Restoration of fertility by gonadotropin replacement in a man with hypogonadotropic azoospermia and testicular adrenal rest tumors due to untreated simple virilizing congenital adrenal hyperplasia

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Abstract

Context: Classical congenital adrenal hyperplasia (CAH), a genetic disorder characterized by 21-hydroxylase deficiency, impairs male fertility, if insufficiently treated.

Patient: A 30-year-old male was referred to our clinic for endocrine and fertility assessment after undergoing unilateral orchiectomy for a suspected testicular tumor. Histopathological evaluation of the removed testis revealed atrophy and testicular adrenal rest tumors (TARTs) and raised the suspicion of underlying CAH. The remaining testis was also atrophic (5 ml) with minor TARTs. Serum 17-hydroxyprogesterone levels were elevated, cortisol levels were at the lower limit of normal range, and gonadotropins at prepubertal levels, but serum testosterone levels were within the normal adult range. Semen analysis revealed azoospermia. CAH was confirmed by a homozygous mutation g.655A/C>G (IVS2-13A/C>G) in *CYP21A2*. Hydrocortisone (24 mg/m^2) administered to suppress ACTH and adrenal androgen overproduction unmasked deficient testicular testosterone production. As azoospermia persisted due to sustained hypogonadotropic hypogonadism, a combined s.c. gonadotropin replacement with human chorionic gonadotropin (hCG) (1500 IU twice weekly) and FSH (human menopausal gondadotropin (hMG) 150 IU three times weekly) was initiated.

Results: Normalization of testosterone levels and a stable low sperm concentration (0.5 mill/ml) with good sperm motility (85% A+B progressive) were achieved within 21 months of treatment. Despite persisting TARTs, while receiving treatment, the patient successfully impregnated his wife twice, the latter impregnation leading to the birth of a healthy girl.

Conclusions: TARTs in unrecognized (simple virilizing) CAH may lead to unnecessary orchiectomy. In hypogonadotropic, azoospermic CAH, a combined treatment with oral corticosteroids and subcutaneously administered hCG and FSH can successfully restore testicular testosterone production and fertility, even if only one hypoplastic and atrophic testis with adrenal rest tumors is present.

Introduction

Classical congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder characterized by adrenal 21-hydroxylase deficiency due to homozygous mutations in the *CYP21A2* gene (MIM 613815). The resulting enzymatic failure causes impairment of adrenal glucocorticoid and mineralocorticoid production. In contrast to the salt-wasting (SW) form, mineralocorticoid production is (partially) preserved in simple virilizing (SV) CAH. Overproduction of adrenal steroids, i.e. 17-hydroxyprogesterone (17-OHP), androstenedione, DHEAS, and testosterone, occurs in both forms, as a consequence of pituitary adrenocorticotropin (ACTH) hypersecretion. Treatment of CAH aims at suppressing ACTH secretion. If this suppression is insufficient, an ACTH-driven proliferation of testicular adrenal rest tumors (TARTs) [1, 2, 3, 4] and progressive spermatogenic tubular atrophy may ensue. These mechanisms, alongside gonadotropin suppression by adrenal androgens, can impair fertility. Herein, we report on a patient with SV CAH who remained undiagnosed and untreated for 30 years before presenting with hypogonadotropic azoospermia and TARTs.

This case report gives insight into the pathophysiology and consequences of CAH on the reproductive tract when it is left untreated for an extended period. Based on this individual case, we weigh and discuss treatment options and propose a new therapeutic approach for the restoration of fertility.

Case report

A 30-year-old Caucasian male was referred to our clinic for endocrine and reproductive evaluation after undergoing left-sided orchiectomy, as histological evaluation of the tumor raised the suspicion of possible CAH.

Methods

Informed consent was provided by the patient for all procedures conducted. Serum testosterone and 17-OHP levels were measured using commercial ELISA kits (DRG Instruments GmbH, Marburg, Germany and IBL International, Hamburg, Germany, respectively). Sex hormone-binding globulin (SHBG) levels and serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were determined using highly specific time-resolved fluoroimmunoassays (Autodelfia, Freiburg, Germany). Free testosterone levels were calculated from SHBG and total serum testosterone levels,

assuming fixed albumin levels, according to the generally accepted procedure as described previously (5). The assessment of semen samples was done according to WHO guidelines (6).

