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Bilateral testicular tumors resulting in recurrent Cushing's syndrome after bilateral adrenalectomy"

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Abstract

Context: Recurrence of hypercortisolism in patients after bilateral adrenalectomy for Cushing's disease is extremely rare.

Patient: We present a rare case of a 27-year-old man who previously underwent bilateral adrenalectomy for Cushing's disease with complete clinical resolution. Cushingoid features recurred 12 years later, along with bilateral testicular enlargement. Hormonal tests confirmed ACTH-dependent Cushing's. Surgical resection of the testicular tumors led to clinical and biochemical remission.

Design and Results: Gene expression analysis of the tumor tissue by qPCR showed high expression of all key steroidogenic enzymes. Adrenocortical-specific genes were 5.1×10^5 (*CYP11B1*), 1.8×10^2 (*CYP11B2*) and 6.3×10^4 (*MC2R*) times higher than non-steroidogenic fibroblast control. This correlated with urine steroid metabolome profiling showing 2-5 fold increases in the excretion of the metabolites of 11-deoxycortisol, 21-deoxycortisol and total glucocorticoids. Leydig-specific genes were 4.3×10^1 (*LHCGR*) and 9.3×10^0 (*HSD17B3*) times higher than control and urinary steroid profiling showed 2-fold increased excretion of the major androgen metabolites androsterone and etiocholanolone. These distinctly increased steroid metabolites were suppressed by dexamethasone, but unresponsive to hCG stimulation, supporting the role of ACTH, but not LH, in regulating tumor-specific steroid excess.

Conclusion: We report bilateral testicular tumors occurring in a patient with recurrent Cushing's disease 12 years after bilateral adrenalectomy. Using mRNA expression analysis and steroid metabolome profiling, the tumors demonstrated both adrenocortical and gonadal steroidogenic properties, similar to testicular adrenal rest tumors found in patients with congenital adrenal hyperplasia. This suggests the presence of pluripotent cells even in patients without CAH.

Introduction

Bilateral adrenalectomy is considered a definitive cure for Cushing's disease, but some patients may have residual adrenal function from post-surgical remnants or ectopic adrenal tissue (1,2). Rarely, patients with Nelson's syndrome (and high ACTH levels) after bilateral adrenalectomy, have been reported to develop testicular tumors, with variable cortisol and androgen production (3-5). Some tumors were removed due to symptoms of mass effects but detailed molecular investigations were not conducted.

In contrast, testicular adrenal rest tumors (TART) are seen in up to 94% of male patients with congenital adrenal hyperplasia (CAH), with increasing prevalence during adolescence (6,7). Elevated ACTH levels, and possibly LH, may play a role in its development (8). We recently demonstrated that TART have both adrenocortical and Leydig cell features, suggesting a pluripotent embryonic cell origin (9).

We report a rare case of bilateral testicular tumors resulting in recurrent hypercortisolism in a patient with Cushing's disease who had previously undergone bilateral adrenalectomy. We characterized the steroidogenic potential of these tumors by mRNA expression analysis and serum and urinary steroid profiling. Our findings demonstrate that the tumor tissue featured both adrenal and gonadal steroidogenic properties, resembling TART tissue found in CAH patients.

Case Report

An 11-year-old boy presented with rapid weight gain, rounded facies, and abdominal striae. He was diagnosed with Cushing's disease, and underwent trans-sphenoidal removal of a corticotrophin-producing pituitary adenoma. Recurrence occurred within the first post-operative year, and after failure of both radiotherapy and ketoconazole to control his symptoms, he underwent bilateral adrenalectomy four years later (age 15 years), resulting in undetectable serum cortisol after ACTH stimulation (<20 nmol/L). Spontaneous pubertal development ensued. One year later, he developed Nelson's syndrome (hyperpigmentation with ACTH 1089 pmol/L) and pituitary apoplexy requiring trepanation and partial extirpation. Subsequently, the pituitary tumor remained stable in size, ACTH levels ranging from 158–2921 pmol/L, while receiving hydrocortisone 25mg and fludrocortisone 0.1mg daily.

