Testicular Adrenal Rest Tumors in Boys and Young Adults with Congenital Adrenal Hyperplasia

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Purpose: Testicular adrenal rest tumors are a well-known complication in males who have congenital adrenal hyperplasia with potential infertility in adulthood. We assessed the prevalence of testicular adrenal rest tumors in infants to young men presenting to a congenital adrenal hyperplasia Comprehensive Care Center.

Materials and Methods: A total of 35 males with congenital adrenal hyperplasia due to 21-hydroxylase deficiency underwent scrotal ultrasonography, including 7 younger than 5 years, 9 who were 5 to 12 years old and 19 who were older than 12 years. Three and 35 patients had classic and nonclassic congenital adrenal hyperplasia, respectively. Bone age x-ray or advanced bone age x-ray history, glucocorticoid dose, fludrocortisone dose, and serum 17-hydroxyprogesterone, testosterone and androstenedione levels within 3 months of ultrasound were also recorded.

Results: Testicular adrenal rest tumors were detected in 5 of 35 patients (14%), including 1 of 9 (11%) who were 5 to 12 years old and 4 of 19 (21%) who were older than 12 years. The tumors were not detected in any patients younger than 5 years, including 1 infant with poor hormonal control. The youngest patient with positive findings was 6.6 years old. All patients with positive findings had bilateral disease and only 1 had suspicious physical findings. The glucocorticoid dose and 17-hydroxyprogesterone did not differ between patients with vs without a testicular adrenal rest tumor. Those with a tumor were more likely to have advanced bone age x-ray results (100% vs 42%, $p = 0.04$) and higher fludrocortisone dose ($p < 0.01$). All males with nonclassic congenital adrenal hyperplasia had negative tumor findings.

Conclusions: Testicular adrenal rest tumors were present in young males with classic congenital adrenal hyperplasia but not in infants or toddlers. These tumors were associated with higher fludrocortisone requirements and a history of advanced bone age x-ray results. However, the tumors did not develop in all poorly controlled males. Longitudinal studies are needed to understand the individual predisposition to testicular adrenal rest tumors and the age at which to begin screening patients with congenital adrenal hyperplasia.

Key Words: testis; adrenal hyperplasia, congenital; adrenal rest tumor; steroid 21-hydroxylase; mass screening

Abbreviations and Acronyms

17OHP = 17-hydroxyprogesterone

ACTH = adrenocorticotropic hormone

CAH = congenital adrenal hyperplasia

GC = glucocorticoid

TART = testicular adrenal rest tumor

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CONGENITAL adrenal hyperplasia is a potentially life-threatening form of primary adrenal insufficiency characterized by cortisol, aldosterone and epinephrine deficiencies as well as androgen excess.[1] It is most commonly caused by a mutation in the *CYP21A2* gene, which encodes the enzyme 21-hydroxylase, of which an absence or deficiency leads to reduced synthesis of cortisol and aldosterone, elevated ACTH, hyperplasia of the adrenal glands, accumulation of steroid precursors and excessive production of androgens (fig. 1).[2]

CAH is typically categorized as classic (severe) or nonclassic (mild, late onset). The classic salt-wasting form, which occurs in 67% of individuals with classic CAH, is characterized by a severe decrease in 21-hydroxylase activity, leading to almost complete deficiencies of cortisol and aldosterone, presentation at or soon after birth with ambiguous genitalia in females and the risk of early adrenal crisis if untreated.[3] The classic simple virilizing form, which occurs in 33% of individuals with classic CAH, is associated with 1% to 2% of normal 21-hydroxylase activity and still carries a risk of adrenal crisis but to a lesser degree than the salt-wasting form.[4] Nonclassic CAH is characterized by 20% to 50% of normal 21-hydroxylase activity, resulting in a milder phenotype.[5] The typical presentation occurs later in childhood or adolescence with findings secondary to androgen excess, including premature pubarche, growth acceleration, advanced bone age in growing children or hirsutism, acne, delayed menarche/menstrual irregularities and infertility among older patients.[6]

TARTs, which are a well-known complication in men with classic CAH due to 21-hydroxylase deficiency, can lead to gonadal dysfunction and infertility in adulthood.[7] The adrenal rest tissue in the testicle expresses ACTH specific receptors and is ACTH responsive, producing steroid hormones, and it can become hyperplastic.[8] A TART develops when there is growth of the adrenal rests within the testicular parenchyma. While the resulting tumors are benign, the location of most TARTs in the rete testis may lead to compressive effects with tubular obstruction and oligozoospermia/azoospermia.[7] In some cases a large TART can compress enough normal tissue to affect spermatogenesis and testosterone production.