Recent medical history ▶ Unilateral orchiectomy was performed on the suspicion of a testicular tumor based on ultrasound imaging. The scans revealed two distinct regions of low echogenicity (8 and 13 mm) in the left testis and two smaller regions of low echogenicity in the right testis. In addition, bilateral testicular atrophy (i.e. testicular volumes of 5 ml) was diagnosed with sperm being absent in the ejaculate, while tests for testicular tumor markers (β-human chorionic gonadotropin (hCG) and AFP) were negative. Examination of fresh frozen sections led to the diagnosis of a 'Leydig cell tumor'. More expansive histopathological evaluation confirmed 'nodular hyperplasia of cells resembling Leydig cells' and tubular atrophy with arrest of spermatogenesis at the spermatocyte stage. The absence of mitotic figures and of Reinke's crystals gave rise to a diagnosis of a TART with a suspicion of underlying CAH.

At the time of examination, the patient was not taking any medication.

Past medical history ▶ During childhood, the patient had been treated with corticosteroids for penile enlargement for a few months. The reason for discontinuing this treatment remained unexplained. He had stopped growing at the age of 11 years. There was no history of other previous illnesses.

Physical examination ▶ Initial assessment revealed short stature (156 cm <5th percentile), obesity (76 kg; BMI: 31 kg/m^2), skin hyperpigmentation, and a single atrophic right testis.

The results of the first endocrine assessments are given in Table 1 (column 1). Specifically, 17-OHP levels were extremely elevated, cortisol levels were at the lower limit of the normal range, and gonadotropins were at low/prepubertal levels, but serum testosterone levels were within the normal range for an adult male. Semen analysis revealed azoospermia.

Molecular genetic testing ▶ Sequencing of the 21-hydroxylase gene (*CYP21A2*) revealed a homozygous mutation g.655A/C>G (IVS2-13A/C>G, often referred to as I2G). Quantitative PCR confirmed the presence of two

Table 1 Hormone values at the diagnosis of CAH and during 5 years of treatment.

	1	2	3	4	5	6	7
	At CAH diagnosis at the age of 30 years	After 6 months of HC treatment	After 11 months of HC treatment	During HC+TU treatment for 2 years	During HC+hCG treatment for 1 year	After 3 months of HC+hCG+ FSH treatment	After 21 months of HC+hCG+ FSH treatment
17-OHP (0.1–4.9 nmol/l)	73 684	2578	153	27–188*	35–158	188	153
Cortisol (50–250 nmol/l)	67.5			77–216			185
Androstenedione (0.7–3.6 ng/ml)	41			10			
DHEAS (770–5280 ng/ml)				2510–2770			
Testosterone (>12 nmol/l)	27.2	16.5	6.4	14–19	10.0	14.3	22
Free testosterone (>250 pmol/l)	881.2	463.9	183		289		662
LH 0 min (2–10 U/l)	0.1	0.2	0.4	<0.1	≪0.1	≪0.1	≪0.1
FSH min (1–7 U/l)	0.3	0.3	1.3	≪0.1	≪0.1	4.7	2.6
Testicular volume (single) (12–35 ml)	5			5		5	7

ND, not determined; HC, hydrocortisone; TU, testosterone undecanoate; 17-OHP, 17-hydroxyprogesterone; hCG, human chorionic gonadotropin.
*, varying

functional *CYP21A2* alleles, a finding also supported by the heterozygous polymorphism g.2691A>G. *CYP21A2* sequencing of the patient's wife revealed no pathogenic mutations. During genetic counseling, the patient and his wife were informed that there was no increased risk of a child with CAH.

Diagnosis, therapy, and results ▶ A combination of the patient's history, endocrine assessment, and molecular genetic testing established the diagnosis of SV CAH.

