Hormonal control during infancy and testicular adrenal rest tumor development in males with congenital adrenal hyperplasia: a retrospective multicenter cohort study

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Abstract: IMPORTANCE Testicular adrenal rest tumors (TARTs), often found in male patients with congenital adrenal hyperplasia (CAH), are benign lesions causing testicular damage and infertility. We hypothesize that chronically elevated adrenocorticotropic hormone exposure during early life may promote TART development. OBJECTIVE This study aimed to examine the association between commencing adequate glucocorticoid treatment early after birth and TART development. DESIGN AND PARTICIPANTS This retrospective multicenter (n = 22) open cohort study collected longitudinal clinical and biochemical data of the first 4 years of life using the I-CAH registry and included 188 male patients (median age 13 years; interquartile range: 10-17) with 21-hydroxylase deficiency (n = 181) or 11-hydroxylase deficiency (n = 7). All patients underwent at least 1 testicular ultrasound. RESULTS TART was detected in 72 (38%) of the patients. Prevalence varied between centers. When adjusted for CAH phenotype, a delayed CAH diagnosis of >1 year, compared with a diagnosis within 1 month of life, was associated with a 2.6 times higher risk of TART diagnosis. TART onset was not predicted by biochemical disease control or bone age advancement in the first 4 years of life, but increased height standard deviation scores at the end of the 4-year study period were associated with a 27% higher risk of TART diagnosis. CONCLUSIONS AND RELEVANCE A delayed CAH diagnosis of >1 year vs CAH diagnosis within 1 month after birth was associated with a higher risk of TART development, which may be attributed to poor disease control in early life.

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Original Research

Hormonal control during infancy and testicular adrenal rest tumor development in males with congenital adrenal hyperplasia: a retrospective multicenter cohort study

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Abstract

Importance: Testicular adrenal rest tumors (TARTs), often found in male patients with congenital adrenal hyperplasia (CAH), are benign lesions causing testicular damage and infertility. We hypothesize that chronically elevated adrenocorticotropic hormone exposure during early life may promote TART development.

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Objective: This study aimed to examine the association between commencing adequate glucocorticoid treatment early after birth and TART development.

Design and participants: This retrospective multicenter ($n = 22$) open cohort study collected longitudinal clinical and biochemical data of the first 4 years of life using the I-CAH registry and included 188 male patients (median age 13 years; interquartile range: 10-17) with 21-hydroxylase deficiency ($n = 181$) or 11-hydroxylase deficiency ($n = 7$). All patients underwent at least 1 testicular ultrasound.

Results: TART was detected in 72 (38%) of the patients. Prevalence varied between centers. When adjusted for CAH phenotype, a delayed CAH diagnosis of >1 year, compared with a diagnosis within 1 month of life, was associated with a 2.6 times higher risk of TART diagnosis. TART onset was not predicted by biochemical disease control or bone age advancement in the first 4 years of life, but increased height standard deviation scores at the end of the 4-year study period were associated with a 27% higher risk of TART diagnosis.

Conclusions and relevance: A delayed CAH diagnosis of >1 year vs CAH diagnosis within 1 month after birth was associated with a higher risk of TART development, which may be attributed to poor disease control in early life.

Keywords: testicular adrenal rest tumors, congenital adrenal hyperplasia, diagnosis, early childhood

Significance

Testicular adrenal rest tumors (TARTs) are common in males with congenital adrenal hyperplasia (CAH) and are an important cause of gonadal dysfunction. Chronically elevated adrenocorticotropic hormone (ACTH) levels in poorly treated patients are considered the main factor in TART development. However, previous studies on long-term CAH control and TART development are equivocal, and it remains unclear why TART occurs less commonly in patients with acquired conditions with chronically elevated ACTH levels. This retrospective study describes the largest cohort of CAH males being evaluated for TART so far and shows that a delayed CAH diagnosis may be associated with a higher risk of TART. This may be attributed to a lack of ACTH suppression during early life and may strengthen the case for implementation of neonatal screening and early effective management of male newborns with CAH.

Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders affecting adrenal steroidogenesis. CAH can be caused by mutations in several genes, including the most commonly affected *CYP21A2* or *CYP11B1* gene, resulting in 21-hydroxylase deficiency (21OHD) and 11β-hydroxylase deficiency (11OHD), respectively.[1,2] Based on residual 21-hydroxylase activity, 21OHD is commonly classified into 3 subtypes; salt-wasting (SW) (0%-1% residual activity), simple virilizing (SV) (1%-5% residual activity), and non-classic (NC) 21OHD (20%-50% residual activity).[1] For both 21OHD and 11OHD, low levels of glucocorticoids lead to an overactivated hypothalamic-pituitary-adrenal axis with chronic hypersecretion of adrenocorticotropic hormone (ACTH). Elevated blood ACTH levels overstimulate the adrenal cortex with increased production of precursor steroids upstream of the enzymatic defect and higher levels of adrenal androgens. Glucocorticoid treatment aims to substitute the low glucocorticoid levels and inhibit the hypersecretion of ACTH and adrenal androgens.[3]

Testicular adrenal rest tumors (TARTs) are a common complication in males with CAH, with a reported prevalence varying between 14% and 86%, depending on the age, method of detection, and type of CAH.[4] These benign tumors are typically located within the rete testis and may cause testicular damage[5] and infertility.[6,7] The etiology of TART is still not fully understood. Chronically elevated ACTH levels are considered important for TART development[8] and growth, as TART expresses the ACTH receptor MC2R[9] and responds to ACTH by means of elevated steroid hormone production *in vitro*.[10] Multiple studies have linked the presence of TART to poor disease control,[4] but a clear relationship between TART development and elevated ACTH levels is not established and an association between longer-term poor disease control and TART development is not consistently observed.[11,12] It could be speculated that ACTH exposure during infancy and childhood, or even during pregnancy, is a prerequisite for the development of TART. Neonatal exposure to high ACTH levels may prevent (complete) regression of adrenal rest tissue[13] or a yet undefined cell population. We, therefore, hypothesize that high ACTH exposure during early life promotes the development of TART during the patient's lifetime. This theory is supported by the clinical observation that TART is uncommon in acquired conditions with elevated ACTH levels in later life.[14] This study aims to investigate the association between hormonal control during infancy and early childhood and TART development during lifetime in a large cohort of males with CAH due to 21OHD or 11OHD. In addition, we aim to verify if an early start of treatment after birth lowers the risk of TART development.

Methods

Data collection

In this retrospective open cohort study, pseudonymized data from the I-CAH registry—an international database on CAH patients with data collected as part of routine clinical care[15]—was analyzed. This registry is approved by the National Research Ethics Service (19/WS/0131) and the deposition of patient information into the registry was preceded by informed consent from patients and/or guardians. The study complies with the Declaration of Helsinki. Retrospective data was collected from individuals with 46, XY karyotype, genetically and/or biochemically confirmed CAH diagnosis due to 21OHD or 11OHD, and at least 1 testicular ultrasound for TART evaluation. Additional data was collected and pseudonymized by the attending physician. Retrospective data on height, biochemical disease control, treatment, and bone age, were collected during 5 visits in the first 4 years of life: at diagnosis and 4 consecutive annual visits. Retrospective data collected after 4.5 years of age (with the exception of data on TART development) were not included. Contemporary data (also obtained after 4.5 years of age) regarding TART development were collected. In

case of missing data, centers were actively approached to add missing information.

Data interpretation

Biochemical disease control was evaluated using interpreted laboratory results for androstenedione and/or 17-hydroxyprogesterone (undertreatment/adequate/overtreatment). Androstenedione concentrations were predominantly (91% of reported sample type) measured in serum and were occasionally reported to be measured in urine (5%), plasma (3%), or saliva (1%). The steroid 17-hydroxyprogesterone was predominantly measured in serum (96%) and occasionally reported to be measured in plasma (5%), or saliva (1%). Adequacy of treatment was monitored according to international guidelines,[16] through measurement of androstenedione and 17-hydroxyprogesterone. Because of differences in sample type, time of measurement with respect to treatment, and center-specific reference values, hormone levels were interpreted by the attending physician or study lead using the center's in-house reference values. For 1 center, including 7 patients, testosterone levels were used to assess biochemical disease control. ACTH levels quantified either in plasma or serum, together with their interpretation (low/normal/high) were collected.

As measures for past long-term hormonal control, data on bone age advancement and height velocity by calculating height for age standard deviation scores (SDS) were assessed for every visit. Bone age advancement was calculated by subtracting the chronological age from the measured bone age, mainly assessed by a pediatric endocrinologist using the Greulich and Pyle method. Height-for-age SDS corrected for target height SDS was calculated for every visit, using international height-for-age references of the World Health Organization;[17] For this, height SDS was calculated using patient's height for age at assessment and the international mean height for age and standard deviation from the WHO. The height SDS was subtracted from the target height SDS, which was calculated using the formula described by Hermanussen and Cole,[18] using paternal and maternal final height, and male and female references of the WHO at age 19.