Standard of care guidelines suggest that periodic screening should begin in adolescence.[9] However, specific recommendations vary and include screening younger children[10,11] with the suggestion that early detection is optimal.[7] While TARTs have been reported in children with CAH at a prevalence of 24% to 33%,[10,11] little is known regarding TART in infants and toddlers with CAH. A study of neonatal autopsies of boys with CAH noted the presence of ectopic adrenal tissue in 3 of 7 younger than 8 weeks and in 14 younger than 14 months.[12]

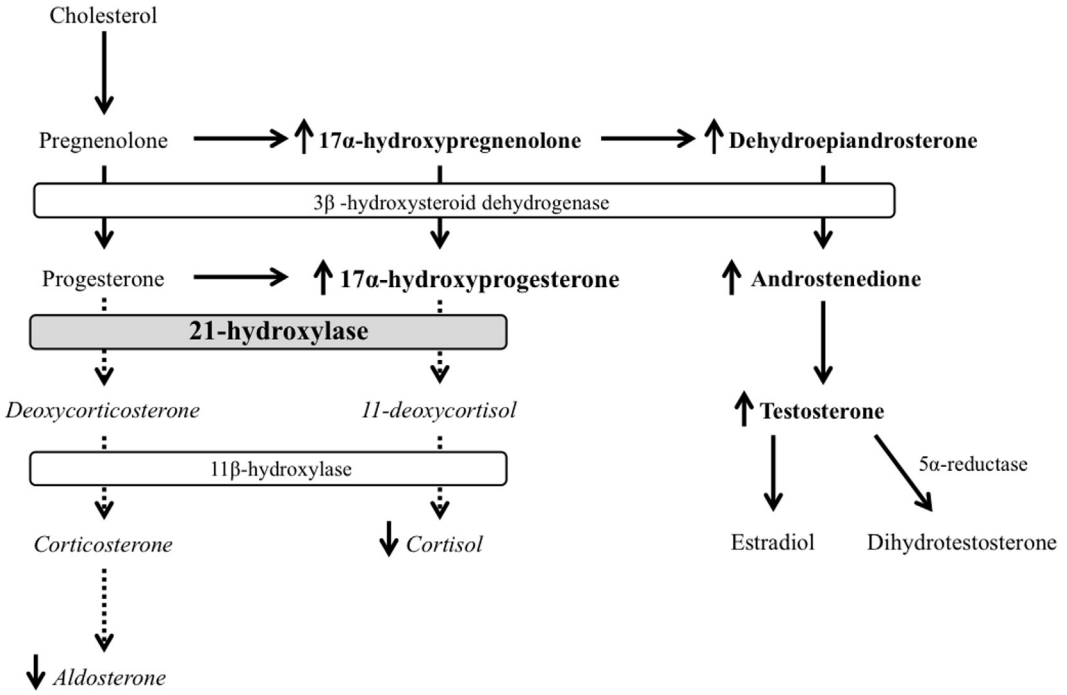

Figure 1. Adrenal gland steroidogenic pathway. Deficiency in enzyme 21-hydroxylase (shaded box) is most common cause of CAH, accounting for 95% of cases and leading to deficiencies in aldosterone and cortisol (dotted pathways). This results in accumulation of steroid precursors and excess androgen production (bold).

However, to our knowledge there have been no ultrasonographic studies of testes in infants with CAH. Therefore, we studied the prevalence of TART in boys over a wide age spectrum from infancy to young adulthood and the association of TART with specific measures of hormonal control. We hypothesized that TART would be found at a lower prevalence in younger patients and poor hormonal control would be associated with a greater prevalence of TART.

MATERIALS AND METHODS

Study Center

We performed a cross-sectional study of males presenting to a CARES (Congenital Adrenal Hyperplasia Research, Education and Support) Foundation designated CAH Comprehensive Care Center, which includes a multidisciplinary care team focused on collaborative surgical and medical management of CAH. Study inclusion criteria were males with classic or nonclassic CAH due to 21-hydroxylase deficiency confirmed by biochemical and/or genetic testing. Those with CAH due to less common enzyme deficiencies, such as 3β-hydroxysteroid dehydrogenase or 11β-hydroxylase deficiency, were excluded.

