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High Prevalence of Testicular Adrenal Rest Tumors, Impaired Spermatogenesis, and Leydig Cell Failure in Adolescent and Adult Males with Congenital Adrenal Hyperplasia

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In male patients with congenital adrenal hyperplasia, testicular tumors, or so-called adrenal rest tumors, have been described, but their presence in well controlled patients is thought to be rare. In this study, the prevalence of testicular tumors in 17 adolescent and adult male patients with congenital adrenal hyperplasia (age, 16–40 yr) was investigated. In 16 of 17 patients, one or more testicular tumors, ranging in maximal length from 0.2–4.0 cm, were found on ultrasonography. In 6 patients, the testicular tumors were palpable. Undertreatment, defined as the presence of a salivary androstenedione level (mean of 6 saliva samples collected over 24 h with intervals of 4 h) above the upper reference morning level, was found in 5 of 17 patients at the time of investigation. The other 12 patients were treated adequately or even overtreated at the time of investigation. Nevertheless, 11 of these 12 patients showed testicular tumors on ultrasonography. Neither the presence of undertreatment at the time of investigation nor characteristics of the therapeutic regimen (daily dose of hydrocortisone equivalents per body surface, the use of glucocorticoid medication either two or three times a day, or the time of taking the highest glucocorticoid dose either in the morning or the evening) could predict tumor size (maximal diameter of largest tumor). In patients who were heterozygous or homozygous for the deletion or conversion of the CYP21 gene, tumor size was significantly larger than in patients who did not have this genotype. Impairment of Leydig cell function as manifested by decreased plasma levels of T was found in 6 of 17 patients. Semen analysis in 11 patients revealed azoospermia in 3 patients and poor semen quality in 4 patients. We conclude that, when carefully sought for, testicular adrenal rest tumors are frequently present in adolescent and adult males with congenital adrenal hyperplasia and are often accompanied by impaired spermatogenesis and Leydig cell failure. (*J Clin Endocrinol Metab* 86: 5721–5728, 2001)

IN PATIENTS WITH congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency, the synthesis of cortisol, and in most cases also of aldosterone, is impaired. Consequently, the secretion of ACTH by the pituitary gland is increased, resulting in hyperplasia of the adrenal cortex and production of high amounts of adrenal androgens. In affected females, prenatal exposure to excess androgens results in virilization of the external genitalia. In both sexes, during childhood excess androgens cause rapid linear growth and bone age advancement resulting in short stature. Treatment of CAH consists of substitution of cortisol and aldosterone, thereby suppressing adrenal androgen overproduction (1).

The presence of testicular tumors, so-called adrenal rest tumors, in male patients with CAH due to 21-hydroxylase deficiency was already described in 1940 by Wilkins *et al*. (2). Histologically, testicular adrenal rest tumors resemble Leydig cell tumors with features on electron microscopy consistent with steroid secreting cells (3). However, unlike Leydig cell tumors they never contain Reinke crystalloids (4, 5).

Testicular adrenal rest tumors can be the first manifestation of CAH. Rutgers *et al.* (6) reviewed 40 cases of testicular adrenal rest tumors in CAH and reported previously undiagnosed CAH in 18% of these cases. Especially in these patients, such tumors can be misdiagnosed as Leydig cell tumors. However, features like a young age, bilateral presence of tumors, absence of metastases, and decrease in size with glucocorticoid therapy may lead to biochemical investigation and DNA analysis confirming the diagnosis of CAH (6). However, in most patients the diagnosis of CAH is made before the testicular tumors become manifest.

The prevalence of testicular adrenal rest tumors in patients with CAH and their impact on fertility are insufficiently known. In the published studies, usually involving only small numbers of patients, prevalence of testicular tumors between 0 and 47% has been reported, dependent on selection of patients and method of detection (palpation *vs.* ultrasonography) (7–11). There are also conflicting results with respect to fertility of male CAH patients. Urban *et al.* (7) described five (periodically) untreated male CAH patients who were normally fertile. Other authors, however, reported poor fertility in male CAH patients (11–15).

These conflicting results prompted us to investigate the prevalence of testicular tumors and their impact on testicular function in a group of 17 adolescent and adult male patients with CAH who were treated in our center. Here, we describe that, when carefully sought for, such tumors are frequently present in these patients and are often accompanied by impaired spermatogenesis and Leydig cell failure.

Abbreviations: CAH, Congenital adrenal hyperplasia; WHO, World Health Organization.