Patients with 21OHD were, based on genotypes, classified into presumed phenotypes (SW, SV, NC, not known).[19-26] Patients with 11OHD were classified as "not known" phenotype for analyses. Adrenal crisis at diagnosis was specified as either a recorded SW crisis or an Addisonian crisis at diagnosis.

Statistical analysis

Data analyses were performed in R.[27] Descriptive statistics are presented as ratios with percentages or median with interquartile range (IQR). Mann–Whitney *U*-tests were used to compare differences in continuous data between patients with or without TART and χ^2 tests were used to test relations between categorical variables when there was no need for normalization for other parameters. The approximate age at diagnosis and start of treatment were obtained or calculated from dates and consequently categorized into groups of <1 month, >1 month but <1 year, and >1 year. Because of the right-censored nature of the data, Cox proportional hazard analyses were performed using the R survival package,[28] providing hazard ratios (HRs) with 95% confidence intervals (CIs). Survival plots were obtained using the R Survminer package.[29] For categorical variables, missing data was incorporated in the analyses as "not known", to evaluate if missing data was informative. If data availability allowed it, the effect of predictors on the risk of TART diagnosis was adjusted for the presumed CAH phenotype. The assumption of proportional hazards was tested using Schoenfeld's test. *P*-values smaller than .05 were considered statistically significant.

Results

Description of the patient cohort

In total, 188 male patients diagnosed with CAH due to 21OHD ($n = 181$) or 11OHD ($n = 7$) were included from 22 centers in 17 countries (Table 1). All patients had at least 1 testicular ultrasound, and 141 (75%) were screened regularly for TART by testicular ultrasound. The median age at most recent testicular ultrasound was 13 years (IQR: 10-17, Table 1). TART was detected in 38.3% (72/188) of the patients. The number of patients included per center ranged from 1 to 27 and the prevalence of TART ranged from 0% to 100%. Sixty-two participants had bilateral TART and 10 had unilateral TART. The youngest patient in which TART were detected was just 2 years old (upon 11OHD diagnosis) and half of the patients (IQR) with detected TART were between 10.8 and 18.3 years old. The median TART size at detection was 9 mm (IQR: 4.8-15.0), with 1 additional patient having a "point-like" tumor. TART were surgically removed in 7 patients from 5 centers. For 11 patients with TART (15%), TART-related complaints were documented, being (testicular) pain ($n = 6$), gonadal dysfunction ($n = 2$), (hypergonadotropic) hypogonadism ($n = 6$), and azoospermia ($n = 3$). Based on the genetic information, most patients with 21OHD were classified as SW (57%), followed by SV (16%), and NC CAH (8%). Five patients (3%) had a heterozygous or homozygous P30L mutation, which is known to

Table 1. General characteristics of the study cohort, grouped by the presence, and absence of TART.

	Overall	TART	No TART
n	188	72	116
21OHD	181 (96%)	68 (94%)	113 (97%)
11OHD	7 (3.7%)	4 (5.6%)	3 (2.6%)
Presumed 21OHD type ($n = 181$)			
SW	103 (57%)	40 (59%)	63 (56%)
SV	29 (16%)	4 (5.9%)	25 (22%)
SV/NC	6 (3.3%)	3 (4.4%)	3 (2.7%)
NC	14 (7.7%)	2 (2.9%)	12 (11%)
Unknown	29 (16%)	19 (28%)	10 (8.8%)
Age at last US	13 (10-17)	15 (11-18)	12 (10-16)
Neonatal screening (21OHD)			
Yes	58 (32%)	9 (13%)	49 (43%)
No	102 (56%)	52 (77%)	50 (44%)
Not known	21 (12%)	7 (10%)	14 (12%)
Adrenal/salt-wasting crisis at diagnosis			
Yes	67 (36%)	35 (49%)	32 (28%)
No	93 (50%)	26 (36%)	67 (58%)
Not known	28 (15%)	11 (15%)	17 (15%)
Age at diagnosis			
<1 month	92 (49%)	24 (33%)	68 (59%)
>1 month and <1 year	38 (20%)	23 (32%)	15 (13%)
≥1 year	51 (27%)	20 (28%)	31 (27%)
Not known	7 (3.7%)	5 (6.9%)	2 (1.7%)

Percentages are calculated relative to all patients with CAH or, if specified, patients with 21OHD.