Outcome Measures

Yearly screening ultrasounds are performed as part of clinical care for male patients at our center. Scrotal studies were done with a real-time Aplio™ 500 scanner using a 14 MHz linear array transducer (fig. 2). Power and spectral Doppler examinations of the testes were performed. Additional followup ultrasounds were performed at 6-month intervals in individuals after TART abnormalities were noted.

Patients were recruited from 2013 through 2015. The presence or absence of TARTs was recorded and diameter measurements in mm were made. In TART positive cases previous ultrasounds were reviewed to pinpoint when TARTs were first detected to identify the index ultrasound for examination of risk factors. Measures of hormonal control were recorded in TART positive cases in relation to the index ultrasound in which TARTs were first detected.

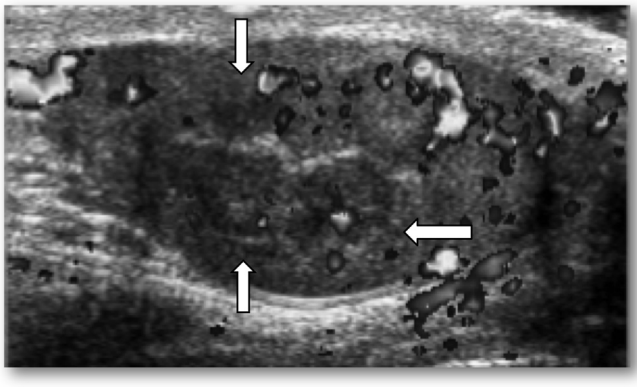

Figure 2. Doppler ultrasound of right testis reveals positive TART findings in 12-year-old male with salt-wasting form of classic CAH, advanced bone age and elevated 17-hydroxyprogesterone levels. TART was 12 × 12 mm in diameter (white arrows) and localized to testicular hilum (hyperechoic midline strip).

Hormonal control at the time of the ultrasound examination was assessed by serum 17OHP androstenedione and testosterone, which were measured elsewhere by liquid chromatography and tandem mass spectrometry. Hormone levels were generally measured prior to routine morning CAH medications. We categorized 17OHP as suppressed—less than 100 ng/dl, normal—100 to 1,200, elevated—greater than 1,200 to 5,000 and very elevated—greater than 5,000. The GC dose was recorded for each patient at the time of the first diagnostic ultrasound. The GC dose was expressed as the hydrocortisone dose in mg/m^2 per day or as calculated hydrocortisone dose equivalents (dexamethasone 1:80 and prednisone 1:5). Androstenedione and testosterone were reported in ng/dl and further categorized as elevated and not elevated based on age specific norms.

Longer term hormonal control was assessed using a history of bone age advancement. A bone age x-ray of the left hand was taken within 6 months of the time of ultrasound. When the growth plates were already fused, only a history of advanced bone age at any time was recorded. A bone age 2 SD scores or greater above chronological age was considered significantly advanced, likely representing substantial prior exposure to postnatal androgens.

Statistical Analysis

Summary statistics are presented as the proportion or percentage, or the mean ± SD. The Student t-test was used for between group comparisons of continuous data, and the Fisher exact and chi-square trend tests were used for between group comparisons with categorical data and trends between groups with categorical data points. The study was reviewed and approved by the Children's Hospital Los Angeles institutional review board.

RESULTS

Study Population

We identified 35 male patients with CAH due to 21-hydroxylase deficiency, of whom 27 (77%) had the salt-wasting form, 5 (14%) had the simple virilizing form and 3 (9%) had the nonclassic form.

TARTs in School-Aged and Older Males

TARTs were found in 5 of 35 patients (14%) overall. Seven patients were older than 5 years and none had a TART. TARTs were found in 1 of 9 patients (11%) 5 to 12 years old and in 4 of 19 (21%) older than 12 years (table 1). No TARTs were detected in males with nonclassic CAH.