Hydrocortisone treatment (12 mg/m^2 p.o. per day, divided into three doses, with the morning dose being highest) was initiated, which markedly reduced adrenal androgen overproduction. A normal level of 17-OHP could not be achieved, despite increasing the dose to 24 mg/m^2 daily. Despite hydrocortisone treatment, gonadotropin levels continued to exhibit a sustained suppression. A drop in serum testosterone levels below the normal adult range unmasked the adrenal origin of the previously normal serum testosterone levels (Table 1, columns 2 and 3). As the patient exhibited symptoms of androgen deficiency, i.e. a decrease in libido and a depressive mood, testosterone undecanoate 1000 mg was injected intramuscularly every 3 months. Consequently, gonadotropin levels remained suppressed (Table 1, column 4). After 2 years of testosterone replacement, the patient expressed his wish for a child. To overcome the azoospermia, testosterone substitution was stopped and, in the absence of adequate serum LH levels, the endogenous testicular testosterone production by the Leydig cells was stimulated by s.c. injections of hCG 1500 IU

twice weekly. This successfully restored testosterone (of testicular origin) to near-normal levels (Table 1, column 6). However, hCG treatment hampered any re-establishment of a normal endogenous gonadotropin secretion by the pituitary (Table 1, column 5). After a year of hCG treatment, FSH (hMG) 150 IU, injected subcutaneously three times weekly, was added to the regimen, and resulted in the initiation of spermatogenesis after 3 months.

Assessment of semen samples, provided after 3–7 days of abstinence, revealed a continuous improvement of
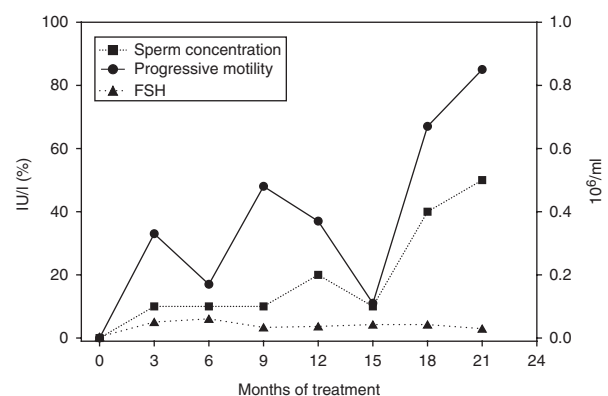
Figure 1

Increase in sperm concentration (mill/ml) and progressive sperm motility (A+B progressive) during 21 months of gonadotropin (hCG and FSH) replacement therapy. Peak sperm count achieved at 21 months was stable during the following 2 years of continued therapy.
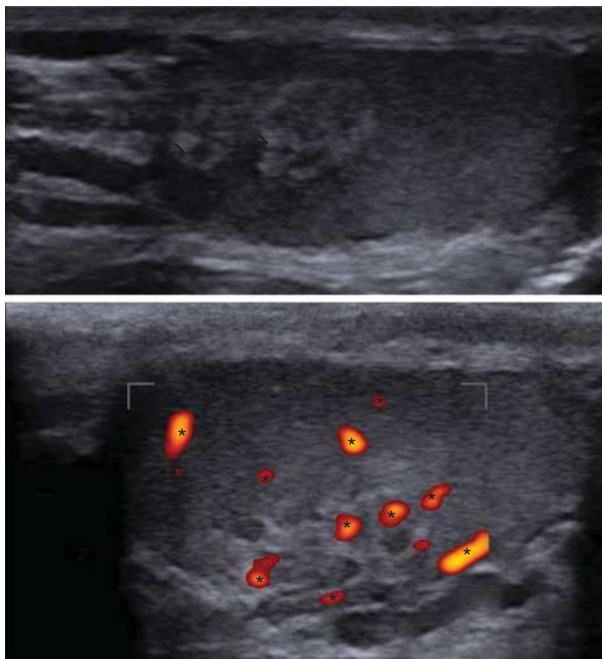
Figure 2

Longitudinal ultrasound image (B-mode) of the remaining right testis (upper panel): TARTs, presenting as hypoechogenic regions with hyperechogenic margins in the rete testis (→). Color Doppler (lower panel): homogenous perfusion of the testis (*), no hyperperfusion in the TARTs. Full colour version of this figure available via http://dx.doi.org/10.1530/EJE-13-0449.

sperm quality (Fig. 1), culminating in a stable but low sperm concentration (0.5 mill/ml) with excellent motility (85% A+B progressive) and normal morphology (11% normal forms) after 21 months of the combined s.c. gonadotropin (hCG/FSH) treatment (Table 1, column 7). Growth of the remaining testis to a volume of 11 ml was observed. Despite the persistence of TARTs (Fig. 2), the patient spontaneously impregnated his wife twice during the course of the third year of gonadotropin treatment. The first pregnancy ended in miscarriage after amniocentesis in the 16th week of gestation, and the second progressed uneventfully, resulting in the birth of a healthy girl.

