Molecular Characterization of Testicular Adrenal Rest Tumors in Congenital Adrenal Hyperplasia: Lesions With Both Adrenocortical and Leydig Cell Features

Evelien E. J. W. Smeets, Paul N. Span, Antonius E. van Herwaarden, Ron A. Wevers, Ad R. M. M. Hermus, Fred C. G. J. Sweep, and Hedi L. Claahsen-van der Grinten

Departments of Pediatrics (E.E.J.W.S., H.L.C.-v.d.G.), Laboratory Medicine (E.E.J.W.S., A.E.v.H., R.A.W., F.C.G.J.S.,), Radiation Oncology (P.N.S.), and Internal Medicine (A.R.M.M.H.), Radboud University Medical Center, 6500 HB Nijmegen, The Netherlands

Context: Testicular adrenal rest tumors (TART) are one of the major long term complications in patients with congenital adrenal hyperplasia. Although several adrenal-like properties have been assigned to these benign lesions, the etiology has not been confirmed yet.

Objective: The aim of this study was to describe TART in more detail by analyzing several (steroidogenic) characteristics that may be classified as adrenal cortex or Leydig cell specific.

Methods: Gene expression analysis by qPCR was performed for 14 genes in TART tissue (n = 12) and compared with the expression in healthy control fibroblasts (nonsteroidogenic control). In addition, a comparison was made with the expression levels in testis tissue (n = 9) and adrenal tissue (n = 13).

Results: Nearly all genes were highly expressed in TART tissue, including all genes that encode the key steroidogenic enzymes. TART expression levels are in the majority almost identical to those found in adrenal tissue. The expression of adrenal cortex specific genes (*CYP11B1, CYP11B2,* and *MC2R*) in both TART and adrenal tissue is approximately 1000–10 000 times higher compared to that in testes samples. In addition, the Leydig cell markers *INSL3* and *HSD17B3* were not only found in testes, but also in TART, both at significantly higher levels than in the adrenal ($p < 0.01$).

Conclusion: Our study shows for the first time that TART have multiple steroidogenic properties, which include not only the expression of adrenal cortex but also of Leydig cell markers. Therefore, the origin of these tumors might be a more totipotent embryonic cell type. **(*J Clin Endocrinol Metab* 100: E524–E530, 2015)**

Congenital adrenal hyperplasia (CAH) is a group of inheritable diseases that are characterized by a disturbed adrenocortical steroid synthesis. In most cases (95%) this is caused by a defect of the *CYP21A2* gene, resulting in 21-hydroxylase deficiency (1, 2). The direct effect of the 21-hydroxylase deficiency is the inadequate production of cortisol by the adrenal cortex and, in some cases, also decreased aldosterone production, which can be life threatening at birth. The lack of negative feedback of cortisol to the hypothalamus and pituitary gland results in increased corticotropin-releasing hormone and adrenocorticotropic hormone (ACTH) levels in blood. Consequently, chronic stimulation of high ACTH levels induces the adrenal cortex to become hyperplastic and to produce high amounts of steroids and steroid precursors that do not require 21-hydroxylase for their synthesis.

Patients are currently diagnosed at an early age and treatment strategies are significantly improved, leading to

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
Copyright © 2015 by the Endocrine Society
Received April 11, 2014. Accepted December 2, 2014.
First Published Online December 8, 2014

Abbreviations: ALC, adult Leydig cell; CAH, congenital adrenal hyperplasia; FLC, fetal Leydig cell; hCG, human chorionic gonadotropin; TART, testicular adrenal rest tumor.

Downloaded from https://academic.oup.com/jcem/article/100/3/E524/2840009 by Reprints Desk user on 14 November 2025

decreased morbidity and mortality (3). Most of these patients reach adulthood without serious life threatening complications and therefore, long-term complications are becoming more important. One of the major complications in male patients is the development of benign testicular tumors (4). These lesions have been reported to be predominantly bilateral and have been linked to infertility because of the mechanical obstruction of the seminiferous tubules (5). Because of functional and histological resemblance with adrenocortical cells, these tumors were named testicular adrenal rest tumors (TART) (6).

Prevalence studies show considerable differences between the prevalence numbers of TART in CAH patients, which can mainly be attributed to patient selection and method of detection (7–9). Studies applying the classical method of palpation report low TART percentages of 0–35% (10–12). However, lesions below 2 cm are generally not palpable because of their typical location within the mediastinum testes. With the use of ultrasonographic examination of the testes, the prevalence was found to be considerably higher, ranking up to 94% in adults (11–13).