At the age of 27 years, twelve years post-adrenalectomy, he experienced increased lethargy and weight gain over a period of 6 months. He had a Cushingoid habitus and abdominal striae. His testes were nodular, hard, and enlarged bilaterally. Corticosteroid replacement was stopped, and recurrent ACTH-dependent Cushing's syndrome was confirmed biochemically (Table 1). MRI imaging showed a small, stable pituitary remnant. Ultrasound and MRI of the testes revealed a single, large right testicular tumor, and multiple left testicular tumors with a small remnant of normal tissue (Fig. 1A+B). Abdominal CT showed a small nodule (<1cm) in the left adrenal region, suggesting incomplete surgical resection or post-surgical scar tissue. Semen analysis showed azoospermia.

He underwent bilateral testicular nodulectomy, with preservation of his left residual normal testicular tissue (Fig. 1C+D). One week after surgery, early-morning 8am cortisol was undetectable

(<20 nmol/L) (Table 1). His symptoms resolved, and he was started on corticosteroid and testosterone replacement.

Materials and Methods

Detailed Materials and Methods are described in the Supplemental Appendix. Written informed consent with permission for publication was obtained from the patient, and the study was approved by the local ethics committee."

Results

Serum and urine steroid metabolite profiling, before and after surgery

Serum and urine were collected at baseline, after high-dose dexamethasone suppression test (HDDST) and after hCG stimulation. Serum cortisol and its precursors (11–deoxycortisol, 17-hydroxyprogesterone), and androstenedione were elevated at baseline, suppressed by HDDST, and unresponsive to hCG stimulation (Table 1). Levels were undetectable after surgery. Testicular vein sampling performed during surgery demonstrated excess steroid production bilaterally (Table 1).

Urine steroid metabolite profiling (Fig. 1E) showed increased excretion of glucocorticoid metabolites, androgen metabolites (etiocholanolone) as well as precursor steroid metabolites from all three steroidogenic pathways. The metabolites of 11-deoxycortisol and 21-deoxycortisol were increased up to 5-fold, while 17-hydroxyprogesterone metabolites were increased >10-fold of the upper reference range. Interestingly, DHEA, reflective of the classic androgen synthesis pathway, was low. In contrast, there was highly increased excretion of the alternative androgen pathway intermediates 3α,5α-17-hydroxyprogesterone (485 μg/24h; control group median 26 (range 5-118) μg/24h) and 11-hydroxy-androsterone (15,766 μg/24h; control 588 (181-1290) μg/24h).

By comparison, urine steroid metabolite profiling of two newly diagnosed and hence untreated adolescent patients with 21-hydroxylase deficiency demonstrated a similar pattern of increased steroid metabolites, in particular those derived from progesterone, 17-hydroxyprogesterone, 21-deoxycortisol and alternative pathway metabolites 3α,5α-17-hydroxyprogesterone and 11beta-hydroxy-androsterone. Conversely, these changes were not observed in two adult patients with Cushing's disease, who predominantly excreted increased amounts of corticosterone, 11-deoxycortisol and in particular cortisol metabolites (Fig.1E). Thus the steroid excretion pattern observed in our patient resembled a combination of both CAH and Cushing's disease.

Gene expression analysis of right testicular tumor tissue

Using qPCR quantification (Fig. 1F), the expressions of adrenal cortex-specific genes were 5.1 x 10^5 (*CYP11B1*), 1.8 x 10^2 (*CYP11B2*), and 6.3 x 10^4 (*MC2R*) times higher in the patient's tumor tissue compared to the non-steroidogenic control (fibroblast), whereas the expression for *AGTR2* was 2 times lower. Genes common to both adrenal and testis tissue were 3.8 x 10^5 (*CYP17A1*), 2.1 x 10^4 (*HSD3B2*) times higher, and Leydig cell genes were 4.3 x 10^1 (LHCGR), 9.3 (*HSD17B3*) times higher compared with non-steroidogenic control. Compared to TART samples from CAH patients that we had previously similarly analyzed (9), the patient's tumor was above the interquartile range (IQR) for

glucocorticoid-related factors (*CYP11B1*, *MC2R*), and below the IQR for androgen-related factors (*LHCGR*, and *HSD17B3*).

Discussion

In this rare case of bilateral testicular tumors causing recurrence of Cushing's syndrome after bilateral adrenalectomy, we were able to demonstrate by gene expression and steroid metabolome studies that the tumor tissue has shared adrenocortical and gonadal steroidogenic properties.

