Figure 2.

(A) Photograph of patient's eye findings; iris mamillations are seen

(B) Coronal and (C) axial fat suppressed T1 weighted post contrast images of the abdomen demonstrate an enhancing mass (1 × 1.4 × 0.9 cm) inferior to the right renal lower pole (arrow).

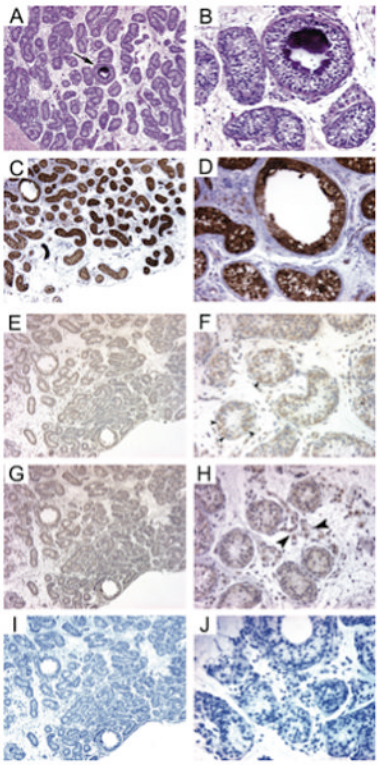

Figure 3.
Immunohistochemical staining of immature testis. (A) Hematoxylin-eosin staining showing a calcification within a seminiferous tubule (arrow, magnification: 4X). (B) Higher magnification of A (40X). (C and D) Inhibin A staining. Note the strong positivity of Sertoli cells in the tubules. (E and F) Aromatase staining. Immunoreactivity was limited to Sertoli cells. (G and H) Both Sertoli (arrowheads) and Leydig cells stained for Luteinizing hormone receptor (LHr). (I and J) Negative controls.

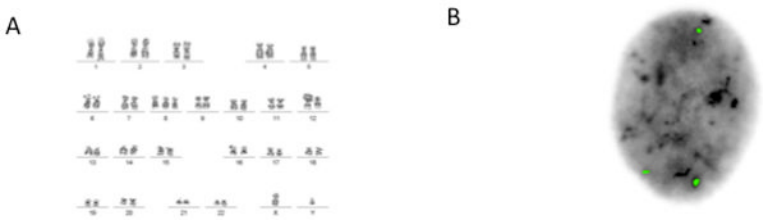

Figure 4.

(A) G-band karyotype of testicular cells indicating trisomy 8.

(B) Fluorescence in situ hybridization performed on testicular tissue utilizing a probe for the centromere of chromosome 8 (CEP8). Three copies of the CEP8 probe were present in 98% of the interphase cells analyzed, whereas 2% of cells had 2 signals per cell. These results demonstrate that the vast majority of cells from the cultured Sertoli cells were trisomic for chromosome 8.

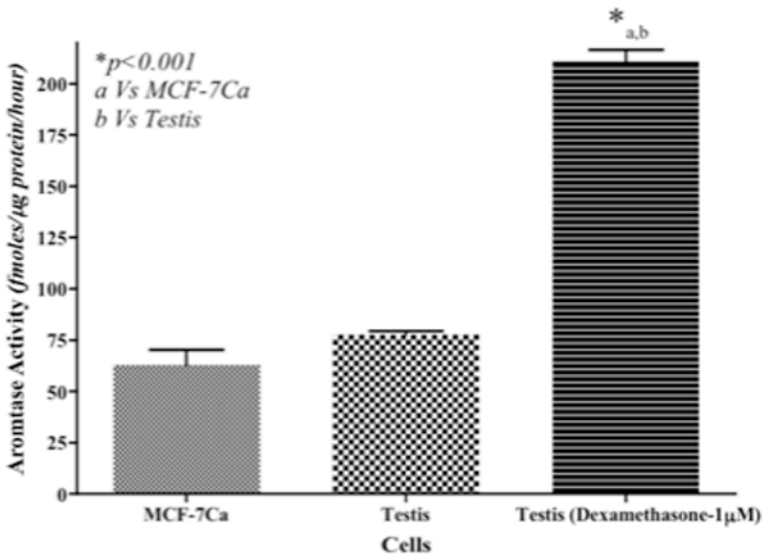

Figure 5.

Radiometric $^{3}H_2O$ release assay to measure intracellular aromatase activity. The graph represents aromatase activity in fmoles/μg protein/hour. The aromatase activity in dexamethasone stimulated testis sample is significantly higher (*p<0.001) than aromatase transfected MCF-7Ca breast cancer cells. MCF-7Ca cells have constitutively active aromatase enzyme.

Table 1

Labs and bone age

Chronological age	Bone age	Total T (ng/dL)	T peak 72 hours after HCG (ng/dL)	Free T (0.15–0.6 pg/mL)	DHEAS (2–37 ug/dL)	17 OH prog. (3–90 ng/dL)	Estradiol (<13 pg/mL)	Estrone (<17 pg/mL)
3 11/12	3	<15			97			
4 3/12		8.5		1.8	149	161		
4 6/12 *	5	6.2		1.2	126			
4 10/12				2.4	164	112		
5 2/12	6	9.9		1.0				
5 11/12	8							
6 11/12 #	9	39					<10	20
7 5/12	10	12		0.3	159	74	<10	17
7 8/12		29	323	0.8	148			
7 11/12 **		12	17	0.2				
8 3/12	11	42	503	0.8	112		<10	14
8 8/12 ***	12	145	428	3.3	155		<10	19
9 3/12	12	9.5		0.2	176		<10	<10

* Leuprolide started #Leuprolide discontinued

** Undescended testis removed

*** Leuprolide and Letrazole started

T = Testosterone

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Table 2

Cortrosyn stimulation tests

	Time	DOC ng/dL	DHEA ng/dL	17OH progesterone ng/dL	Total testosterone ng/dL	17 OH pregnenolone ng/dL	Androstenedione ng/dL
#1 03/2004	0 min	< 10	183	51	9.5	84	66
	60 min	148	783	342	15	1170	185
#2 05/2006	0 min		488	36	12	94	77
	60 min		491	231	20	522	126
Normal ranges							
	0 min		26–153	<120	3–10	10–186	12–43
	60 min	90–251	15–724	75–353	3–10	170–656	24–98

Table 3

Venous sampling

	Peripheral	Left adrenal	Right adrenal	Left testicular	Right testicular
Baseline testosterone (ng/dL)	216	240	222	0	315
Peak testosterone after hCG (ng/dL)	266	278	447	315	8,180