The etiology of TART is not completely understood, but it is thought that these tumors arise from aberrant adrenocortical cells that are stimulated to grow under the influence of high blood ACTH levels. The role of ACTH in TART growth is supported by the fact that TART is mainly described in poorly controlled patients and the observation that a high dosage of glucocorticoids may reduce tumor size in some cases (7, 14). However, tumors are also present in well-controlled patients and intensifying the doses of glucocorticoid treatment does not always result in decreased TART volume, suggesting that there are additional factors involved (15, 16).

Previous studies on TART characteristics report adrenal specific steroids and expression of adrenal specific genes, which suggest the presence of adrenocortical-like properties of TART (6, 17, 18). However, a complete characterization of the TART tissue has not been described and the origin of the tumor cells has never been confirmed. The aim of this study was therefore to map the characteristics of this tissue and compare these to adrenocortical and testis tissue to increase the knowledge on the identity and origin of TART.

Materials and Methods

Sample collection

Twelve TART samples of 6 adult CAH patients (tumors of left and right testis) were collected from surgery as described earlier (6). Written informed consent was obtained from all patients. Tumor tissue was obtained after careful blunt dissection and observed macroscopically and microscopically to be sure no residual testis tissue was included (5). TART biopsies were stored in liquid nitrogen. Frozen tissue sections (5 μm) were stained with hematoxylin and eosin for morphological evaluation. Patient information on phenotype, mutation analysis, tumor weight, and operation indication of the 6 patients is listed in Supplemental Table 1. In addition to the TART samples, 9 samples of normal testis tissue and 13 normal adrenal samples (all male patients) were obtained via the Radboudumc Biobank.

Immunofluorescence

Randomly selected 5-μm tissue sections of TART and normal testis samples were used for fluorescent staining. Sections were fixed in acetone for 10 min at 4°C and rehydrated in 1× phosphate buffered saline. Staining for Insl3 was obtained with overnight incubation at 4°C of an Insl3 antibody (H-035–27, Phoenix Pharmaceuticals) diluted 1:1000 in primary antibody diluent (AbD Serotec), followed by 30 min incubation of a Cy3-conjugated Goat Anti-Rabbit secondary antibody (Jackson Immuno Research Laboratories) at 37°C. All sections were additionally DNA-stained with Hoechst 33342 fluorescent stain and mounted in Fluorostab (ICN Pharmaceuticals). Stained sections were observed and analyzed using a fluorescence microscope (Axioskop, Zeiss) and IPLab software (Scanalytics).

RNA isolation

Frozen tissue sections (10 × 20 μm) were used for RNA isolation, performed with the Total RNA Purification Kit (Norgen Biotek Corporation). The tissue sections were homogenized by addition of lysis buffer and pushing the homogenate through a 21 gauge needle using a 1 mL syringe. Further steps of the isolation were performed according to the manufacturer's protocol. In addition, RNA of healthy control fibroblasts was included as a nonsteroidogenic control. RNA concentrations and quality were determined using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific).

Primer design and optimization

For the characterization of the TART tissue, 14 genes were selected, including the genes encoding 9 key steroidogenic enzymes involved in the adrenocortical and testicular steroid synthesis. Based on the presence or absence of these 9 specific enzymes involved in steroid synthesis, the steroidogenic pathway was divided in four parts: a common, an adrenocortical, a testicular, and a peripheral part (Figure 2A). Early steps in steroidogenesis are common to the adrenal cortex and the Leydig cells of the testis and therefore the enzymes involved in this part (mainly encoded by *STAR*, *CYP11A1*, *CYP17A1*, and *HSD3B2*) can be found both in the adrenal cortex and in the testes. Within the adrenocortical section of the steroidogenic pathway, aldosterone and cortisol and all intermediate products are produced, and therefore the responsible genes (eg, *CYP11B1* and *CYP11B2*) are considered adrenocortical specific. The testis specific conversion of androstenedione to testosterone is catalyzed by 17βHSD type 3, the product of the *HSD17B3* gene (19). Final conversions of androstenedione and testosterone to estrone and estradiol by aromatase (*CYP19A1*) and of testosterone to the dihydroxytestosterone metabolite by 5α-reductase (*SRD5A1*) occur mostly in peripheral regions. In addition to these steroidogenic enzymes, 5 more genes were selected. The common group was expanded with the *NR5A1* gene, encoding for Steroidogenic factor 1 (SF1). Genes encoding the angiotensin II and ACTH receptors (*AGTR2* and *MC2R*) were

Downloaded from https://academic.oup.com/jcem/article/100/3/E524/2840009 by Reprints Desk user on 14 November 2025

added as adrenocortical specific markers. *LHCGR* (encoding the LH/human chorionic gonadotropin [hCG] receptor) and *INSL3* (Insulin-like 3; protein produced by Leydig cells) were chosen for their main functionality in the testes (20).