Discussion

This report illustrates the possible consequences, if no treatment is provided, on the male reproductive tract in SV CAH, namely gonadotropin suppression, but paradoxically normal virilization due to the presence of high levels of adrenal androgens, testicular growth failure, hyperplasia of TARTs, and arrested spermatogenesis. This case also highlights a successful alternative treatment option (i.e. gonadotropin replacement) that can restore fertility, if classical CAH treatment fails.

Genetics

One of the most common CYP21A2 mutations (g.655A/C>G (IVS2-13A/C>G), often referred to as I2G) was found to be homozygous in our patient. It arises through gene conversion from the pseudogene and introduces a new splice acceptor leading to aberrant splicing (7, 8). Although homozygous I2G leads to SW CAH in the majority of cases (9), in our patient it caused only SV CAH.

Clinical aspects of SV CAH

In SV CAH, the defect of 21-hydroxylase enzymatic function does not cause symptomatic mineralocorticoid deficiency. Therefore, our patient was not affected by SW during infancy. The latent glucocorticoid insufficiency was compensated by pituitary ACTH hypersecretion; consequently, despite the lack of treatment, the patient did not suffer from any Addisonian crises. Nevertheless, he did experience precocious pseudopuberty with the attendant precocious growth spurt and a premature epiphyseal closure, which resulted in his short stature.

Normal testosterone levels despite very low gonadotropin levels were the endocrine situation found in our patient at the age of 30 years in the absence of any treatment, indicating that testosterone was of adrenal origin, caused by adrenal androgen overproduction in the presence of 21-hydroxylase deficiency.

Testicular adrenal rest tumors

Our case illustrates that TARTs may be misdiagnosed as a Leydig cell tumor, but may also uncover 21-hydroxylase deficiency. This is consistent with findings that in 18% of patients with no classical CAH, a correct diagnosis is not made until or after the development of a testicular mass (10). The presence of bilateral tumors, absence of metastases, and a decrease in tumor size following glucocorticoid therapy provide clinical clues pointing toward the correct diagnosis (11, 12).

Heterotopic masses of the adrenal cortex in patients with CAH may be present not only in the mediastinum

testis, but also in the spermatic cord and within the epididymis (13, 14). These tumors are characterized as TARTs, developing due to chronic ACTH stimulation (15, 16). Already present in 3.5% of male neonates with CAH (17), TARTs may later present as painful, irregular, firm, or enlarged testes (18), the volume of which may be mistaken for normal pubertal testicular growth (1). Upon ultrasound imaging, they present as unilateral or bilateral (19) hypoechogenic multifocal areas adjacent to the mediastinum, 0.2–4 cm in diameter, with blurred margins, usually avascular, rarely hypervascular. Histologically, TARTs resemble Leydig cell tumors, but do not contain Reinke's crystals (15). The similarities between the conditions are such that they can lead to unnecessary surgical intervention, i.e. orchiectomy.

This case shows that spermatogenesis can recover, even after long-standing uncontrolled CAH and persisting TARTs. This is all the more surprising, considering the description of the histological stages in uncontrolled CAH with TARTs (20), where hypertrophy and hyperplasia of adrenal rest cells are reversible only in the early stages. If left uncontrolled, further growth would lead to the compression of the rete testis, peritubular fibrosis, and hyalinization, with focal lymphocytic infiltrates, indicating an irreversible stage of damage to the testicular parenchyma.