Patients and Methods

Patients

All 17 male patients aged 16 yr or older, who were regularly seen in our center for treatment of 21-hydroxylase deficiency, were included in this study (Table 1). Two of them were monozygous twin brothers (no. 3 and 4), and two others were brothers (no. 9 and 14). Diagnosis of 21-hydroxylase deficiency was confirmed by DNA analysis in all patients. Fourteen patients had the classic salt-wasting form of CAH, characterized by both glucocorticoid and mineralocorticoid deficiency (1, 16). These 14 patients were diagnosed within the first year of life and were treated from the time of diagnosis with glucocorticoids and mineralocorticoids. They were followed up regularly with biochemical and anthropometrical measurements. In the other three patients (no. 2, 11, and 16), CAH was diagnosed later in childhood, because of signs of androgen excess. These three patients were classified as classic simple virilizing CAH patients. Age at diagnosis was 6.2 yr (no. 2 and 16) and 2.9 yr (no. 11), respectively. Glucocorticoid therapy was started from the time of diagnosis, with regular follow-up, as in the classic salt-wasting patients. In patients 2 and 11, treatment with mineralocorticoids was started 5 and 10 yr after initial diagnosis, respectively, after demonstration of increased levels of plasma renin activity (no. 2) and hyponatraemia during salt depletion (no. 11). Two patients (no. 1 and 2) were already known to have testicular tumors before participation in this study. In patient 1, the diagnosis of testicular adrenal rest tumor had been confirmed by pathological examination of a testicular biopsy. Patient 7 had fathered one daughter at the age of 27. The age of the 17 patients was 21.8 ± 6.5 yr (mean ± SD; range, 16.6–40.8 yr). Fifteen of the 17 patients were younger than 24 yr. Height was 1.73 ± 0.07 m (range, 1.61–1.88 m) and body mass index was 24.1 ± 3.2 kg/m^2 (range, 20.0–32.0 kg/m^2). Doses of glucocorticoid and mineralocorticoid substitution at the time of the study are given in Table 1. For comparison, doses of glucocorticoids were converted to hydrocortisone equivalents (30 mg hydrocortisone = 0.75 mg dexamethasone). The daily dose of hydrocortisone equivalents at the time of the study was 25.4 ± 5.7 mg (13.7 ± 3.1 mg/m^2).

Methods

Physical examination and ultrasonography of the testes. All patients underwent physical examination by an experienced endocrinologist to detect palpable testicular tumors. Grayscale and color Doppler ultrasonography were obtained in the longitudinal and transverse planes by using an anterior approach. All ultrasonographic examinations were performed by a staff radiologist (G.J.J.) with experience in scrotal ultrasound. Measurements of testicular size were made on frozen images. Testicular volume was calculated by ultrasonography using the formula: $V = L \times W \times D \times 0.52$, where V is the testis volume (ml), L is the maximal testicular length (cm), W is the maximal width (cm), and D is the maximal depth (cm). The normal range for testicular volume in a population of young adult men is 6.0–31.8 ml (17).

Plasma levels of T, serum levels of LH and FSH, and semen analysis. After overnight fasting, in all patients venous blood sampling was performed at 0900 h to measure basal levels of T, LH, and FSH. Patients had taken their regular doses of glucocorticoids and mineralocorticoids on the day before blood sampling and, in addition, had taken 1 mg dexamethasone at 2300 h. On the day of blood sampling, 0.5 mg dexamethasone was taken at 0730 h instead of the regular morning dose of glucocorticoids. Patients did take their regular mineralocorticoid medication. In 11 patients on a separate day, routine semen analysis was performed according to the World Health Organization (WHO) guidelines (18).

Levels of salivary 17-hydroxyprogesterone and androstenedione. Saliva sampling was done 6 d (median, range 4–20 d) after blood sampling for T, LH, and FSH determinations, at 0800, 1200, 1600, 2000, 2400, and 0400 h (next day), while the patients used their normal glucocorticoid medication. Saliva was collected by salivation into a plastic cup. In all saliva samples, 17-hydroxyprogesterone and androstenedione were determined, and mean levels of 17-hydroxyprogesterone and androstenedione in these six saliva samples were calculated. Undertreatment was defined as the presence of a mean level of salivary androstenedione above the upper reference morning (0800 h) level, *i.e.* more than 0.63 nmol/liter. Overtreatment was defined as the presence of a mean level of salivary androstenedione below the lower reference morning (0800 h) level, *i.e.* lower than 0.14 nmol/liter (19).

Hormone assays. Plasma T was measured by RIA after a paper chromatographic purification step (20). The normal range for plasma T in adult males is 11–45 nmol/liter. Serum LH and FSH were quantitatively determined (AxSYM, Abbott Laboratories, Abbott Park, IL). The normal range for serum LH in adult males is 1.4–8.5 U/liter and for serum FSH is 1.5–11 U/liter. 17-Hydroxyprogesterone and androstenedione in saliva were determined by RIA after a paper chromatographic purification step, as described earlier (19). In males, the normal range for the morning (0800 h) level of salivary 17-hydroxyprogesterone is 0.05–0.36 nmol/liter and for the morning (0800 h) level of androstenedione is 0.14–0.63 nmol/liter (19).