Gene specific primers were retrieved from the RT primerdatabase (21) or, in the case of absent or unsuitable primers, self-designed using online primer-designing tools (NCBI, primer3plus). Genes for which no primers were available from the RTprimerDB were searched in the NCBI database, and primers were devised based on an amplicon size of 70–200 base pairs (bp), a length of 19–23 nucleotides, a GC-content of 35–65% and an exon-exon junction spanning amplicon. The *HPRT* gene was used as a normalizing gene (22). A set of forward and reverse primers for each gene was ordered (Biolegio). Sequences and specifications of all primers are listed in Supplemental Table 2. Primers were tested on cDNA of eight different human tumor cell lines using the SYBR Select Master Mix for CFX (Applied Biosystems, Life Technologies) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories GmbH). The PCR products were checked for quality and product size by melting curve analysis and agarose gel electrophoresis.

Reverse transcription and qPCR

0.5 μg of total RNA in a volume of 20 μL was used for cDNA synthesis using the GoScript Reverse Transcription System (Promega). cDNA synthesis was performed according to the manufacturer's protocol with a 2720 Thermal cycler (Applied Biosystems).

For qPCR, the cDNA samples were diluted 10 times and 5 μL was added to 7.5 μL Sybr Select Master Mix for CFX (2×; Applied Biosystems), in a total amount of 15 μL on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories GmbH). All values were normalized to the corresponding *HPRT* value and then to the nonsteroidogenic control.

Data analysis

The mRNA expression of all 14 genes was calculated using the delta-delta C_t method ($2^{-\Delta\Delta Ct}$). Data were converted to GraphPad Prism 5 for further analysis. Expression levels of TART, testes and adrenal samples were calculated relative to the corresponding value of the nonsteroidogenic control. For each gene, the distribution of the samples was tested for normality (D'Agostino and Pearson omnibus normality test). Differences between the different tissues (TART, adrenal, testis) were tested for significance with the Kruskal-Wallis test for nonparametric data, followed by a Dunn's post hoc test. Normally distributed data were analyzed using the One-way analysis of variance (ANOVA) with an additional Tukey's multiple comparison test. Values of $P < .05$ (*) or $P < .01$ (**) were considered statistically significant.

Results

Morphological evaluation and Insl3 immunofluorescent staining of TART samples

Macroscopically, the TART tissues were found to be firm and multilobular nodules with a yellow to tan surface color and narrow bands of fibrous tissue. These tumors were sharply demarcated but unencapsulated and could easily be removed from the surrounding testis tissue. Microscopically evaluated sections showed that no normal testicular cells were present within the TART tissue, including Leydig cells. Immunofluorescent staining with the Leydig cell marker Insl3 in the testis samples showed a specific expression located between the seminiferous tubules, corresponding with the expected Leydig cell pattern (Figure 1A). In the TART samples, no Insl3 staining was observed, indicating that there are no normal Leydig cells present (Figure 1B).

Characterization of TART steroidogenic properties and a comparison with adrenal and testis mRNA levels

Expression levels of steroidogenic tissue-specific enzymes (Figure 2A), were quantified by qPCR in the TART samples (red dots, Figure 2B) as the ratio of expression in the nonsteroidogenic fibroblast control (horizontal dotted line); all delta CT values are listed in Supplemental Table 3. The five genes that are typically expressed in both adrenal and testis tissue, thus belonging to the common group (gray section), are all highly expressed in TART with a median of 8.1×10^4 (*STAR*), 3.6×10^2 (*CYP11A1*), 7.9×10^5 (*CYP17A1*), 3.9×10^4 (*HSD3B2*), and 1.4×10^6 (*NR5A1*) times higher, respectively, than the nonsteroidogenic control. With a median expression of 3.1×10^4 (*CYP11B1*), 2.8×10^1 (*CYP11B2*), 2.8×10^0 (*AGTR2*), and 7.4×10^2 (*MC2R*), the expression levels of adrenocortical-specific genes (pink section) are also higher in TART than in the nonsteroidogenic control. The Leydig cell genes *LHCGR*, *INSL3*, and *HSD17B3* (blue section) have an expression in TART of 1.3×10^3, 5.2×10^2, and 1.5×10^2 times higher than the nonsteroidogenic control. Expression of *CYP19A1* and *SRD5A1* is relatively