cause a phenotype in between SV and NC,[24] and was, therefore, classified as SV/NC. Another patient with a P453S and I2G mutation was also classified as SV/NC.[30] For 29 (16%) patients with 21OHD, genetic information was either not available ($n = 23$) or could not be classified into presumed phenotypes ($n = 6$). SW phenotype was associated with a 3.3 (1.2-9.1) times higher risk of TART diagnosis compared with the SV phenotype (Figure 1A; Table 2). Remarkably, the SV/NC patients had a significantly higher risk of TART diagnosis than SV patients. The low number of patients with this subtype ($n = 6$) rendered a wide 95% CI (1.9-38.5). TART was diagnosed in 2 of 14 patients (14%) with presumed NC CAH (Table 1), who both had homozygous V281L mutations. Age at diagnosis and TART development.

Diagnosed children were predominantly treated with hydrocortisone, but prednisolone or "other" glucocorticoids were occasionally prescribed during the first 4 years of life. Of the treated patients with available data, the median hydrocortisone(-equivalent)[31] doses during the first 4 years of life were 5.5 mg (IQR = 5.0-7.5), 7.5 mg (5.0-10), 7.5 mg (6.9-10), and 9.0 mg (7.5-12), around each birthday respectively. Normalized for body surface area (calculated with the formula of Mosteller[32]), this corresponded to 27.9 mg/day/m^2 (22.9-37.9), 20.7 mg/day/m^2 (16.8-29.4), 19.7 mg/day/m^2 (16.5-24.7), and 17.2 mg/day/m^2 (15.1-20.8), respectively. To investigate if poor disease control with concomitant high ACTH exposure in early life affected the risk of TART diagnosis, we first evaluated whether a delayed diagnosis—and, therefore, a longer untreated period—increased the risk of TART diagnosis during lifetime. Unadjusted, a delayed CAH diagnosis of >1 year, compared with a diagnosis within 1 month, did not significantly affect TART development (HR = 1.8; $P = .06$). However, as patients with SW 21OHD (which have a 3.3-times higher risk of TART development) are typically diagnosed earlier than SV or NC 21OHD patients (data not shown), the association between age at diagnosis and TART development is underestimated if left unadjusted for the negative confounding effect of CAH phenotype. When adjusted for CAH phenotype, a delayed CAH diagnosis (or presentation) of >1 year, compared with a diagnosis within 1 month, was associated with 2.6 times higher risk of TART diagnosis (Table 2). A slightly delayed CAH diagnosis between 1 month and 1 year of age was not significantly associated with a higher risk of TART diagnosis. When solely focusing on patients with SW 21OHD ($n = 98$; events = 35), a significant impact of age at diagnosis on

