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Testicular Adrenal Rest Tumors and Leydig and Sertoli Cell Function in Boys with Classical Congenital Adrenal Hyperplasia

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Context: Infertility observed in adult males with congenital adrenal hyperplasia (CAH) has been associated with testicular adrenal rest tumors (TART) that may originate during childhood.

Objective: Our objective was to describe the prevalence of TART and Sertoli and Leydig cell function in a group of boys aged 2–10 yr with CAH and to compare prevalence with that of a control group.

Design: From August 2005 to January 2007, 19 patients with classical CAH (CAH group) were referred from seven endocrinology centers.

Methods: We studied 19 subjects in the CAH group and, as a control group, 13 boys from the community that did not have testicular diseases. A complete physical exam was performed. High-resolution ultrasound was used to determine TART prevalence. Inhibin B and anti-Müllerian hormone were used as Sertoli cell markers. The ratio between basal testosterone levels and testosterone levels 72 h after β-human chorionic gonadotropin (5000 U/m^2) treatment [(T_{72} − T_0)/T_0] was used to evaluate Leydig cell response.

Results: CAH and control groups were comparable in chronological age (5.9 *vs.* 5.6 yr; $P = 0.67$) and bone age/chronological age ratio (1.09 *vs.* 1.03; $P = 0.09$). TART prevalence was four of 19 (21%) in the CAH group. Lower values for inhibin B (49.2. *vs.* 65.2 pg/ml; $P = 0.018$), anti-Müllerian hormone (70.1 *vs.* 94.2 ng/ml; $P = 0.002$), and (T_{72} − T_0)/T_0 (5.6 *vs.* 13.6; $P < 0.01$) were observed in the CAH group.

Conclusion: TART in prepubertal males with classic CAH could be found during childhood. We also report differences in markers of gonadal function in a subgroup of patients, especially in those with inadequate control. **(*J Clin Endocrinol Metab* 92: 4583–4589, 2007)**

FERTILITY RATES OF males with congenital adrenal hyperplasia (CAH) are reduced compared with the normal population (1). Available data suggest that the most frequent cause of infertility in CAH patients is associated with testicular adrenal rest tumors (TART) (2). TART have been hypothesized to be adrenal in origin. Development of the primitive adrenal cortex occurs close to the gonads, and TART is considered to be an aberrant adrenal tissue that has descended with the testes (3). In some animal models, both LH and ACTH can regulate testicular steroidogenesis in fetal Leydig cells (4). Recently, Val *et al.* (5) found that adrenal-like cells that migrate into the testis respond to ACTH and express Cyp11b1 and Cyp21. Pretumor development and growth of these cells is assumed to be ACTH dependent, and under-treatment may play an important role in tumor development. TART have also been reported in other conditions that exhibit high levels of ACTH, such as Addison's (6) and Nelson's (7) syndromes.

In CAH patients, not only have anatomical lesions been found, but impaired function of the testes and hypogonadotropic hypogonadism due to chronic suppression of gonadotropin secretion, both caused by overproduction of adrenal androgens, have also been observed (8). Most fertility problems in CAH could have originated during childhood. Otten *et al.* (9) suggested that prevention of subfertility should be implemented as a treatment goal in pediatric endocrinology at early puberty.

To optimize the fertility of adults with CAH, it is necessary to improve knowledge of the natural history of TART and testicular function during childhood. In this article, we describe the prevalence of TART and Leydig and Sertoli cell function in a group of 19 prepubertal boys with classical CAH.

Subjects and Methods

Subject group (CAH)

From August 2005 to January 2007, 19 patients from seven endocrinology centers with classical CAH were invited to participate. All male patients were 2–10 yr old and were nonconsanguineous. Three patients had prenatal diagnoses of CAH. In 15 patients, the diagnosis was suspected during hypovolemic shock associated with hyponatremia and hyperkalemia where 17-hydroxyprogesterone (17-OHP) was more than 2000 ng/ml at the median age of 21 d (range, 11–57 d); in one patient (no. 7), CAH was diagnosed at the age of 2 yr because of signs of androgen excess and cyclic vomiting syndrome.

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Abbreviations: A2, Androstenedione; AMH, anti-Müllerian hormone; BA, bone age; BMI, body mass index; CA, chronological age; CAH, congenital adrenal hyperplasia; CV, coefficients of variation; DHEA-S, dehydroepiandrosterone sulfate; β-hCG, β-human chorionic gonadotropin; HSDS, height SDS; Inh-B, inhibin B; 17-OHP, 17-hydroxyprogesterone; PRA, plasma renin activity; SDS, SD score; T, testosterone; TART, testicular adrenal rest tumors; THSDS, target height SDS; US, ultrasonography.