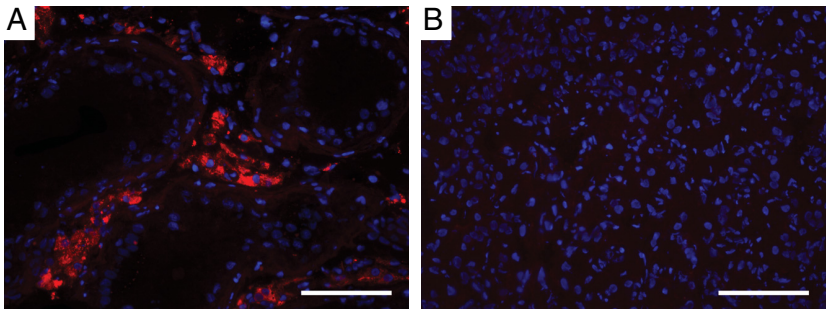

Figure 1. Immunofluorescent staining of Insl3 showed that this protein was specifically stained in the Leydig cells of normal testis (A), whereas no staining was observed in TART tissue (B). Scale bar = 100 μm.

Downloaded from https://academic.oup.com/jcem/article/100/3/E524/2840009 by Reprints Desk user on 14 November 2025

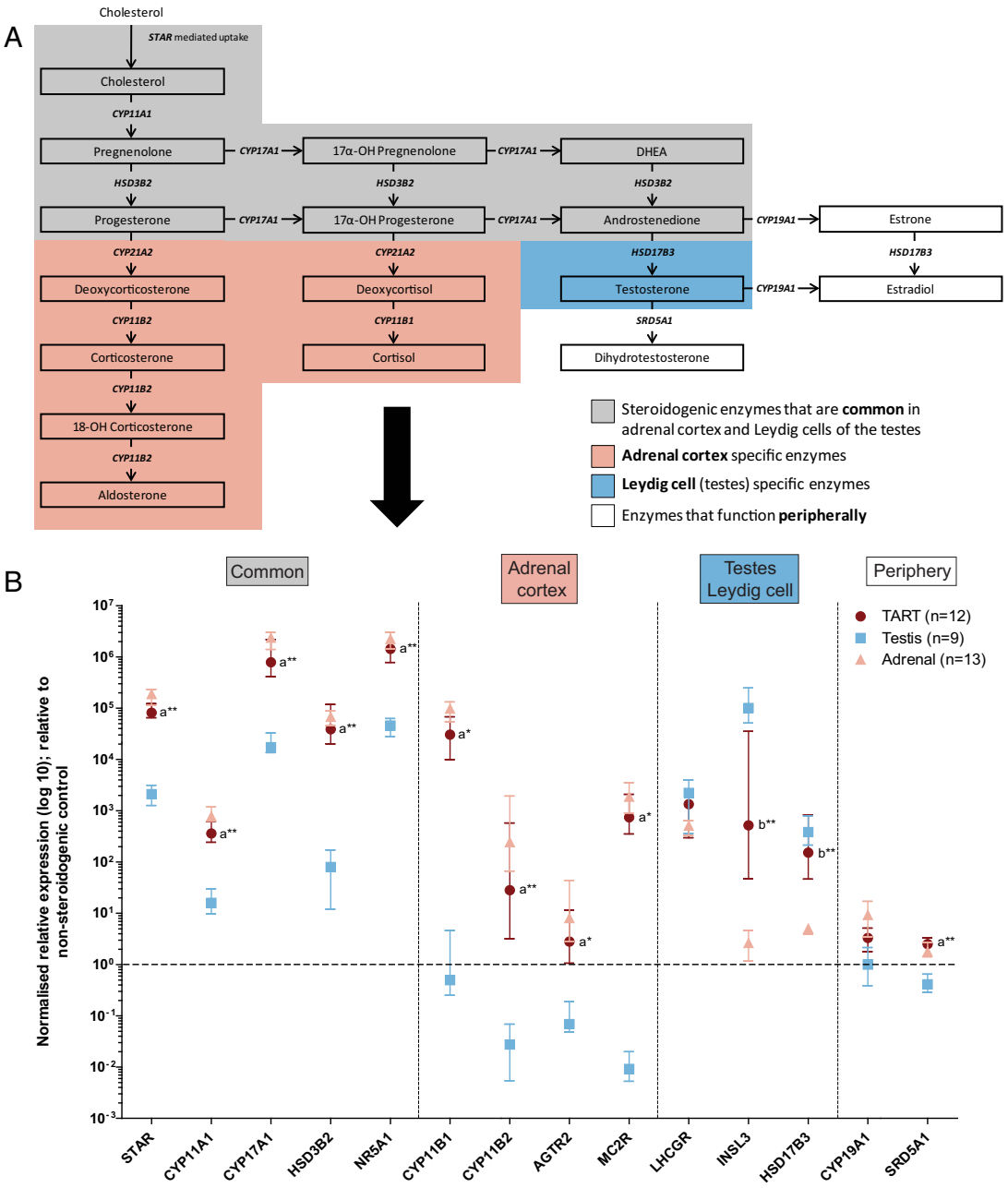

Figure 2. A. Overview of steroidogenesis with main products and corresponding enzyme-coding genes divided in a common part for both adrenal cortex and Leydig cells of the testis (gray), a group of adrenocortical specific genes (pink), and a Leydig cell specific part (blue). B. Gene expression profile of 14 markers measured in TART (red dots), adrenal (pink triangles), and testes tissue (blue squares). The genes were subdivided in the same groups as in panel A. 9 out of 14 genes encode the corresponding enzymes of the steroidogenic pathway. The symbols and error bars in the graph represent median and 25th and 75th percentile of all normalized relative expression values. Differences between TART and adrenal or testis tissue were tested for significance with the Kruskall-Wallis test. a: significantly different from testis; *, $P < .05$; **, $P < .01$; b: significantly different from adrenal; *, $P < .05$; **, $P < .01$.

low with a median of 3.3- and 2.5- fold increase compared to the nonsteroidogenic control sample.

In addition to the TART samples, expression of the 14 genes in 4 groups was measured in 13 adrenal samples (pink triangles) and 9 testes samples (blue squares) for comparison (Figure 2B). For all genes, the difference in expression between the adrenal and testes samples is statistically significant ($P < .05$ for *LHCGR*; $P < .01$ for *MC2R* and *SRD5A1*; $P < .001$ for