Statistical analysis. Statistical analyses were performed using Mann-Whitney *U* test (*P* values denoted by *P*) and Spearman's rank correlation test (*P* values denoted by *P**). Mean values ± SD are given. $P < 0.05$ was considered significant.

Results

Physical examination

In 6 of the 17 patients, palpable testicular tumors were found, either solitary or multiple nodules or irregular nodular masses (no. 1–6). These tumors were bilaterally palpable in 4 patients (no. 1, 2, 3, and 5) and unilaterally palpable in 2 patients (no. 4 and 6). In 11 patients, there were no palpable tumors (patients 7–17).

Ultrasonography of the testes

Results of testicular ultrasound investigations are presented in Table 2 and shown in Figs. 1 and 2. Testicular volume ranged from 4.2–26.7 ml (mean ± SD, 11.7 ± 5.2 ml). In 16 patients, testicular size was within the normal range, and in 1 patient (no. 11) the size of the left testis was below the normal range.

Table 2 shows the sizes of testicular tumors on ultrasonography. All tumors detected by palpation were confirmed by ultrasonography. The maximal diameters of these palpable tumors ranged from 2.8–4.0 cm. In the two cases with only one abnormal testis on palpation (no. 4 and 6), a tumor was also found in the other testis. In addition, ultrasonography revealed tumors in 10 of 11 patients in whom palpation was negative, resulting in a 37.5% sensitivity of clinical evaluation against ultrasonography. The maximal diameter of the nonpalpable tumors ranged from 0.2–1.6 cm. In four cases, these nonpalpable tumors were bilateral. In five of the six patients with nonpalpable unilateral tumors, the tumor was located in the left testis. In all 16 patients, the tumors seemed to originate from the mediastinum testis, although large tumors were not confined to this region and covered the major part of the testis.

Plasma levels of T and serum levels of LH

Plasma T levels were below the normal range in 6 patients and normal in the 11 other patients (Table 3). Basal serum LH levels were decreased in 4 patients (no. 3, 4, 10, and 15). Administration of 0.1 mg GnRH caused no response of serum LH in patient 3, whereas in patients 4, 10, and 15 maximal increases of serum LH of 4.6, 22.0, and 25.7 U/liter, respectively, were observed.

TABLE 1. Age at the time of investigation, height, phenotype, results of DNA analysis and glucocorticoid and mineralocorticoid therapy at the time of investigation in 17 male patients with 21-hydroxylase deficiency

Patient no.	Age (yr)	Height (m)	Phenotype	Allele 1[a]	Allele 2[a]	Daily glucocorticoid therapy (mg/m^2)[b]	Daily mineralocorticoid therapy (μg)[c]
1	34.8	1.78	SW	Del/con	IVS2-13A/C>G	10.2 (D 0.125 + 0.375 mg)	62.5
2	23.0	1.81	SV	Del/con	1001T>A (I172N)	11.9 (HC 15 + 10 mg)	125.0
3	22.9	1.69	SW	Del/con	Del/con	17.8 (HC 20 + 10 mg)	62.5
4	22.9	1.71	SW	Del/con	Del/con	17.1 (HC 10 + 20 mg)	125.0
5	17.3	1.73	SW	Del/con	IVS2-13A/C>G	9.0 (HC 8 + 4 + 4 mg)	62.5
6	16.6	1.64	SW	Del/con	Del/con	18.6 (HC 10 + 10 + 15 mg)	125.0
7	40.8	1.74	SW	Del/con	Del/con	13.7 (D 0.5 + 0.125 mg)	187.5
8	21.6	1.61	SW	Del/con	Del/con	12.7 (HC 10 + 5 + 5 mg)	312.5
9	21.3	1.88	SW	708-715del8	1382T>A (I236N), 1385T>A (V237E), 1391T>A (M239K)	12.1 (HC 15 mg + D 0.25 mg)	125.0
10	20.7	1.69	SW	IVS2-13A/C>G	IVS2-13A/C>G	12.3 (HC 10 + 10 + 5 mg)	62.5
11	19.7	1.73	SV	1001T>A (I172N)	2110C>T (R356W)	15.9 (HC 10 + 20 mg)	125.0
12	19.3	1.69	SW	2110C>T (R356W)	2110C>T (R356W)	8.2 (HC 6 + 3 + 6 mg)	125.0
13	18.9	1.75	SW	IVS2-13A/C>G	2110C>T (R356W)	16.2 (HC 10 + 10 mg + D 0.3 mg)	62.5
14	18.3	1.80	SW	708-715del8	1382T>A (I236N), 1385T>A (V237E), 1391T>A (M239K)	15.1 (HC 10 + 5 + 15 mg)	62.5
15	17.9	1.75	SW	1382T>A (I236N), 1385T>A (V237E), 1391T>A (M239K)	2110C>T (R356W)	15.8 (HC 7 + 7 + 14 mg)	187.5
16	17.0	1.65	SV	IVS2-13A/C>G	1001T>A (I172N)	14.9 (HC 10 + 5 + 10 mg)	
17	16.9	1.72	SW	IVS2-13A/C>G	IVS2-13A/C>G	11.3 (HC 10 + 5 + 5 mg)	93.75

SW, Classic salt wasting; SV, classic simple virilizing; HC, hydrocortisone; D, dexamethasone.