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Eighteen patients had the classic salt-wasting form of CAH, characterized by both glucocorticoid and mineralocorticoid deficiency. Patient 7 presumably had a classic simple virilizing form of CAH; however, he had persistently elevated plasma renin activity, suggesting the need for extra salt and mineralocorticoid therapy. For this reason, all patients were treated with a median cortisol dose of 12.6 mg/m^2·d (range, 7.8–17) and a median 9-fluorohydrocortisone acetate dose of 0.1 mg (range, 0.05–0.2). Patient 19 did not receive mineralocorticoid treatment during the 6 months before this study for financial reasons.

None of the patients had been previously evaluated for TART.

Control group

The control group included boys from the community; many of them were classmates or neighbors of patients in the subject group. The exclusion criteria were consanguinity with the patients, testicular or inguinal pathology, or treatment with cortisol or any drugs that could interfere with steroidogenesis.

Study protocol

The boys were evaluated at the Pediatrics Endocrinology Office at the Pontificia Universidad Catolica de Chile, with a complete physical exam performed by a pediatric endocrinologist. Height was measured using a wall-mounted stadiometer. Weight was measured using a manual scale with a 10-g gradation (Seca). Height and body mass index (BMI) were converted into SD scores (SDS) to adjust for chronological age (CA) using National Center for Health Statistics growth reference charts (10), which are applicable to the Chilean population (11). The height SDS (HSDS) was corrected for target height SDS (THSDS): HSDS − THSDS. Pubarche was assessed using the method of Tanner (12). Testicular volume was calculated with a Prader orchidometer and testicular palpation. Penis length (cm) was measured from the pubic ramus to the tip of the glans penis by placing the end of a straight edge ruler against the pubic ramus.

At baseline, a hand radiograph was obtained to determine bone age (BA) using the method of Greulich and Pyle (13). Color Doppler ultrasonography (US) was performed by two experienced pediatric radiologists (C.G. and R.P.) using a high-resolution 12-mHz transducer. Measurements of testicular size were made from frozen images. Testicular and tumor volumes were calculated by US using the ellipse formula: $V = L \times W \times D \times 0.52$, where V is the volume (ml), L is the maximal length (cm), W is the maximal width (cm), and D is the maximal depth (cm). This reading was performed by two observers who were blind to the patient's status (C.G. and R.P.).

The tests began between 0800 and 0900 h. A basal blood sample was taken for analysis of 17-OHP, androstenedione (A2), dehydroepiandrosterone sulfate (DHEA-S), FSH, LH, testosterone (T), plasma renin activity (PRA), inhibin B (Inh-B), and anti-Müllerian hormone (AMH).

Serum Inh-B and AMH were used as markers of Sertoli cell function. Interstitial Leydig cell function was evaluated by measuring serum T under basal conditions (T_0) and 72 h (T_{72}) after im administration of 5000 IU/m^2 β-human chorionic gonadotropin (β-hCG) (Pregnyl; Organon, Roseland, NJ), as previously described (14). The T level at 72 h after Pregnyl (T_{72}) treatment was determined in part by the basal level of T (T_0); a high basal level may indicate inadequate control and give a smaller response to β-hCG and lower ratio $(T_{72} - T_0)/T_0$. For this reason, we presented both the maximal values and the ratio $(T_{72} - T_0)/T_0$ to evaluate how many times the testicular steroidogenic response increased over the basal level. Blood samples were separated immediately after collection, and the plasma was stored at −70 C until analysis.

Hormone assays

Serum Inh-B was assayed using an ELISA kit (DLS-10-84100; Diagnostic Systems Laboratories, Webster, TX). This assay has a sensitivity limit of 7 pg/ml, and the interassay and intraassay coefficients of variation (CV) were 6.7 and 4.6%, respectively. Serum AMH was assayed using the AMH/MIS ELISA kit (DLS-10-14400; Diagnostic Systems), and this assay has a sensitivity limit of 0.006 ng/ml. The interassay and intraassay CV were 6.5 and 3.4%, respectively. FSH and LH were measured by immunoradiometric assays (ACS-Centaro; Bayer, Tarrytown, NY). The detection limits were 0.6 and 0.8 mIU/ml, respectively. The interassay CV were 5.54% for FSH and 7.6% for LH. The serum 17-OHP level was determined by RIA (Diagnostic Products Corp., Los Angeles, CA). The detection limit was 0.1 ng/ml. The interassay CV was 8%. The serum DHEA-S level was determined by a competitive chemiluminescence immunoassay (Immulite 2002; Diagnostic Products). The detection limit was 0.3 μg/ml, and the interassay CV was 8.56%. Serum A2 was determined by RIA (Diagnostic Systems). The detection limit of the RIA was 0.1 ng/ml, whereas the interassay CV was 7.6%. Serum T was determined by an electrochemiluminescent immunoassay (Modular Analytics E 170; Roche, Indianapolis, IN), which had a detection limit of 10 ng/dl and an interassay CV of 3.4%. PRA was determined by RIA (DiaSorin, Stillwater, MN). The detection limit of the PRA was 0.2 ng/ml·h, whereas the interassay CV was 6.7%.