all other genes). For the genes in the common group, the TART expression levels are nearly equal to the levels found in adrenal cortex tissue. For these five genes, the levels in the testes are approximately 10–100 times lower compared to the adrenal and TART levels ($P < .01$). For the four adrenocortical specific

Downloaded from https://academic.oup.com/jcem/article/100/3/E524/2840009 by Reprints Desk user on 14 November 2025

genes, the expression in TART is also comparable to the adrenal data. For these genes, the difference between the TART samples and the testes samples is statistically significant (TART-testes $P < .05$ for *CYP11B1*, *AGTR2*, and *MC2R*; $P < .01$ for *CYP11B2*). The expression of these "adrenocortical" genes in the testes samples is approximately the same as measured in the nonsteroidogenic control (or even less) and can therefore be considered as negative. For the Leydig cell markers, the relative expression in the three tissues has a different pattern than the common and adrenal cortex specific genes. Here, the adrenal expression levels are relatively low and for the testes these are relatively high, except for *LHCGR*, which has expression levels that are nearly equal in all samples. In this part, the TART levels are in the same range or near the testes levels and are significantly different ($P < .01$) from the adrenal expression for *INSL3* and *HSD17B3*. The expression of *CYP19A1* and *SRD5A1* is equally low in all three tissues. In summary, TART tissue shows gene expression levels that are equal to adrenal tissue for genes that are classified as common for the adrenal cortex and Leydig cell of the testes and genes that are adrenocortical specific. In addition, expression levels of Leydig cell markers were comparably high in both TART and testes tissue.