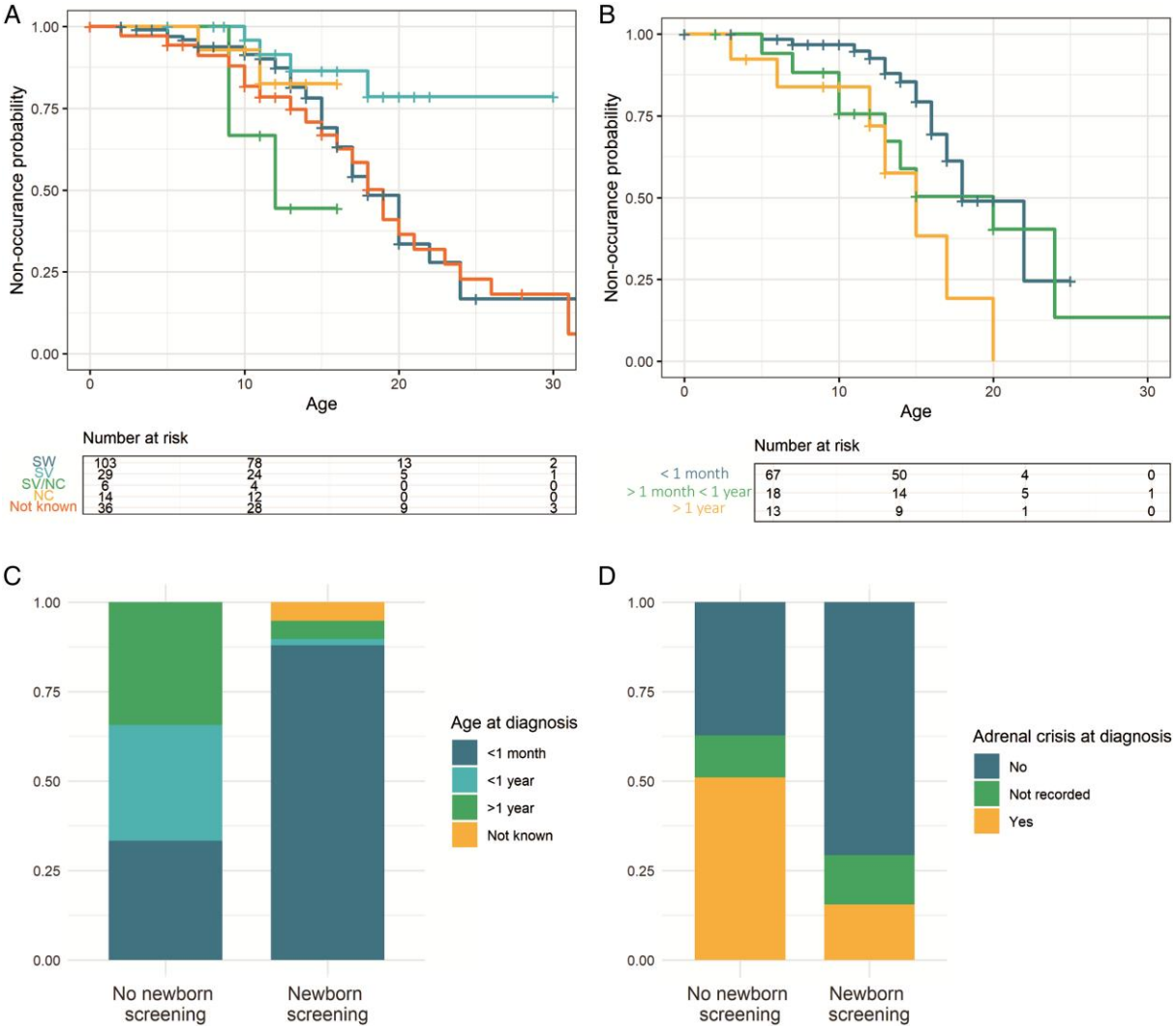

Figure 1. Predicted non-occurrence probability of TART in men with 21OHD or 11OHD stratified by CAH phenotype (A) or grouped age at CAH diagnosis (B) and the effect of neonatal screening on age at 21OHD diagnosis (C) and occurrence of adrenal crisis at 21OHD diagnosis (D).

Table 2. Putative risk factors for TART diagnosis expressed as HR with 95% CIs.

	Schoenfield's *p*	Events	HR	CI	*P*-value
CAH type					
SW vs SV	0.867	72/188	3.250	1.158-9.121	.03
SV/NC vs SV			8.457	1.857-38.522	<.01
NC vs SV			2.603	0.467-14.519	.28
Not known vs SV			3.604	1.236-10.507	.02
Age diagnosis grouped[a]					
<1 year >1 month vs <1 month	0.993	72/188	1.438	0.784-2.639	.24
>1 year vs <1 month			2.645	1.374-5.091	<.01
Not known vs <1 month			0.945	0.318-2.806	.92
Neonatal screening[a]					
No vs yes	0.057	72/188	3.242	1.551-6.779	<.01
Not known vs yes			1.098	0.382-3.156	.863
Biochemical control visit 2 (year 1)[a]					
Overtreatment vs adequate	0.288		0.717	0.228-2.252	.57
Undertreatment vs adequate			0.863	0.214-3.479	.84
Not known vs adequate			0.873	0.363-2.097	.76
Biochemical control visit 3 (year 2)[a]					
Overtreatment vs adequate	0.562		0.957	0.269-3.409	.95
Undertreatment vs adequate			1.842	0.590-5.750	.29
Not known vs adequate			1.288	0.532-3.122	.58
Biochemical control visit 4 (year 3)[a]					
Overtreatment vs adequate	0.186		0.664	0.193-2.279	.52
Undertreatment vs adequate			2.161	0.720-6.488	.17
Not known vs adequate			0.970	0.427-2.204	.94
Biochemical control visit 5 (year 4)[a]					
Overtreatment vs adequate	0.187		0.626	0.188-2.087	.45
Undertreatment vs adequate			1.413	0.449-4.450	.56
Not known vs adequate			1.001	0.478-2.096	1.00
Corrected height SDS visit 5	0.062	37/98	1.271	1.062-1.521	<.01
Bone age advancement (years) visit 5	0.080	21/58	1.115	0.927-1.341	.25

The assumption of proportional hazards was tested using Schoenfield's test.
[a]Adjusted for CAH type.