Molecular methods

Genomic DNA was isolated from peripheral blood leukocytes of CAH patients by standard procedures. The mutations Pro30Leu, I2 splice, G110Δ8nt, Ile172Asn, Cluster E6, Val281Leu, Leu306 + 1nt, Gln318stop, Arg356Trp, and Pro453Ser were genotyped by allele-specific PCR, as previously described; in Chilean patients with classical CAH, about 80% of the mutated alleles are identified with this method (15).

Ethics

The protocol for this study was approved by the Ethical Committee of the Pontificia Universidad Catolica de Chile. Parents of all children participating in the study gave signed informed consent.

Statistical analysis

Results are expressed as medians and ranges. Statistical analyses were performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). Differences between CAH and control groups were assessed by nonparametric tests (Mann-Whitney *U* tests) for variables that were not normally distributed. Spearman's rho was used to determine the associations among Leydig cell function, 17-OHP, Inh-B, and AMH. P values < 0.05 were considered statistically significant.

Results

Thirty-two boys were examined, 19 of whom were patients with CAH. Clinical characteristics and DNA analyses of the patients in the CAH group at the beginning of the study are shown in Table 1.

Anthropometrics and physical examinations

At recruitment, the CA (in years) and heights (SDS) were not different between the two groups. In the CAH group, the BMI (SDS) and penis length were greater than those in the control group. No tumors were detected by palpation. Three patients with CAH had pubarche (patient 7, Tanner II; patient 9, Tanner III; patient 18, Tanner II) (Table 2).

Testicular US

The median testicular volume was 0.6 ml (with a range of 0.27–1.29 ml) and 0.78 ml (with a range of 0.35–1.29 ml; $P = 0.065$) in the CAH and control groups, respectively. In the CAH group, ultrasound found seven lesions in four of the 19 patients (bilateral lesions in three subjects). The prevalence of TART in CAH was 21%. No masses were found in the control group. The maximal diameter of lesions varied from 0.5–1.0 cm. They were all located adjacent to the mediastinum testis and were all hypoechoic and had ill-defined margins (Fig. 1). Patient 6 had right and left testicular volumes of 0.96 and 0.42 ml, respectively; TART were identified in the

TABLE 1. Phenotype, CA, BA, HSDS, results of DNA analysis, and glucocorticoid and mineralocorticoid therapy at the time of investigation in 19 male patients with CAH

Patient no.	Phenotype	CA (yr)	BA (yr)	HSDS	HSDS − THSDS[a]	DNA analysis	TART by US	HC ($mg/m^2 \cdot d$)	Florinef (mg/d)
1	SW	2.8	2.2	0.03	1.31	Arg356Trp/ND	No	9.8	0.10
2	SW	3.7	5.7	1.32	1.79	ND/ND or ND/Del or LGC[b]	No	12.0	0.10
3	SW	2.8	3.1	0.77	0.54	Gln318stop/ND	No	13.0	0.15
4	SW	6.3	7.5	−0.58	0.31		No	12.0	0.10
5	SW	6.5	7.0	−0.98	−0.72		No	7.8	0.15
6	SW	8.5	11.5	−0.13	1.38	ND/ND or ND/Del or LGC[b]	Yes	10.0	0.10
7	SV	7.3	7.9	−0.15	1.91	I2 splice/ND	Yes	12.0	0.10
8	SW	7.6	8.2	−0.35		ND/ND or ND/Del or LGC[b]	No	10.8	0.10
9	SW	6.8	12.5	2.21	3.72	G110Δ8nt/G110Δ8nt or G110Δ8nt/Del or LGC[b]	Yes	12.6	0.20
10	SW	2.8	2.3	−0.86	0.93	ND/ND or ND/Del or LGC[b]	No	11.6	0.05
11	SW	5.4	6.3	0.92	0.20	Arg356Trp/Arg356Trp o Arg356Trp/Del or LGC[b]	No	13.0	0.15
12	SW	5.9	6.8	1.13	−0.30	Gln318stop/ND	No	15.9	0.10
13	SW	3.7	6.2	0.51	1.22	G110Δ8nt/G110Δ8nt o G110Δ8nt/Del or LGC[b]	No	15.0	0.10
14	SW	4.3	4.5	0.60	1.83	Gln318stop/Gln318stop o Gln318stop/Del or LGC[b]	No	13.3	0.10
15	SW	4.1	6.0	1.08	3.28	I2 splice/ND	No	14.3	0.10
16	SW	6.7	6.0	−0.79	0.86	Gln318stop/ND	No	15.0	0.10
17	SW	6.7	7.2	0.25	0.16	Gln318stop/ND	No	9.3	0.10
18	SW	9.6	12.5	0.99	0.83	ND/ND or ND/Del or LGC[b]	Yes	13.0	0.05
19	SW	5.1	5.0	−1.22	−0.40	I2 splice/ND	No	17.0	

Patients 6, 7, 9, and 18 had TART. Patient 19 had his mineralocorticoid treatment suspended by his father for financial reasons. Patients 4 and 5 have not yet undergone molecular study. In patient 8, the target height was not available. Del, Deletion; HC, hydrocortisone; LGC, large gene conversion; ND, not detected; SV, classic simple virilizing; SW, classic salt wasting.