[a] Nucleotides are numbered according to the Higashi's functional CYP21 sequence (37).

[b] When dosed twice daily, glucocorticoid medication was taken before 0900 h and between 1800 and 2300 h; when dosed three times a day, glucocorticoid medication was taken before 0900 h, between 1200 and 1630 h, and between 2000 and 2300 h.

[c] Mineralocorticoid medication (9-α-fluorohydrocortisone acetate) was taken in one to three doses (total dose given in table).

TABLE 2. Testicular volumes and sizes of testicular tumors (both determined by ultrasonography) in 17 male patients with 21-hydroxylase deficiency

Patient no.	Testis volume (ml)[a]		Tumor size (cm)[b]	
1	R: 9.4	L: 9.9	R: 2.8 × 1.4 × 1.4	L: 2.8 × 1.4 × 1.3
2	R: 13.7	L: 11.7	R: 3.2 × 2.3 × 1.9	L: 3.7 × 2.3 × 1.4
3	R: 9.6	L: 11.7	R: 3.0 × 2.0 × 2.0	L: 4.0 × 2.5 × 2.0
4	R: 12.9	L: 11.2	R: 3.9 × 1.9 × 1.3	L: 2.7 × 2.2 × 1.7
5	R: 12.8	L: 9.8	R: 3.9 × 2.2 × 2.2	L: 3.8 × 3.8 × 2.0
6	R: 9.4	L: 8.2	R: 3.0 × 0.8 × 0.8	L: 1.8 × 0.7 × 0.6
7	R: 8.8	L: 9.1	R: 0.5 × 0.3 × 0.3	L: 1.1 × 0.5 × 0.5
8	R: 9.4	L: 9.6	R: —	L: 0.2, 0.2 and 0.2 (3 tumors)
9	R: 24.2	L: 20.3	R: —	L: 1.2 × 0.6 × 0.4
10	R: 14.2	L: 13.9	R: 0.9 × 0.7 × 0.5	L: 1.6 × 0.5 × 0.5
11	R: 6.9	L: 4.2	R: —	L: 0.3
12	R: 14.2	L: 14.7	R: 1.5 × 0.7 × 0.5	L: —
13	R: 9.7	L: 8.2	R: —	L: 0.2
14	R: 24.9	L: 26.7	R: —	L: —
15	R: 7.5	L: 9.3	R: 0.6	L: 0.4, 0.3 and 0.2 (3 tumors)
16	R: 6.8	L: 8.9	R: 0.5 × 0.5 × 0.3	L: 0.7 × 0.5 × 0.3
17	R: 7.7	L: 8.8		L: 0.4 × 0.2 × 0.2 and 0.2 × 0.2 × 0.2 (2 tumors)

R, Right; L, left; —, no tumor present.
[a] Testicular volumes were calculated as described in *Patients and Methods*. The normal range for testicular volume is 6.0–31.8 ml (17).
[b] Sizes of testicular tumors are given in centimeters (length × width × depth), except for patients no. 8, 11, 13, and 15, where only the maximal diameter (centimeters) of the tumor(s) is given.

There was a significant positive correlation between serum LH levels and plasma T levels ($r = 0.57$; $P^* < 0.02$). All four patients with a serum LH level below the normal range had plasma T levels below the normal range. Two other patients with plasma T levels below the normal range had normal serum LH levels.

Semen analysis and serum levels of FSH

Semen analysis could be performed in 11 patients (Table 4). Azoospermia was found in three patients, all of them had palpable tumors. In four patients, semen analysis revealed oligoasthenoteratozoospermia. In these four patients, the maximal diameter of testicular tumors ranged from 0.2–3.7 cm. Normozoospermia was found in four patients (no. 8, 9, 14, and 15). Three of these patients had testicular tumors, ranging in maximal diameter from 0.2–1.2 cm. The best result on semen analysis was found in patient 14, who did not have any testicular tumors on ultrasonography.

Serum FSH levels were within the normal range in 12 patients (Table 3). Patients 3 and 4 had serum FSH levels below the normal level (<0.2 and 0.6 U/liter, respectively). In both patients, administration of 0.1 mg GnRH caused no response of serum FSH. Both patients had serum LH levels below normal as well. Serum FSH levels above the normal range were found in patients 1, 6, and 7.

All three patients with azoospermia had abnormal serum FSH levels, either decreased (no. 3) or increased (no. 1 and 6). All four patients with normozoospermia had normal FSH levels. Of four patients with oligoasthenoteratozoospermia, only one (no. 7) had an abnormal (elevated) serum FSH level.