TART development was observed; Patients with a delayed diagnosis of >1 year faced a 3.4-fold increase in risk of TART development ($P < .01$) compared with patients diagnosed within the first month. Figure 1B illustrates the non-occurrence of TART over time in patients with SW CAH, stratified for age at diagnosis. A diagnosis of CAH within the first month of life did not prevent TART development in all patients but seemed to overall delay the presence of TART. No effect of age at diagnosis on TART development was observed when separately analyzing the patients with SV 21OHD (HR = 0.7; $P = .8$), potentially because of the small sample size ($n = 27$, events = 4).

The neonatal screening was performed in at least 32% of the patients with 21OHD and resulted as expected in earlier 21OHD diagnosis (Figure 1C; χ^2 test; $P < .001$); 68 of the 102 patients (67%) without neonatal screening for 21OHD were diagnosed after the first month of life, of which 35 patients (34%) were diagnosed after 1 year of age, while only 4 patients neonatally screened for 21OHD (7%) were diagnosed after the first month of life. Of these 4 patients, 3 patients had a false negative result, and 1 patient was diagnosed just after 1 month because of a delayed result. Adjusted for CAH phenotype, patients diagnosed by a neonatal screening program faced a 3.2 times lower risk of TART development compared with CAH patients not diagnosed by a neonatal screening program ($P < .01$). Neonatal screening was also associated with lower occurrence (17% vs 58%) of adrenal/SW crisis at diagnosis in patients with 21OHD (Figure 1B; χ^2 test; $P < .001$). Two of 4 patients prenatally treated with dexamethasone developed TART. The exact timeframe of dexamethasone treatment is not known.

Biochemical disease control and TART development

ACTH is not routinely measured in clinical practice and retrospective data on ACTH levels were available only for a total of 145 assessments (120 with interpretation) of 69 patients (52 with interpretation). Patients who were categorized as undertreated had significantly higher blood ACTH levels in contrast to patients classified as adequately treated or overtreated when all assessments were combined (pairwise Mann–Whitney *U*-tests with Benjamini–Hochberg adjustment). Of the 52 ACTH assessments that were classified as "high", 20 patients were classified as undertreated, while 26 patients were classified as adequately treated, and 6 patients were even classified as overtreated.

Data on interpreted biochemical disease control (undertreatment, adequate treatment, or overtreatment) were available for 44 patients at a visit between 0.5 and 1.5 years of age, for 52 patients at a visit between 1.5 and 2.5 years of age, 47 patients at a visit between 2.5 and 3.5 years of age, and 54 patients at a visit between 3.5 and 4.5 years of age. Of the 59 patients with multiple (available) biochemical assessments during their first 4 years of life, 40 patients had variable biochemical assessments over the years. Undertreatment vs adequate or overtreatment at yearly visits during the first 4 years of life did not predict TART diagnosis later in life (Table 2).

Growth acceleration, bone age advancement, and TART development

To evaluate if chronic poor disease control during early life was associated with TART development, the relation between

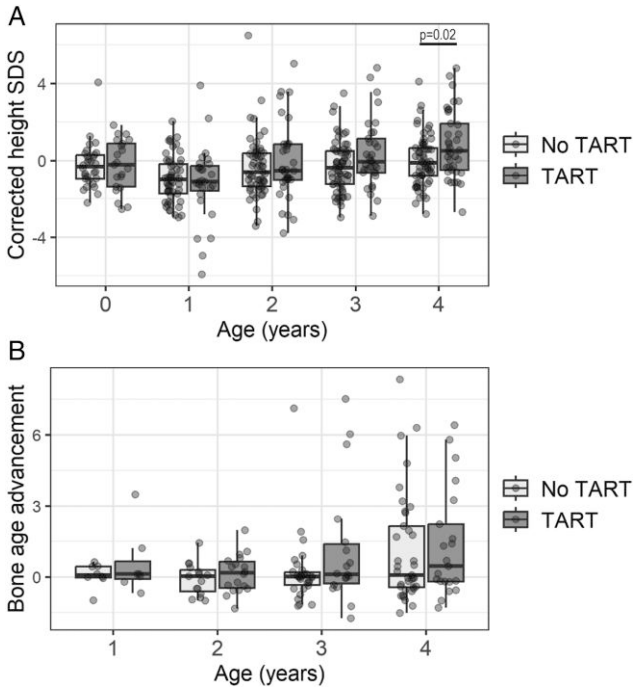

Figure 2. Retrospective height for age SDS corrected for target height SDS (A) and bone age vs chronological age (B) of assessments during the first 4 years of life in patients with and without TART diagnosis in their lifetime.