[a] Height is expressed as SDS and corrected for THSDS (HSDS − THSDS).

[b] The method cannot be used to distinguish between a deletion and a large gene conversion.

right testis, with a maximal diameter (and volume) of 0.7 × 0.2 × 0.1 cm (0.007 ml). Patient 7 had right and left testicular volumes of 0.75 and 0.7 ml, respectively; TART were identified in both testes with a maximal diameter (and volume) of 1.0 × 0.3 × 0.4 cm (0.06 ml) and 0.8 × 0.3 × 0.3 cm (0.04 ml), respectively. Patient 9 had right and left testicular volumes of 1.38 and 1.2 ml, respectively; TART were identified in both testes with a maximal diameter (and volume) of 0.6 × 0.3 × 0.4 cm (0.04 ml) and 0.5 × 0.3 × 0.3 cm (0.02 ml), respectively. Patient 18 had right and left testicular volumes of 0.7 and 0.9 ml, respectively; TART were identified in both testes with a maximal diameter (and volume) of 0.6 × 0.8 × 0.4 cm (0.1 ml) and 0.8 × 0.8 × 0.4 cm (0.13 ml), respectively.

Hormone profile

17-OHP was higher in the CAH group [6.6 (range, 0.1–556) ng/ml)] compared with the control group [0.8 (0.2–1.1) ng/ml; $P = 0.007$; Fig. 2A]. No significant differences were found in basal T (however, all the individuals in the control group had basal T < 10 ng/dl) [10 (10–163) *vs.* <10 ng/dl; $P = 0.32$; Fig. 2B], A2 [0.2 (0.1–15.8) *vs.* 0.3 (0.1–0.4) ng/ml; $P = 0.97$], DHEA-S [<0.3 *vs.* 0.3 (0.3–0.97) μg/ml; $P = 0.28$], FSH [0.6 (0.6–2.2) *vs.* 0.6 (0.6–2.9) mIU/ml; $P = 0.97$], LH [<0.8 *vs.* <0.8 mIU/ml; $P = 1.0$], or PRA [1.5 (0.2–75) *vs.* 2.9 (1.3–11) ng/ml·h; $P = 0.108$] between the CAH and control groups.

In the CAH group, four patients were currently under control as characterized by basal elevated T (in pubertal range) and high levels of 17-OHP (patients 2, 6, 9, and 13), two of them had TART (patients 6 and 9). The other two subjects with TART (patients 7 and 18) had a basal T less than 10 ng/dl with a 17-OHP of 2.7 and 62.5 ng/ml, respectively. The accelerated BA in patient 18 (Table 1) suggests that he was probably under control for a long time.

Leydig cell function

After 72 h of β-hCG treatment, the maximal T level was lower in the CAH group than in the control group [78 (33–403) *vs.* 143 (60–413) ng/dl; $P = 0.02$; Fig. 2B]. Moreover, the ratio between $(T_{72} - T_0)/T_0$ was lower in the CAH group [5.6 (−0.26–39.3)] compared with the control group [13.3 (5.0–40); $P < 0.01$; Fig. 3A].

Serum levels of Inh-B and AMH

Serum Inh-B was significantly lower in the CAH group [49.2 (17.7–89.9) pg/ml] compared with the control group

TABLE 2. Anthropometrics and data from physical examinations at recruitment

	CA (yr)	BA/CA ratio	HSDS	HSDS − THSDS[a]	BMI (SDS)	Pubarche (yes)	Penis length (cm)	Median TV (ml)
CAH (n = 19)	5.9 (2.8–9.6)	1.09 (0.76–1.85)	0.25 (−1.2–2.1)	0.9 (−0.7–3.7)	1.28 (−0.2–4.4)	3	5.4 (3.6–7.0)	2.5 (1.5–3.0)
Control (n = 13)	5.6 (2.5–9.2)	1.03 (0.8–1.6)	0.12 (−2.1–2.9)	1.16 (−0.9–1.7)	0.09 (−0.97–1.56)	0	4.0 (3.0–5.3)	2 (2.0–3.0)
P value	0.677	0.092	0.570	0.780	0.01	0.19	<0.001	0.448

Results are expressed as median (range). *P* values are from Mann-Whitney *U* or Fisher tests. TV, Testicular volume as determined by physical exam.