TART are historically considered to originate from adrenal rests descending together with the testes during embryonic development (8). However, we recently found evidence that they develop from pluripotent cells within the testes with adrenal and Leydig cell features (9). Our patient's tumor shared morphological and biochemical features with adrenal tissue, Leydig cell tumors, and virilizing adrenocortical tumors, illustrating the difficulty of differentiating adrenocortical cells from Leydig cells.

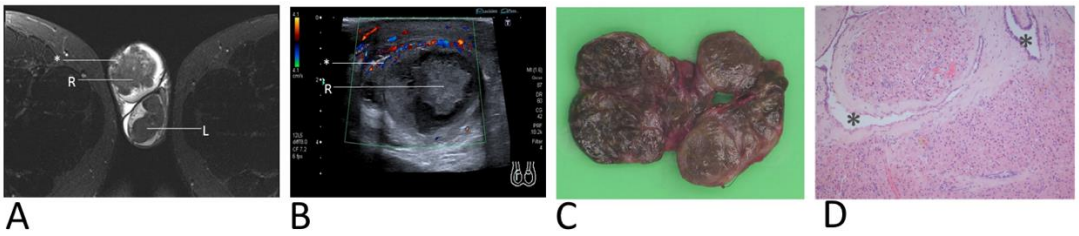
In a previous similar case, Hamwi (10) demonstrated via *in vitro* studies that the testicular tumor in a patient with recurrent Cushing's was able to produce cortisol and cortisone, although it did not produce androgens. We now demonstrated expression of adrenal-specific genes in the tumor tissue, and correlated this with increased glucocorticoid excretion. In addition, androgen excretion from both classic and alternative synthesis pathways (11,12) was significantly increased, although relative gene expression of Leydig cell-specific genes was only slightly increased. Interestingly, the steroid excretion profile of the TART tumor in this patient resembled a combination of steroid excretion in CAH and Cushing's disease with ACTH-mediated stimulation of intact steroidogenesis.

It is postulated that chronically elevated ACTH, LH and angiotensin II contribute to differentiation of the early cell (even pre-natally), and TART development (8,13,14). In our patient, response to dexamethasone, and high *MC2R* expression support the role of ACTH, that was persistently elevated. However, in this case, exposure was only post-natal, suggesting the presence of pluripotent cells within the testes also in post-natal life and adulthood. Conversely, no cases of post-bilateral adrenalectomy TART development have been described in adults older than 23 years, suggesting that regression of pluripotent cells ensues shortly after puberty.

There was relatively higher expression of *CYP11B1* and *MC2R*, but lower expressions of *HSD17B3* and *LHCGR*, compared to TART in CAH patients (9). This suggests that our patient's tumor was more adrenal-like, while TART in CAH patients are more Leydig cell-like, similar to other findings (3,10). It has also been suggested that LH surge during puberty is important for TART development and progression in CAH patients (6). Although our patient had low relative expression of *LHCGR,* and no response to hCG, he entered into puberty soon after adrenalectomy, where he was exposed to high LH levels. Hence, it remains conceivable that early pre-pubertal LH stimulation of testicular tissue contributed to TART development.

In conclusion, we present a rare case of recurrent Cushing's disease after bilateral adrenalectomy from testicular tumors with adrenal and Leydig cell-specific features. Endocrinologists should consider this possibility if symptoms recur after adrenalectomy, while patients are on maintenance

steroids. Further research is needed to understand the mechanism of development of these testicular tumors.