Discussion

TART is a typical finding in male adult CAH patients with a high prevalence in adulthood and can potentially lead to infertility and irreversible damage of the testicular tissue (5, 12). Knowledge about the etiology and the functional features is important to develop strategies for treatment and prevention of these tumors. Previous studies have mainly revealed adrenocortical specific characteristics and therefore an adrenal origin of TART was considered (17, 23). Our study shows for the first time that TART also have typical Leydig cell features based on the expression of *HSD17B3*, *LHCGR*, and *INSL3*. To our knowledge, 17βHSD and Insulin-like 3 expression has never been measured before in TART tissue. The present study expands the knowledge about the steroidogenic characteristics of TART tissues obtained in earlier studies (6, 17, 18) by additionally measuring common steroidogenic genes, adrenocortical genes, and Leydig cell genes. The presence of all common steroidogenic enzymes, measured by mRNA, indicates a capability of "basal" steroidogenesis of TART tissue. Notably, the expression of the common genes was lower in the testes samples compared to the adrenal samples, which may be explained by the small fraction of Leydig cells in testes compared to the number of steroidogenic adrenocortical cells in the adrenal gland (24, 25). The presence of adrenocortical specific *CYP11B1* and *CYP11B2* mRNA and mRNA measurements of ACTH and AII receptor corresponds well with previous results of our group (6). In the current study, we found mRNA levels of the LH/hCG receptor, as well as Leydig cell specific *HSD17B3* and *INSL3* levels highly expressed in TART tissue. The presence of LH/hCG receptors in TART tissue has been indicated before (26). Although others failed to measure these receptors (23), we found mRNA levels that are in the same range as the ACTH receptor. Although the presence of LH/hCG receptor mRNA indicates testis-like features of TART, it has to be noted that these receptors were also found in normal adrenocortical tissue, but its functionality is not clear (27). This corresponds with our analysis of LH/hCG receptor mRNA expression in adrenal tissue, which is very close to the levels found in testis and TART tissue. The presence of residual viable Leydig cells contaminating TART tissue was considered unlikely because the testis tissue surrounding the tumors was irreversibly damaged (5). This possibility was further excluded by careful macroscopic and microscopic evaluation and the immunofluorescence staining of INSL3. In normal testis tissue Leydig cells account for approximately 5–10% of total cells (25). In the case of a Leydig cell "contamination" of TART, a maximum of 10% Leydig cells is expected that would then account for the Leydig cell specific features within the investigated tissue. In contrast to the Leydig cell, specific immunofluorescent staining that was seen in the testis tissue, and no INSL3 staining was observed in TART tissue, indicating that the Leydig cell characteristics that were measured by qPCR belong to the tumor cells rather than contaminated Leydig cells. It is, however, interesting that no functional INSL3 protein was measured, while there has been mRNA measured in TART tissue. A possible explanation for this might be the differences in phenotypes of the TART and testis tissue. The testis consists of a low percentage of Leydig cells that account for the high *INSL3* expression measured by qPCR. In contrast, TART tissue consists of a uniform mass of TART cells that might have a low *INSL3* expression, but altogether show a high cumulative *INSL3* expression. In qPCR, the amount of total RNA is used and is more sensitive than immunofluorescence. It is therefore possible that a large part of the TART cells produce INSL3 protein, but in such low amounts that it cannot be detected by immunofluorescence. Another possibility is that there might be differences in translation efficiency of the INSL3 protein between TART and testis tissue. In TART, the steroidogenic cells have a different phenotype than the Leydig cells of the testis and this might include that in TART less protein is made or more protein is degraded from the same amount of RNA compared to the testis.

The etiology of TART is not yet understood. An earlier hypothesis was that in the embryological period cells des-

Downloaded from https://academic.oup.com/jcem/article/100/3/E524/2840009 by Reprints Desk user on 14 November 2025

tined to become adrenocortical cells migrate together with the descending testes. These adrenocortical cells may grow in the presence of growth promoting factors, such as ACTH and AII, thus explaining the high incidence of TART in classic CAH patients (28). Val et al suggest that adrenal-like cells in the testis originate from a different cell type that migrate from the mesonephros into the developing gonad (29). Other studies propose that TART may derive from pluripotent cells within the testes (30). The results of our TART gene expression analysis shows that these tumors possess both adrenal cortex and Leydig cell features, indicating a more totipotent embryological origin.

In mice, it has been reported that the Leydig cell lineage consists of several types that differ in time of presence, functional features, and origin (31). The fetal Leydig cell (FLC) population is present in the embryonic testis during development and declines shortly after birth (32, 33). These cells originate from the adrenogonadal primordium, the common precursor for the steroid producing adrenocortical and gonadal cells. It is thought that the FLCs are replaced by the adult Leydig cells (ALCs), which originate from a different cell type, that expand during puberty and remain functional during adult life. In contrast to the ALC, the FLC has features of both Leydig cells and adrenocortical cells (34, 35). O'Shaughnessy found that the melanocortin type-2 receptor (MC2R or ACTH receptor) is expressed in fetal mice testes and that ACTH incubation resulted in a clear increase of androgen production in fetal but not in adult testes (36). This indicates that the FLC contains ACTH receptors beside LH/hCG receptors, and that they can be stimulated by two pituitary hormones, LH and ACTH. For the human testicular development there is not much known about the role of the FLC, so differences with the murine type have to be taken into account. There are conflicting data about the *HSD17B3* expression in rodents (37, 38), so it still remains uncertain whether the FLC is capable of testosterone production or solely androstenedione production.