[a] Height is expressed as SDS and is corrected for target height SDS (HSDS-THSDS). Target height from patient was not available.

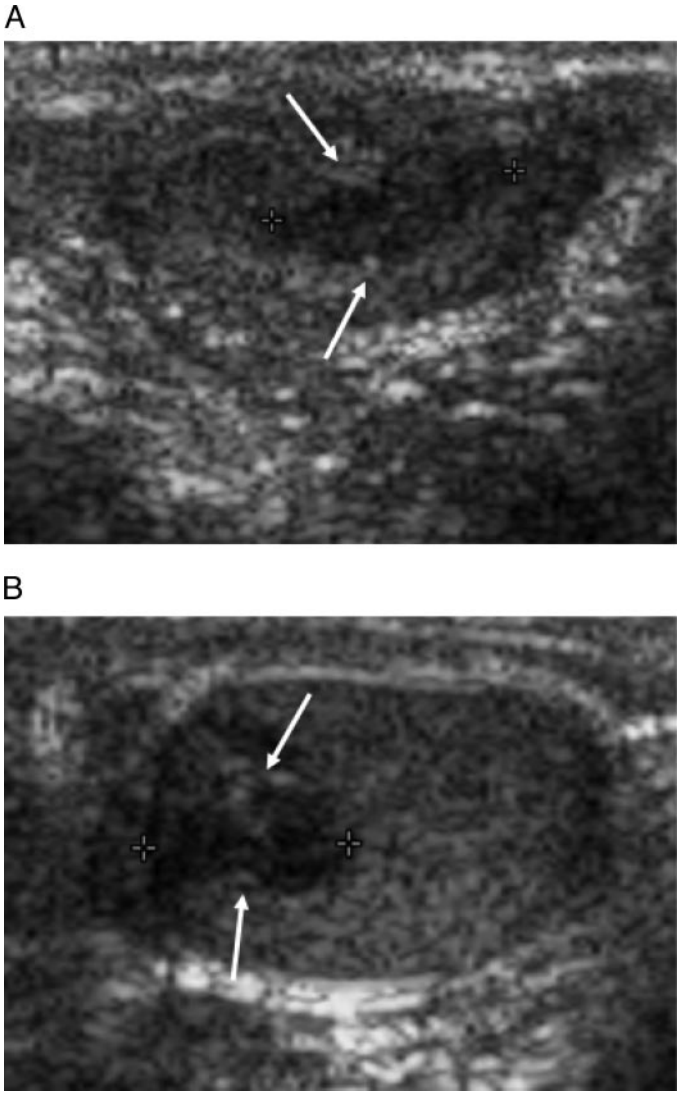

FIG. 1. Patient 18. Sagittal (A) and transverse (B) US of the right testicle show an intratesticular, hypoechoic, and ill-defined lesion, measuring 0.8 × 0.6 × 0.4 cm, that is located around the mediastinum testis (*arrows*).

[65.2 (27.2–122) pg/ml; $P = 0.018$; Fig. 3B]. Also, AMH was lower in the CAH group [70.1 (2.1–140) ng/ml] than in the control group [94.2 (47.5–307.7) ng/ml; $P = 0.002$; Fig. 3C]. In the CAH group, Inh-B and AMH were directly associated (rho = 0.556; $P = 0.013$).

Correlations between Inh-B and AMH with Leydig cell function

The associations between Leydig cell function and Inh-B (rho = 0.490; $P = 0.004$) and AMH (rho = 0.485; $P = 0.005$), as defined by the ratio $(T_{72} - T_0)/T_0$, were directly proportional in all groups (CAH and control; Fig. 4). Lower values of Inh-B and AMH were associated with lower Leydig cell function, especially in the CAH group, particularly the ones with TART. In the CAH group, a significant inverse correlation was found between Leydig cell function and 17-OHP (rho = −0.622; $P = 0.004$) but not with Inh-B (rho = −0.047; $P = 0.85$) and AMH (rho = −0.204; $P = 0.401$).