Mean TART diameter at initial detection was 6.79 ± 6.14 mm (range 3 to 20). All patients with TART positive findings had bilateral lesions and

Table 1. *Testicular adrenal rest tumor prevalence in males with congenital adrenal hyperplasia due to 21-hydroxylase deficiency, and phenotypic subclassification and medication dose in those with and without tumor*

	Age (yrs)			
	Less than 5	5–12	Greater than 12	Overall
No. pts	7	9	19	35
No. TART (%)*	0	1 (11)	4 (21)	5 (14)
No. CAH type:				
Salt-wasting	6	17	14	27
Simple virilizing	1	1	3	5
Nonclassic	0	1	2	3
Mean ± SD hydrocortisone dose (mg/m^2/day):				
TART neg	16.2 ± 4.7	15.7 ± 3.1	15.4 ± 6.3	15.5 ± 5.2
TART pos	—	19.7	16.6 ± 2.2	17.2 ± 2.4
Mean ± SD fludrocortisone dose (mg/day):				
TART neg	0.08 ± 0.04	0.08 ± 0.04	0.06 ± 0.05	0.07 ± 0.05
TART pos	—	0.2	0.15 ± 0.06	0.16 ± 0.05†

* Age trend did not reach statistical significance ($p = 0.16$).
† Significantly higher vs patients without testicular adrenal rest tumor ($p < 0.01$).

none had a palpable lesion. One patient had bilaterally firm testes at diagnosis.

Glucocorticoid and Fludrocortisone Medication Doses

The GC dose most proximate to the index ultrasound was not statistically different between patients who were TART positive vs negative (mean 17.2 ± 2.4 vs 15.5 ± 5.2 mg/m^2 per day, $p = 0.49$, table 1). The fludrocortisone dose was higher in TART positive than negative cases (mean 0.16 ± 0.05 vs 0.07 ± 0.05 mg per day, $p < 0.01$).

Control of Congenital Adrenal Hyperplasia Hormone Levels

The 17OHP levels were elevated in 3 of 5 TART positive cases (60%) at the time of ultrasound and in 12 of 30 (40%) that were TART negative (table 2). Overall, males with TART positive findings were not more likely than those with TART negative findings to have elevated 17OHP ($p = 0.63$). Mean 17OHP levels were also similar between males who were TART positive and negative ($p = 0.85$).

Androstenedione was similar in TART positive and negative cases (mean 242 ± 265 vs 124 ± 216 ng/dl,

Table 2. *Testicular adrenal rest tumors and hormonal control in males with classic congenital adrenal hyperplasia*

	Age (yrs)			
	Less than 5	5–12	Greater than 12	Overall
No. pts	7	9	19	35
Mean ± SD testosterone (ng/dl):				
TART neg	17.6 ± 26	80.7 ± 205	384.6 ± 267	232.8 ± 276
TART pos	—	54	326.3 ± 376	258.2 ± 336
Mean ± SD androstenedione (ng/dl):				
TART neg	33.3 ± 51	36.4 ± 32	211.3 ± 274	124.3 ± 216
TART pos	—	542	142.6 ± 214	242.5 ± 265
Mean ± SD 17OHP (ng/dl):				
TART neg	5,782 ± 12,410	905 ± 1,123	4,049 ± 7,425	3,778 ± 7,958
TART pos	—	9,944	2,989 ± 3,146	4,380 ± 4,135
No. suppressed 17OHP (less than 100 ng/dl):				
TART neg	2	1	2	5
TART pos	—	0	1	1
No. normal 17OHP (100–1,200 ng/dl):				
TART neg	2	4	6	12
TART pos	—	0	1	1
No. elevated 17OHP (1,200–5,000 ng/dl):				
TART neg	2	3	4	9
TART pos	—	0	0	0
No. very elevated 17OHP (greater than 5,000 ng/dl):				
TART neg	1	0	3	4
TART pos	—	1	2	3
No. advanced bone age (2 SD or greater)/total No.:				
TART neg	1/3	4/8	7/13	10/24*
TART pos	—	1/1	4/4	5/5*

Other hormonal control measures were statistically similar between groups.
* Significantly associated with presence of TART ($p = 0.04$).

$p = 0.32$), as was testosterone (mean 258 ± 336 vs 233 ± 276 ng/dl, $p = 0.87$). Males with TART positive findings were no more likely to have elevated levels of androstenedione (positive vs negative 50% vs 24%, $p = 0.30$) or testosterone (positive vs negative 25% vs 20%, $p = 0.99$).

Longer Term Hormonal Control and Advanced Bone Age

All patients with TARTs had an advanced bone age (2 SD or greater of normal). Only 10 of 24 patients with TART negative findings (42%) had an advanced bone age ($p = 0.04$, table 2).