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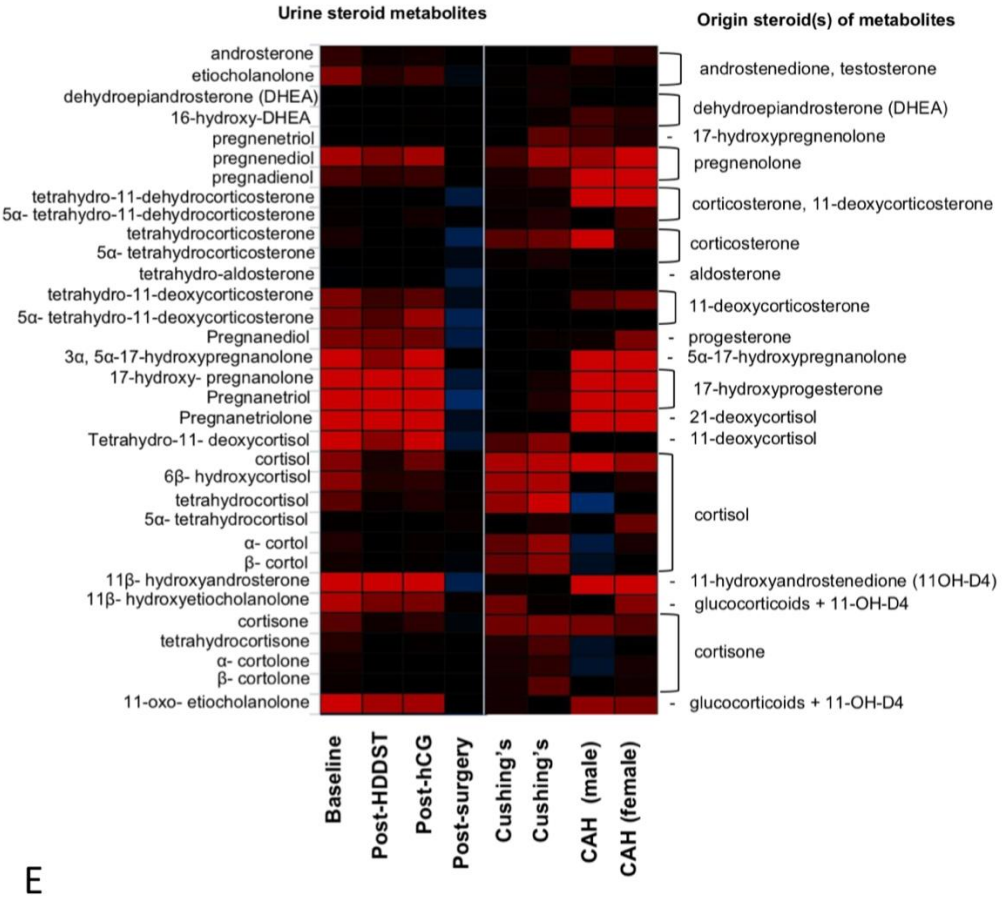

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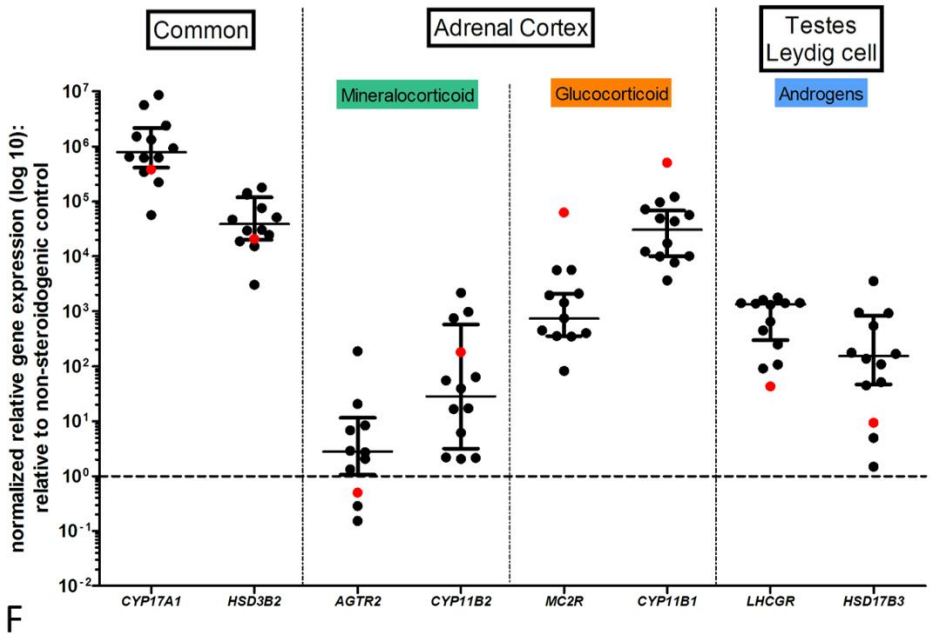

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Figure 1.

A. MRI Axial T2 turbo inversion recovery magnitude (TIRM) image showing the irregular tumor in the right testis (R), surrounded by a rim of normal testicular tissue (*) and the tumor in the left testis (L).

B. Sagittal ultrasound image showing the irregular mass in the right testis (R), surrounded by a rim of normal testicular tissue (*).