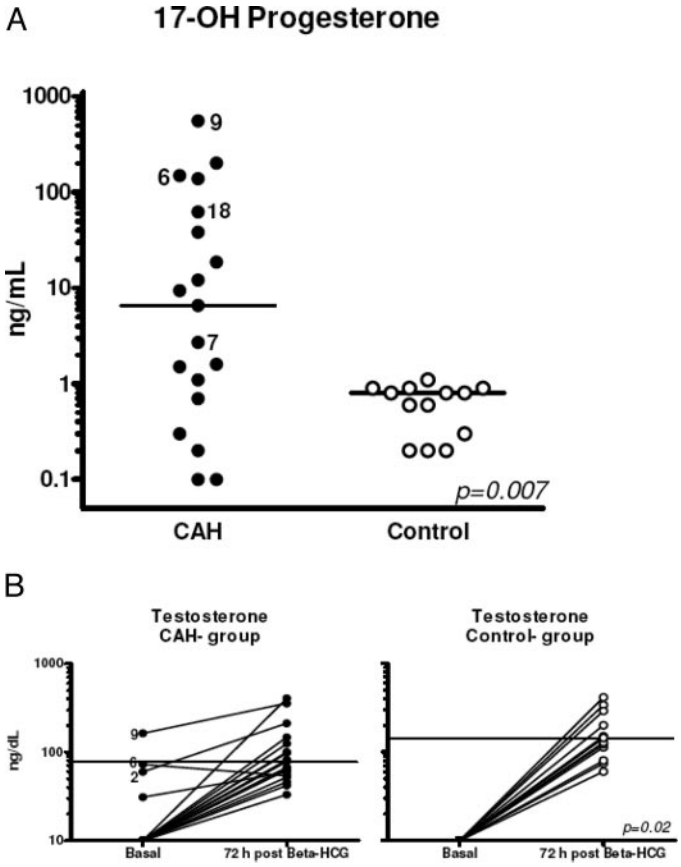

FIG. 2. 17-OHP and basal and post-β-hCG treatment levels of T in the CAH and control groups. Data are represented as individual values of 17-OHP on a scatter dot plot graphic. The *solid lines* are at the median (A). Basal T and post-β-hCG treatment levels of T are presented as *symbols* and *lines* in the graphic: ●, CAH group; ○, control group. *Lines* are at the median (B). *P* values are from the Mann-Whitney *U* test. Patients 6, 7, 9, and 18 had TART.

Clinical and hormonal status in the CAH group with and without TART

In the CAH group, the boys with TART were older than the boys without TART [7.8 (7.7–9.6) *vs.* 4.7 (2.7–6.7) yr old; $P = 0.002$] and had higher plasma levels of 17-OHP [105.7 (2.7–556) *vs.* 4.1 (0.1–201) ng/ml; $P = 0.049$]. Of the four patients with TART, three (patients 6, 9, and 18) had extremely high plasma levels of 17-OHP (148, 556, and 62 ng/ml, respectively; Fig. 2A). The BA/CA ratio [1.3 (1.1–1.9) *vs.* 1.1 (0.8–1.7); $P = 0.08$] and HSDS − THSDS [1.6 (0.8–3.7) *vs.* 0.7 (−0.7–3.3) SDS; $P = 0.079$] were similar in both subgroups.

There were no differences in the ratio $(T_{72} - T_0)/T_0$ [2.4 (−0.26–8.8) *vs.* 5.7 (0.9–39.3); $P = 0.185$] or serum Inh-B [41.9 (37.9–58.5) *vs.* 52.6 (17.7–89.9) pg/ml; $P = 0.721$] between the CAH group with TART compared with the CAH group without TART. AMH was significantly lower in the CAH group with TART compared with the CAH group without TART [40.7 (2.1–45.6) *vs.* 73.6 (34–140) ng/ml; $P = 0.009$]. Patient 9 had the lowest AMH (2 ng/ml) and the highest basal T level (163 ng/dl) in the whole group; this subject had bilateral TART and poor adherence to treatment.