Levels of salivary 17-hydroxyprogesterone and androstenedione

Mean values of the six salivary 17-hydroxyprogesterone and androstenedione determinations in the individual patients are shown in Table 3. The mean levels of salivary 17-hydroxyprogesterone and androstenedione were highly correlated ($r = 0.88$; $P^* < 0.001$). At the time of the investigation, five patients (no. 2, 3, 4, 10, and 17) appeared to be undertreated, and three patients (no. 7, 9, and 13) were overtreated. The other nine patients were treated adequately.

Relationship between hormonal control and the tumor size

Neither the presence of undertreatment at the time of investigation nor characteristics of the therapeutic regimen (daily dose of hydrocortisone equivalents per body surface, the use of glucocorticoid medication either two or three times a day, or the time of taking the highest glucocorticoid dose in either the morning or the evening) could predict tumor size (maximal diameter of largest tumor).

Relationship between suppression of salivary androstenedione and levels of plasma T, serum LH, and serum FSH

The relationship between the mean level of salivary androstenedione and the level of plasma T, serum LH, and serum FSH is represented in Fig. 3, A–C. Figure 3A shows a negative correlation between salivary androstenedione levels and plasma T levels ($r = -0.58$; $P^* < 0.02$). In four of the five patients with a mean salivary androstenedione level above the normal range, T levels were below normal (3.2–7.0 nmol/liter). Figure 3B shows a negative correlation between salivary androstenedione levels and serum LH levels ($r = -0.54$; $P^* < 0.03$). Of the five patients with an elevated mean salivary androstenedione level, three had LH levels below the normal range. Figure 3C shows a negative correlation between salivary androstenedione and serum FSH levels ($r = -0.55$; $P^* < 0.03$). Increased levels of FSH were found in three patients with levels of salivary androstenedione within the normal range. Decreased FSH levels were seen in two patients with elevated salivary androstenedione levels.

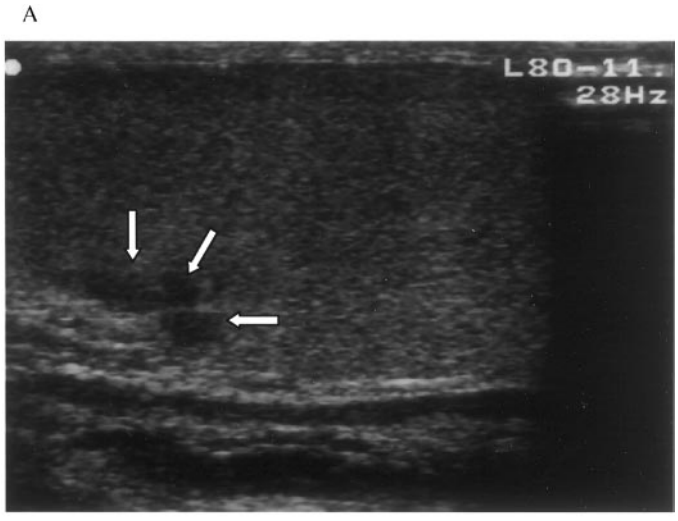

FIG. 1. Small adrenal rest tumors in a 17.9-yr-old male patient (no. 15) with 21-hydroxylase deficiency. A, Longitudinal ultrasonographic image of the left testis in the plane of the mediastinum showing three small well delineated conflating tumors (*arrows*). The tumors are hypoechoic compared with the normal testicular tissue. Their maximal lengths were 0.4, 0.3, and 0.2 cm, respectively. B, Ultrasonographic image obtained in the transverse plane. Note that the tumors are located around the mediastinum, which is visible as a small white line (*curved arrow*).