Because of the functional similarity between FLCs and TART we hypothesize that a totipotent embryological cell type (such as the FLC) may be the main progenitor of adrenal-like rests within the testes of CAH patients. Since these FLCs contain ACTH receptors, chronic exposure to ACTH as occurs during fetal life in patients with classic forms of CAH may not only lead to adrenal hyperplasia, but may also prevent regression of FLCs and cause proliferation of these cells depending on the duration and concentration of ACTH. In addition to the ACTH receptor, we found AII and LH/hCG receptor expression in tumor samples of our patients suggesting that TART might also be sensitive to AII and LH (39). The presence of the LH/hCG receptor on tumor tissue may explain the significant increase in prevalence of TART during puberty, even in well-controlled patients (10, 40). During puberty, the rise in LH may further stimulate the growth of the persistent FLCs in the testes of CAH patients. Although our results only focused on the mRNA level, it seems that TARTs have multiple steroidogenic features and possess characteristics that are known as both adrenocortical or Leydig cell specific.

In summary, our study indicates that TART consists of steroidogenic cells that have both Leydig cell and adrenocortical features and contain ACTH, AII, and LH/hCG receptors supporting the hypothesis of totipotent cells. These tumor cells resemble features of embryological cells (such as FLCs), which may be the main source of TART. Further studies have to be performed to investigate the functional features of TART on mRNA and protein level.

Acknowledgments

We thank Joop Heuvel for technical assistance, Dr Egbert Oosterwijk for providing testis and adrenal tissue, and Dr Christina Hulsbergen-van de Kaa for evaluation of the tissue sections.

Address all correspondence and requests for reprints to: Hedi Claahsen-van der Grinten, MD PhD, Department of Pediatric Endocrinology, Radboud University Medical Center (804), P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. E-mail: Hedi.Claahsen@radboudumc.nl.

This work was supported by the International Fund Congenital Adrenal Hyperplasia (2012 IFCAH grant).

Disclosure Summary: The authors have nothing to disclose.

References

1. **White PC, Speiser PW.** Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev*. 2000;21:245–291.
2. **Speiser PW, White PC.** Congenital adrenal hyperplasia. *N Engl J Med*. 2003;349:776–788.
3. **Thil'en A, Nordenström A, Hagenfeldt L, von Döbeln U, Guthenberg C, Larsson A.** Benefits of neonatal screening for congenital adrenal hyperplasia (21-hydroxylase deficiency) in Sweden. *Pediatrics*. 1998;101:E11.
4. **Reisch N, Arlt W, Krone N.** Health problems in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Horm Res Paediatr*. 2011;76:73–85.
5. **Claahsen-van der Grinten HL, Otten BJ, Hermus AR, Sweep FC, Hulsbergen-van de Kaa CA.** Testicular adrenal rest tumors in patients with congenital adrenal hyperplasia can cause severe testicular damage. *Fertil Steril*. 2008;89:597–601.
6. **Claahsen-van der Grinten HL, Otten BJ, Sweep FC, et al.** Testicular tumors in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency show functional features of adrenocortical tissue. *J Clin Endocrinol Metab*. 2007;92:3674–3680.
7. **Aycan Z, Bas VN, Cetinkaya S, Yilmaz Agladioglu S, Tiryaki T.** Prevalence and long-term follow-up outcomes of testicular adrenal

Downloaded from https://academic.oup.com/jcem/article/100/3/E524/2840009 by Reprints Desk user on 14 November 2025