Of the four patients with TART, three of them had poor

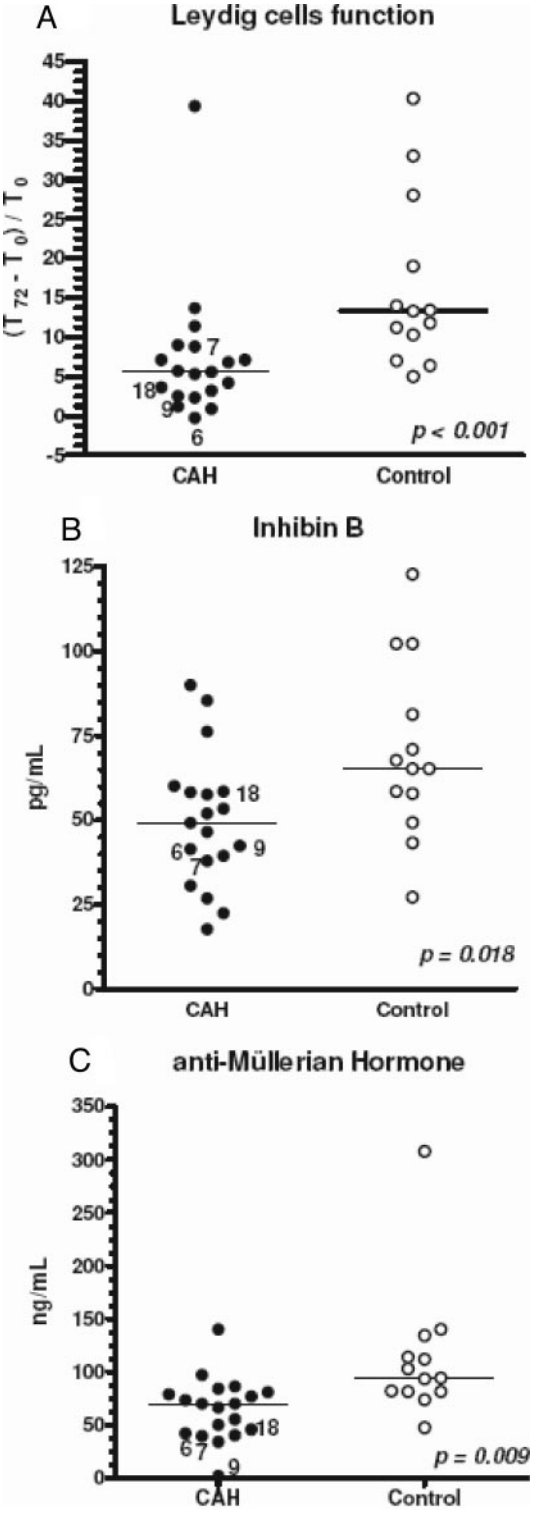

FIG. 3. Leydig and Sertoli cell function in the CAH and control groups. Data are shown as a scatter dot plot graphic. Individual values of the ratio between $(T_{72} - T_0)/T_0$ (A), Inh-B (B), and AMH (C) are shown. ●, CAH group; ○, control group. *Lines* indicate the median value. *P* values are from the Mann-Whitney *U* test. Patients 6, 7, 9, and 18 had TART.

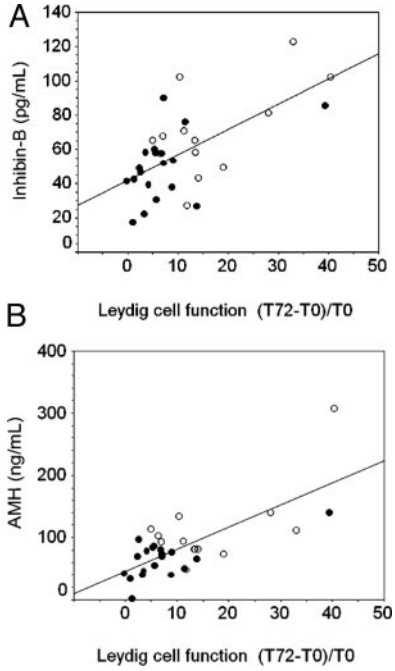

FIG. 4. Correlations between Leydig cell function and Inh-B (A) and AMH (B) in all groups. ●, CAH group; ○, control group.

response to β-hCG treatment (under the minimum range of the control group; Fig. 3A), which was associated with a high level of 17-OHP (patients 6, 9, and 18), and two subjects had elevated basal T levels (patients 6 and 9; Fig. 2B). Patient 7 had a basal T less than 10 ng/dl with a 17-OHP of 2.7 ng/ml with a $(T_{72} - T_0)/T_0$ that was comparable with the control group.

Discussion

Our results show that TART can be detected from childhood in boys with classical CAH and appear to be associated with inadequate control of CAH. The differences in markers of gonadal function occurring in children with CAH suggest a possible dysfunction in a subgroup of these patients, especially among boys with high basal androgen levels.

In 1940, Wilkins *et al.* (16) reported the presence of testicular masses in male patients with CAH due to 21-hydroxylase deficiency, so-called TART. Histologically, by electron microscopy, TART resemble Leydig cell tumors with features that are consistent with steroid-secreting cells. However, unlike Leydig cell tumors, they never contain Reinke crystalloids. The most likely pathological diagnosis is Leydig cell tumor. The associated clinical and laboratory features, including a high frequency of bilaterality and a decrease in the size of the tumor with corticosteroid therapy, are important in the diagnosis of TART (17).

Rutgers *et al.* (17) proposed that these masses could originate from hilar pluripotent cells, which proliferate as a result of elevated levels of ACTH. Recently, an interesting study identified and characterized a novel population of adrenal-like cells that appear in mouse testes during embryonic development and are found in adulthood. This experiment provides evidence that these cells exhibit mixed adrenal and Leydig cell properties; they express the adrenal markers Cyp11b1, Cyp21, and Cyp17

and respond to ACTH and hCG stimulation, but they do not express the Leydig cell-specific marker insulin-like factor 3. This indicates that they are a population of steroidogenic cells distinct from Leydig cells that migrate from the gonad/mesonephros border during development and that they are likely equivalent to precursors of the adrenal rest tumors found in patients with CAH (5).

Depending on the detection method (palpation *vs.* US), the prevalence of TART varied from 0–95% (8). US is considered the method of choice for the study of TART, because it is as sensitive as magnetic resonance imaging but is more accessible (18).