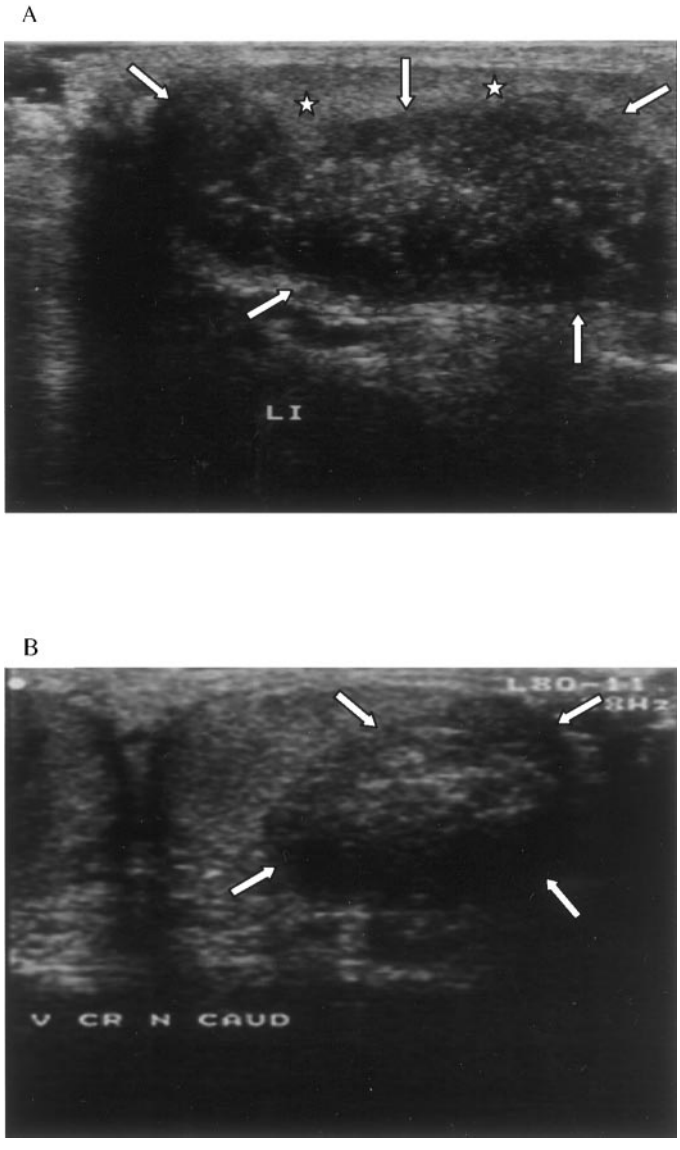

FIG. 2. Large palpable adrenal rest tumor in a 17.3-yr-old male patient (no. 5) with 21-hydroxylase deficiency. A, Longitudinal ultrasonographic image of the left testis showing a well delineated mass with marked irregular echo texture (*arrows*). Note that only a small rim of normal testicular tissue is visible (*stars*). B, Ultrasonographic image obtained in the transverse plane. The tumor measured 3.8 × 3.8 × 2.0 cm.

Relationship between genotype and tumor size

Table 1 shows the results of genotyping. In patients who were heterozygous or homozygous for the deletion or conversion of the CYP21 gene, tumor size was significantly larger than in patients who did not have this genotype ($P < 0.02$).

Discussion

In this study we have demonstrated that the prevalence of testicular tumors in adolescent and adult male patients with CAH is surprisingly high. Palpation of the testes revealed testicular tumors in 6 of 17 patients. These palpable tumors were bilateral in 4 of them and were already found in a patient as young as 16 yr old. In 10 of the 11 patients without palpable abnormalities, ultrasonography revealed tumors ranging in diameter from 0.2–1.6 cm. Therefore, the absence of palpable tumors does not imply that no testicular adrenal rest tumors are present.

In prenatal life, the adrenals develop in the immediate vicinity of the gonads, and separation of both does not evolve until the adrenal groove becomes prominent. Before that moment, adrenal cortical tissue may adhere to the gonad. This aberrant adrenal tissue may then descend with the testis or ovary along the course of their supplying arteries (21–24). Therefore, it is possible that the testicular tumors of CAH originate from aberrant adrenal tissue. Biochemical studies *in*

TABLE 3. Plasma levels of testosterone, serum levels of LH and FSH, mean salivary levels of 17-hydroxyprogesterone (17-OHP) and androstenedione (adione) in 17 male patients with 21-hydroxylase deficiency

Patient no.	Plasma T (nmol/liter)	Serum LH (U/liter)	Serum FSH (U/liter)	Mean salivary 17-OHP (nmol/liter)[a]	Mean salivary adione (nmol/liter)[a]
1	27.0	7.2	12.1	0.01	0.15
2	6.6	4.0	5.7	3.87	1.75
3	3.3	<0.2	<0.2	7.37	3.38
4	3.2	0.8	0.6	5.90	3.48
5	10.0	2.6	4.5	0.71	0.23
6	12.0	7.2	15.2	1.08	0.20
7	14.0	3.8	14.4	0.02	0.12
8	37.0	2.1	3.4	0.57	0.29
9	12.0	1.8	1.9	0.01	0.06
10	7.0	0.9	2.3	4.91	1.23
11	12.0	5.6	10.8	0.78	0.15
12	11.0	1.8	2.2	0.42	0.26
13	19.0	7.4	6.1	0.04	0.09
14	11.0	2.2	2.3	1.25	0.34
15	10.0	1.2	5.2	0.08	0.17
16	16.0	1.9	5.3	0.53	0.24
17	18.0	2.2	3.9	4.02	1.00
Normal values	11.0–45.0	1.4–8.5	1.5–11.0		

[a] Salivary levels of 17-OHP and adione are mean levels from six samples (see *Patients and Methods*).