Hormonal changes and fertility impairment in poorly controlled male CAH

Our case further illustrates that if untreated for an extended period, CAH can result in an impairment of the hypothalamic–pituitary–gonadal axis. The patient's testes were peripubertal in size; therefore, it can be assumed that the impairment was already present during adolescence, resulting in absent testicular growth and spermatogenesis. Nevertheless, there were no symptoms of androgen deficiency, as the excess of adrenal steroids counterbalanced T deficiency. There are conflicting reports regarding the consequences of CAH on male reproductive function, ranging from normal fertility despite nonadherence to medication (21) to poor fertility (3, 22, 23, 24). In parallel to gonadotropin suppression by excessive adrenal androgens (21, 25, 26), there is evidence that endogenous estrogens (such as estrone) that originate from the peripheral aromatization by adrenal androgens (such as androstenedione) (27) may also act on the hypothalamus to inhibit GNRH secretion and on the pituitary to decrease the responsiveness to GNRH (28, 29).

Hormonal treatment and outcome

Our case illustrates that even after adrenal androgen overproduction in CAH is restored to near-normality by a 'standard' glucocorticoid therapy, gonadotropin suppression and TARTs can persist, causing symptoms of androgen deficiency and persisting azoospermia.

Similar findings of low serum testosterone levels in the presence of low LH levels after reduction of adrenal androgen production by hydrocortisone replacement have been reported in CAH patients who were appropriately treated previously, but after periods of insufficient glucocorticoid therapy were again placed on controlled stringent replacement regimens (22). However, if high gonadotropin levels are found, they can be indicative of a testicular failure (22, 30). Whereas some investigators found that TARTs regressed and spermatogenesis was reactivated when glucocorticoid therapy was instituted or intensified (11, 31), others report that patients remain azoospermic (27, 32). The use of dexamethasone (0.25–0.5 mg p.o. administered in a single late-evening dose) with or without additional hydrocortisone doses (12, 33) and/or the addition of fludrocortisone are other current treatment options. The first options were not chosen due to our patient's fear of weight gain and other symptoms of Cushing's syndrome. Fludrocortisone was not considered, as an elevation of the patient's blood pressure was a concern.

Gonadotropin (hCG/FSH) replacement

Our case illustrates that low testicular testosterone production due to sustained LH suppression can be successfully normalized by hCG therapy, despite the persistence of TARTs and even at a very late stage of disease after 30 years with no treatment. The LHCG receptor in Leydig cells is stimulated by hCG due to its structural homology to LH (34). However, by exerting a negative feedback on the pituitary, this treatment hampered the reappearance of endogenous gonadotropin secretion. Therefore, a combined s.c. hCG and FSH treatment was required to fully replace the deficient gonadotropins and to initiate spermatogenesis. This therapy needed to be performed for a period of 21 months to reach a stable sperm count in the ejaculate, the sperm quality remaining unchanged with a persisting low sperm count after 21 months of continued gonadotropin replacement. The low sperm concentration, found in our patient, seemed to be compensated by enhanced sperm quality, as it enabled a spontaneous conception twice,

without any need for assisted reproductive techniques. Remarkably, this fertility was achieved from a single hypoplastic and atrophic testis and even though TARTs regressed only partially.

Surgery

Surgical removal of TARTs by testis-sparing surgery, to decompress the mediastinum testis and to abolish obstruction of the seminiferous tubules, has been shown to be unsuccessful in improving semen quality (35).

Artificial reproductive technology

In cases where patients remain azoospermic, a combination of testicular sperm extraction and ICSI can constitute a viable treatment option (36).

Conclusions

Untreated SV 21-hydroxylase deficiency has to be kept in mind as a cause of hypogonadotropic hypogonadism. In male patients with CAH, attention needs to be paid to testicular function not only during childhood and adolescence but throughout life, in concert with a sustained ACTH-suppressive and thus 17-OHP-suppressive treatment. The inhibition of adrenal androgen overproduction may prevent the impairment of gonadotropin secretion and thus prevent testicular growth failure during puberty and azoospermia. Moreover, adequate CAH treatment may prevent the development of adrenal rest tumors. However, our case illustrates that if an adequate treatment is established only at a late stage of untreated or insufficiently treated disease, additional gonadotropin substitution may be an option to restore fertility and to enable spontaneous conception.

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