This study of 19 male patients with CAH showed TART in four patients. The TART incidence in children is less then that reported for adolescents and adults (8), which, according to their lower age, is expected. In our study, boys with CAH and TART were older and had higher levels of 17-OHP than boys with CAH without TART. It is possible that there is a higher prevalence in adolescents because adults and poor control patients are more likely to have hyperplastic cells due to LH- and ACTH-mediated stimulation.

Stikkelbroeck *et al.* (19) described that all TART in their patients were located adjacent to the mediastinum testis and that small TART (<2 cm) were hypoechoic; the same characteristics were found in our CAH group with TART.

Many authors have suggested that infertility in patients with CAH could originate during the prenatal stages or during childhood (8, 20, 21). Studying testicular activity during the prepubertal period is difficult because it has classically been considered a quiescent phase. However, the combined analysis of Sertoli cell markers and T in basal and hCG-stimulated conditions enhances our ability to diagnose the underlying endocrine defect, as in our study (22).

Different mechanisms have been proposed to explain infertility in males with CAH. The location of TART near the testicular mediastinum can lead to the obstruction of seminiferous tubules. The steroids produced by the tumors may reach the circulation and interfere with the secretion of FSH and LH by the pituitary, or there could be a steroidogenic enzyme defect due to paracrine toxicity. However, some of these theories are supported only by case reports (23, 24).

The response of T to β-hCG has long been used to successfully evaluate the presence or absence of testicular tissue and to elucidate defects of T biosynthesis or function (25–27). Work by Combes-Moukhovsky *et al.* (24) showed no response to β-hCG in a prepubertal boy with TART and postulated that, in this case, the normal testicular tissue was either nonfunctional or was destroyed. In adolescents and adults with CAH, Leydig cell dysfunction has also been reported (8). In our study, we show that some patients with CAH have a lower testicular response to β-hCG stimulation, which was inversely proportional to the basal level of 17-OHP. Lower LH receptor responses to β-hCG could be associated with Leydig cell dysfunction in a subgroup of patients with CAH or with suppression of the gonadostat by endogenous adrenal steroids and may not necessarily indicate testicular damage *per se*.

Inh-B is accepted as a marker of Sertoli cell function in children (28–30) and adults (31–33). Measurements of AMH have been used to determine testicular status in prepubertal children with nonpalpable gonads, thus differentiating anorchia from undescended testes in boys with bilateral cryptorchidism, and serve as a measure of testicular integrity in children with intersexual anomalies (34). These new markers may contribute to a better understanding of the regulation of hypothalamic-pituitary-gonadal axis function and the physiopathology of the mechanisms involved in sexual differentiation and/or fertility disorders (35).

Around the onset of puberty, Sertoli cells undergo a radical change in their morphology and function. There is also an increase in T concentration at puberty, coincident with androgen receptor expression in Sertoli cells. Together, all of these factors induce a change from an immature, proliferative state (which produces AMH) to a mature, nonproliferative state (36). After puberty, AMH becomes low or undetectable due to down-regulation of AMH production by T (37). In patients with either central or gonadotropin-independent precocious puberty, the hormonal regulatory mechanisms of AMH secretion are androgen mediated and are independent of gonadotropins, as we observed in one of our patients (38). We did not perform an LHRH test to evaluate central precocious puberty; however, boys with TART and lower AMH levels also had lower Inh-B, which usually increases with the progression of puberty in boys.

The lower levels of Inh-B and AMH observed in the CAH group could reflect gonadal dysfunction in a subgroup of these children, associated with inadequate control and TART. We could postulate that in patients with uncontrolled CAH, high levels of T could stimulate the androgen receptor and induce premature maturation of immature Sertoli cells, which could result in a decrease in AMH. If premature maturation of Sertoli cells impacts future fertility, it must be investigated.

In this study, the percentage of identified alleles was lower than previously reported in Chilean patients with CAH (15). Other methods are being implemented to further analyze the patients with unidentified alleles, including sequencing of the CYP21 gene and MPLA for detection of deletions and duplications.

We suggest studying TART with testicular ultrasound in all prepubertal patients with inadequately treated classical CAH and in all patients at the onset of puberty, due to its potential impact on future fertility. In those patients with documented TART, glucocorticoid therapy must be optimized. The suggested follow-up should include periodic assessment of testicular volume with ultrasound and testicular function with measurements of serum Inh-B levels. If TART persists accompanied by an increase in the relation between tumor/testicular parenchymal tissue and/or a lack of increase in serum Inh-B, more aggressive glucocorticoid therapy and eventually testis-sparing surgery must be considered.

In conclusion, in our series, we show that TART could be detected since childhood in boys with classical CAH. We also report differences in markers of gonadal function in these boys compared with the control group, which could reflect a gonadal dysfunction in a specific subgroup of patients with CAH. Its physiopathology could be explained by the paracrine regulatory mechanism of local T produced in TART and Leydig and Sertoli cell functions. More studies are necessary

to determine the reversibility of this phenomenon and its impact on future fertility.

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