TABLE 4. Results of semen analysis, according to 1999 WHO guidelines in 11 male patients with 21-hydroxylase deficiency

Patient no.	Semen volume (ml)[a]	Sperm concentration ($\times 10^6$/ml)[a]	Motile spermatozoa (%)[a,b]	Normal spermatozoa (%)[a,c]	Classification[d]
1	0.6	0			A
2	2.3	10	65	10	OAT[e]
3	0.7	0			A
6	1.5	0			A[e]
7	4.3	1.8	45	23	OAT[e]
8	4.7	65	50	23	N[e]
9	3.0	50	60	47	N
13	0.8	7	0	12	OAT[e]
14	2.2	>250	40	49	N
15	3.0	35	75	27	N
16	1.8	4	4	20	OAT[e]
Normal values	>2.0	>20	>50	>15	

[a] According to 1999 WHO guidelines (18).
[b] Motile spermatozoa: the percentage of rapidly and slowly moving spermatozoa (grades a and b) to the total number of spermatozoa counted, normal value 50% or more.
[c] Normal spermatozoa: the percentage of morphologically normal spermatozoa to the total number of spermatozoa counted, normal value 15% or more.
[d] Classification: A, azoospermia; OAT, oligoasthenoteratozoospermia; N, normozoospermia.
[e] Confirmed with minimal two analyses.

vitro and *in vivo* support this hypothesis by showing adrenal specific 11β-hydroxysteroids in the testicular tumors (4, 25–27).

Testicular adrenal rest tumors are ACTH-dependent in that they may develop during periods of sustained elevation of plasma ACTH levels and may regress when glucocorticoid therapy is instituted or intensified (6, 28, 29). Another argument for ACTH dependency of these tumors is the fact that they may also occur in other patient groups characterized by elevated plasma ACTH levels, such as Nelson's syndrome (30, 31) and Addison's disease (32). These observations have led to the hypothesis that poor hormonal control and inadequate suppression of ACTH secretion is a dominant etiological factor in the development of the testicular masses in CAH. Observations about poor therapeutic compliance in many CAH patients with testicular adrenal rest tumors are in line with this hypothesis (3, 4, 6, 26–29, 33).

In the present study, however, 11 patients showed testicular adrenal rest tumors, despite adequate treatment or even overtreatment at the time of investigation. Although the latter finding does not prove that hormonal control was adequate over the years, we feel that our patients have been well treated, considering their intensive follow-up regimen and their acceptable final height. The present study and other reports showing development of testicular tumors despite good hormonal control (8, 9, 34, 35) suggest that undertreatment is not the only cause of the high prevalence of testicular tumors in these patients.

To find other factors that might influence the development and growth of testicular adrenal rest tumors, the effects of parameters like the daily dose of hydrocortisone equivalents, the use of glucocorticoid medication either two or three times a day, or the time of taking the highest glucocorticoid dose in either the morning or the evening on tumor size were