growth acceleration or bone age advancement and TART development was assessed. Exposure to high androgen levels leads to growth acceleration and bone age advancement and patients with high androgen levels presumably also have high ACTH levels. Data on patient's height and their biological mother's and father's final height was available for 51 patients with an assessment within the first 6 months of life (age 0) and for 81, 90, 96, and 98 patients around their first, second, third, and fourth birthday, respectively (Figure 2A). Corrected height SDS at the first 4 height assessments did not differ between patients who developed or did not (yet) develop TART (Figure 2A). However, at the last assessment, around the fourth birthday, the median (IQR) height SDS was significantly higher (Wilcoxon signed rank test; $P = .02$) in patients with TART [0.49 SDS (−0.5-1.9)] vs without TART [−0.1 SDS (−0.8-0.7)] (Figure 2). Cox proportional hazard analysis showed that patients with an increased corrected height SDS of 1 SDS around the fourth birthday had a 27% higher risk of a positive TART screening (Table 2). Data on bone age was available for 15, 33, 48, and 58 patients around the first, second, third, and fourth birthday, respectively. No statistically significant difference in bone age vs chronological age during yearly visits of the first 4 years of life between patients that developed TART vs patients who did not develop TART was observed (Figure 2B). Bone age advancement during the first 4 years of life did not predict TART detection (Table 2).

Discussion

This retrospective open cohort study describes the largest cohort of CAH males being evaluated for TART. Using the I-CAH registry, this study was able to include a total of 188 patients. This study investigated the association between biochemical disease control during infancy and early childhood and the development of TART in male patients with 21OHD or 11OHD. It was hypothesized that high ACTH exposure during early life promotes the development of TART. Postnatal trophic stimulation of adrenal(-like) tissue by ACTH may prevent physiological reduction of the relatively large-sized neonatal adrenal gland,[33,34] as well as regression of adrenal rest tissue[13] or a yet undefined cell-population in the testes. Neonatal trophic stimulation of cells within the testes may even increase the pool of ACTH-sensitive cells that could grow into significantly sized TART during periods of poor hormonal control. When adjusted for CAH phenotype, a delayed CAH diagnosis of >1 year after birth was associated with a higher risk of TART development. This relation might be attributed to chronically ACTH exposure of cells within the infantile testes. Neonatal screening for CAH resulted in earlier diagnosis and may therefore help improve early CAH treatment and lower the risk of TART development. Previously, a large epidemiological study by Falhammar et al.[35] reported that fertility outcomes (number of men that fathered a child) normalized for men with CAH after the introduction of neonatal screening. Although a potential role of TART could not be established in this study, this study could possibly complement our results. It should be noted that early CAH diagnosis and the consequent start of treatment within the first month of life did not prevent TART development in all patients. TART has also been described in patients with acquired conditions with elevated ACTH-hypersecretion,[36-40] suggesting that neonatal (or prenatal) ACTH overexposure is not a prerequisite for TART development. However, TART occurrence is rare in acquired endocrinopathies,[14] despite high ACTH exposure during adulthood. Therefore, it could be speculated that chronic ACTH exposure during infancy in patients with CAH may promote and accelerate TART development by preventing or inhibiting regression of adrenal rest cells or a yet undefined cell population and by facilitating expansion of these cells that could develop into detectable TART earlier on, within a shorter period of poor disease control.

A potential confounder of our study should, however, be discussed. Although the number of patients per center was low and prevalence per center could not be properly estimated, the difference in prevalence of TART between centers has been reported previously[4] and there might be a center-effect on the risk of TART development. For centers with less experience in CAH care or a lower quality health-system in general, patients might be at higher risk for delayed CAH diagnosis (less CAH awareness and/or no neonatal screening) and for (earlier) TART development. Difference in TART prevalence between centers advocates for improvement and uniformity of CAH care and guidelines to prevent this long-term complication. The number of patients per center did not allow normalization for center-specific effects, nor did it allow for subanalyses per center. Previous smaller studies have not found differences in age at diagnosis or treatment onset between patients with or without TART.[41-43] However, 2[41,42] of the 3 studies did not specifically evaluate this relation. Even more importantly, in contrast to the previous studies, the current study adjusted for disease severity (which is essential as age at CAH diagnosis varies with disease severity[43] and interferes with risk of TART development) and for the right-censored nature of the data. Of note, patients with the SW 21OHD are generally diagnosed and treated earlier than patients with SV 21OHD (data not shown). Still, patients with SW phenotype faced a 3-fold higher risk of TART

development in comparison to patients with the SV phenotype. Similar results were reported by Reisch et al.[12] This observation probably stresses that the effect of CAH disease severity is larger than the effect of a delayed CAH diagnosis on the risk of TART development. Moreover, the absence of a detected association between a delayed CAH diagnosis between 1 month and 1 year, compared with a CAH diagnosis within the first month of life, and the development of TART might indicate that a considerable delay in CAH diagnosis is needed in order to observe an impact on TART development.