DISCUSSION

The main finding of our study is that TARTs may develop in males as young as school age with classic CAH due to 21-hydroxylase deficiency. However, these lesions were not detected in infants and toddlers with CAH by sonographic techniques. Our data support the finding of others that the TART prevalence seems to increase with age[10,13] with an overall prevalence of 14% in our cohort. Although some reports exist of TARTs and oligospermia in men with nonclassic CAH,[14] they are more commonly associated with classic CAH. We did not detect TARTs in any nonclassic CAH cases.

The presence of TARTs carries significance for gonadal function and fertility in adulthood[7] with TARTs potentially leading to infertility by obstructing the seminiferous tubules and destroying adjacent testicular tissue. Thus, it is concerning that we and others are finding TARTs in young children.[10,11,13] Although 3 infants with CAH have previously been reported to be TART positive on autopsy,[12] to our knowledge there are no published reports in which TARTs have been found by ultrasonography in infants with CAH.

In contrast, the incidence of testicular adrenal rests in newborns without CAH appears low (3.5%) based on an examination of autopsy material.[15] The incidence is similar to that in children without CAH who are younger than 16 years.[16] The predisposition to TART development could vary among males, for example due to differing levels of ACTH exposure during gestation[15] with TARTs observed more frequently in male patients with poorly controlled CAH.[7] However, our study demonstrates that the risk factors for TART development in males with CAH are not yet fully understood.

While all males with TARTs exhibited a significantly advanced bone age, not all had abnormal 17OHP levels around the time of the diagnostic testicular ultrasound. Notably, 1 infant did not have sonographic evidence of TARTs on 3 consecutive scrotal ultrasounds despite consistently poor hormonal control during infancy (17OHP 14,764 to 33,821 ng/dl). Conversely, there were males who were TART negative with signs of poor hormonal control, including advanced bone age and/or elevated 17OHP. Another study showed no correlation between adrenal hormones and TART overall, although boys with TARTs were more likely to have higher androstenedione.[11] More patients with CAH need to be studied to better assess the relationship between hormonal control and the development of TART over time.

All TARTs in the current study were found in 5 of the 27 patients with the salt-wasting form of classic CAH and none were found in the 5 with simple virilizing and the 3 with nonclassic forms. In patients with salt-wasting CAH on inadequate cortisol replacement relatively higher levels of ACTH stimulation during long periods could provide the necessary environment for TART to develop in susceptible individuals.

There are several limitations to our study. The cross-sectional design could not account for hormonal control between visits. It would be ideal to follow patients longitudinally from infancy to adulthood. Ultrasound was used to identify TART but histological verification of these lesions could not be performed. We were also unable to measure Sertoli cell markers (eg inhibin B) and gonadotropins to assess gonadal dysfunction. These analytes could be important in males of any age with CAH as decreases in functional testicular volume have been found to correspond to lower inhibin B and sperm concentration in adults with sperm concentration further correlating with inhibin B and higher follicle-stimulating hormone levels.[17] Also, prepubertal males with CAH have shown lower levels of inhibin B and antimüllerian hormone, correlating with decreased Leydig cell function, especially in those with TARTs.[13]

There is growing evidence that scrotal ultrasound starting at or near school age in males with classic CAH due to 21-hydroxylase deficiency could be prudent in the clinical setting. We recommend beginning to screen males with classic CAH at ages 4 to 6 years, which is the age that we and others have detected TARTs on scrotal ultrasound. The prevalence of TARTs increases with age and, thus, initiating screening during adolescence is a minimum with more frequent ultrasounds in males who are TART positive. Variability among individuals with regard to risk factors for TARTs and the baseline presence of adrenal rest tissue merits further study in patients with CAH.

CONCLUSIONS

Males with classic CAH due to 21-hydroxylase deficiency are at risk for TARTs as young as age

6 years, although infants and toddlers do not appear to exhibit sonographic evidence of TARTs. Markers of hormonal control were often elevated in our patients with TART positive findings and advanced bone age was significantly more prevalent in TART positive males. Similarly, higher fludrocortisone doses were associated with TARTs, suggesting that more severe salt-wasting occurred in patients with TARTs. Males with the mildest form of CAH (nonclassic) did not show evidence of TARTs. Since not all patients with markers of poor hormone control had evidence of TARTs, specific individual risk factors for TARTs need to be further elucidated.

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