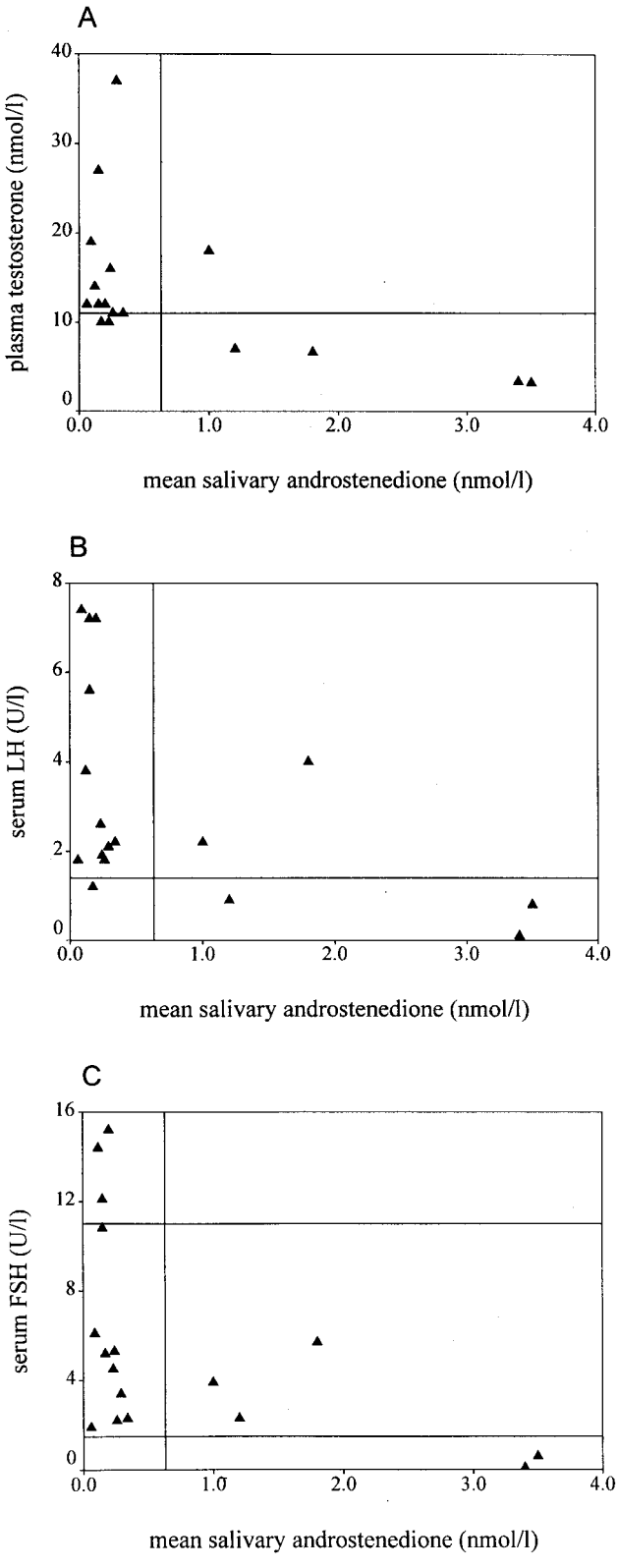

FIG. 3. Relationship between mean levels of salivary androstenedione (see *Patients and Methods*) and plasma levels of T (A), serum levels of LH (B), and serum levels of FSH (C). *Reference lines* represent the upper normal morning level of salivary androstenedione (A–C), the lower normal level of plasma T (A) and serum LH (B), and the upper and lower normal levels of serum FSH (C).

analyzed, but none of these parameters could predict tumor size. However, a significant effect on tumor size was demonstrated for genotype; in patients who were homozygous or heterozygous for deletion or conversion of the CYP21 gene, tumor size was significantly larger than in patients who did not have this genotype. Deletion or conversion of the CYP21 gene is associated with complete absence of enzyme activity (1). The functional significance of this finding is unclear, but one might hypothesize that when the CYP21 gene is absent in both alleles, resulting in complete absence of enzyme activity, plasma ACTH levels are already extremely elevated in early prenatal life, possibly contributing to the development of the testicular adrenal rest tumors.

In our CAH patients, not only anatomical lesions but also impaired function of the testes was found. Testicular dysfunction was demonstrated by decreased levels of plasma T in 6 of 17 patients, and poor semen quality was demonstrated in 7 of 11 patients. This could be caused by the testicular tumors themselves interfering directly with the function of normal testicular tissue in a mechanical way or by local steroid production (5, 26). Alternatively, at the hypothalamic-pituitary level, the secretion of gonadotropins may be suppressed by high levels of adrenal androgens that are aromatized peripherally or in the central nervous system to estrogens (5, 13, 14, 16, 34). In our patients, we found indications for both mechanisms. The combination of an elevated salivary androstenedione level and decreased levels of serum LH and plasma T, suggesting suppression of the pituitary-gonadal axis at the level of the hypothalamus or the pituitary gland, was found in three patients. Two patients, who had large palpable tumors, had a decreased level of plasma T and normal serum LH levels. In these patients, a local negative effect of the tumor on the normal testicular tissue possibly contributed to impaired Leydig cell function.

Sperm production was impaired in 7 of 11 patients tested, 3 patients even showing azoospermia. In two of the azoospermic patients, serum FSH levels were increased, indicating primary testicular dysfunction. In the other patient with azoospermia, serum levels of both LH and FSH were undetectably low, both before and after GnRH administration, suggesting hypogonadotropism as the cause of testicular dysfunction. Our data demonstrate that normal serum levels of FSH do not imply normal semen production in these patients. Thus, to assess the semen production, measurement of serum FSH is not sufficient, and semen analysis should be proposed to these patients. When azoospermia is found in combination with a large testicular tumor on ultrasonography, it is likely to have a mechanical cause, especially when the tumor is located in the mediastinum. At this location, large tumors can easily compress the rete testis and cause obstructive azoospermia (36).

The preferred method of treatment of testicular adrenal rest tumors and/or impaired spermatogenesis in patients with CAH is intensifying glucocorticoid therapy. This may lead to decrease of tumor size and improvement of testicular function. When the tumor is unresponsive to steroid therapy, surgical treatment should be considered, preferably by a testis-sparing procedure, instead of orchiectomy (5). Also, cryopreservation of the semen can be offered, because fertility prognosis is yet uncertain.

We conclude that the prevalence of testicular tumors in male CAH patients is high, despite adequate treatment. Testicular function, both semen production and T secretion, may be impaired in these patients, especially when large testicular tumors are present. Early detection and treatment of testicular adrenal rest tumors should be of primary concern, especially in patients who are heterozygous or homozygous for deletion or conversion of the CYP21 gene, because they are most at risk for developing large tumors. Ultrasonography to detect testicular tumors should be proposed to them and to other male patients with CAH, and semen analysis should be performed when this is appropriate for age.

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