Besides neonatal ACTH exposure, fetal or embryological ACTH exposure or faulty mechanisms occurring during fetal development have been proposed as being key in TART development,[12,44] and may also explain the predisposition to TART development in patients with CAH vs patients with later-onset ACTH-hypersecretion; Turcu et al.[44] noticed that "adrenal remnants' were more prevalently observed in neonates and infants with 21OHD[45] vs unaffected neonates.[46] Although the exact timeframe of prenatal dexamethasone treatment was unknown and the number of patients is limited, the current study showed that prenatal dexamethasone treatment did not prevent TART development, as 2 of 4 prenatally treated patients still developed TART.

Previous studies have been aiming to relate TART (development) to measures of hormonal control, measured either at the moment of TART detection or in a certain period before TART detection.[8,12,41-44,47-54] ACTH is not commonly measured as part of clinical care and multiple retrospective studies, including this study, used other measures of disease control, assuming that these parameters reflect ACTH levels. In previous studies, TART has been reported in obviously "well-controlled" patients.[47,53,54] Data on ACTH levels in these patients were not available or reported. The goal of conventional glucocorticoid therapy in CAH is to suppress the adrenal androgen concentrations, but not 17OHP levels (and likely ACTH levels) in order to prevent glucocorticoid overtreatment.[16] Novel therapies allowing more efficient suppression of ACTH without the need for supraphysiological glucocorticoids doses, such as the use of corticotropin-releasing hormone (CRH) antagonists or ACTH receptor antagonists, are, therefore, promising strategies to lower the prevalence of TART.[3] This study showed that ACTH levels could indeed still be elevated in patients classified as adequately treated or even overtreated. The use of other steroid hormones to define disease control as an estimate of ACTH exposure is therefore an important limitation. This study also collected data on ACTH levels during early childhood, but no sufficient data was available to study associations with TART development. The sometimes elevated ACTH levels in adequately or overtreated patients, but also the degree of missing data on biochemical disease control may explain why we—but also other studies—did not find an association between biochemical disease control (during the first 4 years of life) and TART detection. In addition, yearly biochemical assessments are only a snapshot of disease control during that year. Therefore, this study also assessed growth velocity and bone age advancement, as a result of chronic androgen overexposure and, likely, ACTH overexposure. At the end of the 4-year study-period, an increased height for age SDS, reflecting poor past disease control,[55] was associated with higher risk of TART development. The difference between chronological age and bone age seemed higher for patients who developed TART at the end of the study period, but advanced bone age at the end of the follow-up period did not significantly predict TART diagnosis. The discrepancy between bone age advancement and increased height for age SDS can probably be explained by the lower number of patients with available bone age data. Increased growth velocity or advanced bone maturation may not yet be expected to be substantial in poorly treated patients with CAH during the first 3 years of life, at least not during the first year of life in untreated SV patients.[56] Nonetheless, together the data suggests that poor disease control during the first 4 years of life is associated with increased risk of TART development, already in childhood. Clinicians should be vigilant for TART already in early childhood. Although we were interested in the relation between ACTH exposure during early childhood and TART diagnosis later in life, it would be of interest to also review data after 4 years of life, in order to verify if poor disease control during early life reflects poor disease control after this 4-year period, affecting TART development. While the size of this international patient cohort is a strength, potential variation between centers with respect to patient care or experience in testicular ultrasonography could be a limitation. A prospective multicenter study with harmonized evaluation of hormonal disease control and treatment compliance at set timepoints along with standardized evaluation of TART is of interest to verify our findings. Nonetheless, this international study gives a clear image of the prevalence of TART and its medical impact on males with CAH and the potential impact of delayed diagnosis and poor disease control on TART development.

In conclusion, adjusted for CAH phenotype, a delayed CAH diagnosis was associated with a higher risk of TART development, which might be due to longer and higher neonatal ACTH exposure, but may also reflect the differences in TART risk across centers.

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