C. Macroscopically, both right and left tumors were nodular brown homogeneous tumors, partly encapsulated, measuring up to 5 respectively 6 cm.

D. Microscopically, the tumors were located in and near the rete testis (asterix). They had a multinodular aspect consisting of sheets or confluent cords of large polygonal cells with abundant eosinophilic cytoplasm, which infiltrated in between seminiferous tubules, into the rete testis and sporadically intravascular. No other criteria for malignancy (mitosis, necrosis) were met. Incomplete spermatogenesis with maturation arrest at the primary spermatocytic level was seen in the surrounding seminiferous tubules.

E. Heat map depicting results of 24h urine steroid metabolite profiling at baseline, after high-dose dexamethasone suppression test (HDDST), after hCG stimulation, and after surgical removal of testicular tumors. Red indicates excretion amounts higher than the adult male reference range, and blue indicates decreased levels. For comparison, 24 urine steroid profiling from two adult male patients with ACTH-dependent Cushing's syndrome and two adolescent patients (male on the left, female on the right) with non-classic 21-hydroxylase deficiency are also displayed.

F. Gene expression profile of 8 markers measured in Testicular Adrenal Rest Tumors (TART) of congenital adrenal hyperplasia (CAH) patients (9) (black) and the right testis tumor of the patient (red). The genes were subdivided into common genes for both adrenal cortex and Leydig cells of the testis, adrenocortical specific genes (including mineralocorticoids and glucocorticoids), and Leydig cell specific genes. Symbols and error bars in the graph represent median and 25^{th} and 75^{th} percentile of all normalized relative expression values.

Table 1 . Serum hormones and steroids during dynamic tests, testicular venous sampling and post-surgery.

	Baseline [a]	Dynamic Testing			Testicular Venous sampling [d]			Post-Surgery [e]	Reference range
		Baseline	Post-HDDST [b]	Post- hCG[c]	Peripheral vein	Left testicular vein	Right testicular vein		
24hr urinary free cortisol	1020								20 – 135 nmol/24hr
Midnight salivary cortisol	5.6								<2.2nmol/L
ACTH	193	293	181	282				1010	2.2 – 13.2 pmol/L
Renin		27	46	78				54	4.4 – 85 mU/L
FSH		0.18	0.15	0.19				4.9	1.5 – 11 U/L
LH		<0.10	<0.10	<0.10				2.8	1.4 – 8.5 U/L
Inhibin B		63.1	60.9	69.6				<10	150 – 400 ng/L
Progesterone		3.8	1.0	4.8	2.1	15	69	0.020	<1.3 nmol/L
Corticosterone		14.1	9.2	19.2	27.4	35.5	337	2.6	5.8 – 56 nmol/L
Aldosterone		<0.09	0.12	0.10	0.43	NA [f]	0.89	<0.03	0.08 – 0.69 nmol/L
17-hydroxy-pregnenolone		4.17	3.15	3.33	NA [f]	NA [f]	NA [f]	NA	0.9 – 10.5 nmol/L
17-hydroxy-progesterone		126	38	165	85	880	1780	<0.070	2.0 – 10.8 nmol/L
11-deoxycortisol		6.1	1.5	6.6	4.0	111	136	<0.17	0.2 – 4.3 nmol/L
Cortisol		530 [g]	180 [g]	570 [g]	230	2730	2650	<20	190 – 550 nmol/L
DHEA		2.4	1.3	2.4	1.7	2.1	15.1	<0.2	15 – 45 nmol/L
DHEAS		3.8	2.8	4.2	2.8	NA [f]	4.1	<0.41	1 – 7 umol/L
Androstenedione		58	21	63	36	390	790	<0.060	1.15 – 4.7 nmol/L
Testosterone		27.0	10.1	27.1	16.9	658	443	<0.1	11.0 – 45.0 nmol/L
5α-dihydro-testosterone		2.19	1.39	2.39	1.61	NA [f]	5.31	<0.098	1 – 2.9 nmol/L
Estrone		470	370	430	NA [f]	NA [f]	NA [f]	NA	80 – 250 pmol/L
Oestradiol		80	67	57	81	74	210	25	75 – 220 pmol/L
11deoxy-corticosterone		NA	NA	NA	<0.30	0.43	11.7	NA	

[a] Baseline tests were done after exogenous glucocorticoids and mineralocorticoids had been stopped for a week.

[b] Dexamethasone was administrated orally as 2mg every 6 hours for 48 hr, with investigations done at 8am on the third day, 6 hours after last dose.

[c] Daily IM injections of 1500 U hCG were administered subcutaneously on 3 consecutive days at 8 am

[d] Testicular vein sampling done during surgery. Testicular veins were cannulated prior to surgical removal. Peripheral sample was taken from a cubital vein during the sampling

[e] taken 1 week after surgery, 24 hr after last hydrocortisone dose.

[f] levels not taken due to inadequate serum volume collected.

[g] cortisol levels done with immunoassay. Other cortisol levels are measured with LCMSMS

ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; NA not available

References

1. Kemink L, Hermus A, Pieters G, Benraad T, Smals A, Kloppenborg P. Residual adrenocortical function after bilateral adrenalectomy for pituitary-dependent Cushing's syndrome. The Journal of clinical endocrinology and metabolism 1992; 75:1211-1214
2. Assie G, Bahurel H, Coste J, Silvera S, Kujas M, Dugue MA, Karray F, Dousset B, Bertherat J, Legmann P, Bertagna X. Corticotroph tumor progression after adrenalectomy in Cushing's Disease: A reappraisal of Nelson's Syndrome. The Journal of clinical endocrinology and metabolism 2007; 92:172-179
3. Johnson RE, Scheithauer B. Massive hyperplasia of testicular adrenal rests in a patient with Nelson's syndrome. American journal of clinical pathology 1982; 77:501-507
4. Krieger DT, Samojlik E, Bardin CW. Cortisol and androgen secretion in a case of Nelson's syndrome with paratesticular tumors: response to cyproheptadine therapy. The Journal of clinical endocrinology and metabolism 1978; 47:837-844
5. Ntalles K, Kostoglou-Athanassiou I, Georgiou E, Ikkos D. Paratesticular tumours in a patient with Nelson's syndrome. Hormone research 1996; 45:291-294
6. Claahsen-van der Grinten HL, Dehzad F, Kamphuis-van Ulzen K, de Korte CL. Increased prevalence of testicular adrenal rest tumours during adolescence in congenital adrenal hyperplasia. Hormone research in paediatrics 2014; 82:238-244
7. Stikkelbroeck NM, Otten BJ, Pasic A, Jager GJ, Sweep CG, Noordam K, Hermus AR. High prevalence of testicular adrenal rest tumors, impaired spermatogenesis, and Leydig cell failure in adolescent and adult males with congenital adrenal hyperplasia. The Journal of clinical endocrinology and metabolism 2001; 86:5721-5728
8. Claahsen-van der Grinten HL, Otten BJ, Stikkelbroeck MM, Sweep FC, Hermus AR. Testicular adrenal rest tumours in congenital adrenal hyperplasia. Best practice & research Clinical endocrinology & metabolism 2009; 23:209-220
9. Smeets EE, Span PN, van Herwaarden AE, Wevers RA, Hermus AR, Sweep FC, Claahsen-van der Grinten HL. Molecular characterization of testicular adrenal rest tumors in congenital adrenal hyperplasia: lesions with both adrenocortical and leydig cell features. The Journal of clinical endocrinology and metabolism 2015; 100:E524-530
10. Hamwi GJ, Gwinup G, Mostow JH, Besch PK. Activation of Testicular Adrenal Rest Tissue by Prolonged Excessive Acth Production. The Journal of clinical endocrinology and metabolism 1963; 23:861-869
11. Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, Chalder SM, Borucka-Mankiewicz M, Hauffa BP, Malunowicz EM, Stewart PM, Shackleton CH. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet 2004; 363:2128-2135
12. Swart AC, Storbeck KH. 11beta-Hydroxyandrostenedione: Downstream metabolism by 11betaHSD, 17betaHSD and SRD5A produces novel substrates in familiar pathways. Molecular and cellular endocrinology 2015; 408:114-123
13. Benvenga S, Smedile G, Lo Giudice F, Trimarchi F. Testicular adrenal rests: evidence for luteinizing hormone receptors and for distinct types of testicular nodules differing for their autonomization. European journal of endocrinology / European Federation of Endocrine Societies 1999; 141:231-237
14. Griswold SL, Behringer RR. Fetal Leydig cell origin and development. Sex Dev 2009; 3:1-15