rest tumours in children and adolescent males with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)*. 2013;78:667–672.
8. **Delfino M, Elia J, Imbrogno N, et al.** Testicular adrenal rest tumors in patients with congenital adrenal hyperplasia: prevalence and sonographic, hormonal, and seminal characteristics. *J Ultrasound Med*. 2012;31:383–388.
9. **Shanklin DR, Richardson AP Jr., Rothstein G.** Testicular hilar nodules in adrenogenital syndrome. The nature of the nodules. *Am J Dis Child*. 1963;106:243–250.
10. **Claahsen-van der Grinten HL, Sweep FC, Blickman JG, Hermus AR, Otten BJ.** Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Eur J Endocrinol*. 2007;157:339–344.
11. **Arlt W, Willis DS, Wild SH, et al.** Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab*. 2010;95:5110–5121.
12. **Stikkelbroeck NM, Otten BJ, Pasic A, et al.** High prevalence of testicular adrenal rest tumors, impaired spermatogenesis, and Leydig cell failure in adolescent and adult males with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2001;86:5721–5728.
13. **Reisch N, Flade L, Scherr M, et al.** High prevalence of reduced fecundity in men with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2009;94:1665–1670.
14. **Claahsen-van der Grinten HL, Otten BJ, Sweep FC, Hermus AR.** Repeated successful induction of fertility after replacing hydrocortisone with dexamethasone in a patient with congenital adrenal hyperplasia and testicular adrenal rest tumors. *Fertility and Sterility*. 2007;88:705.e5–705.e8.
15. **Walker BR, Skoog SJ, Winslow BH, Canning DA, Tank ES.** Testis sparing surgery for steroid unresponsive testicular tumors of the adrenogenital syndrome. *J Urol*. 1997;157:1460–1463.
16. **Stikkelbroeck NM, Hermus AR, Suliman HM, Jager GJ, Otten BJ.** Asymptomatic testicular adrenal rest tumours in adolescent and adult males with congenital adrenal hyperplasia: basal and follow-up investigation after 2.6 years. *J Pediatr Endocrinol Metab*. 2004;17:645–653.
17. **Bercovici JP, Fiet J, Gibault L, et al.** Testicular adrenal rest tumours in salt wasting congenital adrenal hyperplasia (in vivo and in vitro studies). *J Steroid Biochem Mol Biol*. 2005;93:67–72.
18. **Franco-Saenz R, Antonipillai I, Tan SY, McCorquodale M, Kropp K, Mulrow PJ.** Cortisol production by testicular tumors in a patient with congenital adrenal hyperplasia (21-hydroxylase deficiency). *J Clin Endocrinol Metab*. 1981;53:85–90.
19. **Day JM, Tutill HJ, Purohit A, Reed MJ.** Design and validation of specific inhibitors of 17beta-hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis. *Endocr Relat Cancer*. 2008;15:665–692.
20. **Ivell R, Wade JD, Anand-Ivell R.** INSL3 as a biomarker of Leydig cell functionality. *Biol Reprod*. 2013;88:147.
21. **Pattyn F, Robbrecht P, De Paepe A, Speleman F, Vandesompele J.** RTPrimerDB: the real-time PCR primer and probe database, major update 2006. *Nucl Acids Res*. 2006;34:D684–688.
22. **de Kok JB, Roelofs RW, Giesendorf BA, et al.** Normalization of gene expression measurements in tumor tissues: comparison of 13 endogenous control genes. *Lab Invest*. 2005;85:154–159.
23. **Clark RV, Albertson BD, Munabi A, et al.** Steroidogenic enzyme activities, morphology, and receptor studies of a testicular adrenal rest in a patient with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1990;70:1408–1413.
24. **Ross MH, Pawlina W.** *Histology: A Text and Atlas - With Correlated Cell and Molecular Biology*. Baltimore, MD: Lippincott Williams, Wilkins; 2010.
25. **Grinspon RP, Loreti N, Braslavsky D, et al.** Sertoli cell markers in the diagnosis of paediatric male hypogonadism. *J Pediatr Endocrinol Metab*. 2012;25:3–11.
26. **Benvenga S, Smedile G, Lo Giudice F, Trimarchi F.** Testicular adrenal rests: evidence for luteinizing hormone receptors and for distinct types of testicular nodules differing for their autonomization. *Eur J Endocrinol*. 1999;141:231–237.
27. **Pabon JE, Li X, Lei ZM, Sanfilippo JS, Yussman MA, Rao CV.** Novel presence of luteinizing hormone/chorionic gonadotropin receptors in human adrenal glands. *J Clin Endocrinol Metab*. 1996; 81:2397–2400.
28. **Hamwi GJ, Gwinup G, Mostow JH, Besch PK.** Activation of testicular adrenal rest tissue by prolonged excessive ACTH production. *J Clin Endocrinol Metab*. 1963;23:861–869.
29. **Val P, Jeays-Ward K, Swain A.** Identification of a novel population of adrenal-like cells in the mammalian testis. *Dev Biol*. 2006;299: 250–256.
30. **Rutgers JL, Young RH, Scully RE.** The testicular "tumor" of the adrenogenital syndrome. A report of six cases and review of the literature on testicular masses in patients with adrenocortical disorders. *Am J Surg Pathol*. 1988;12:503–513.
31. **DeFalco T, Takahashi S, Capel B.** Two distinct origins for Leydig cell progenitors in the fetal testis. *Dev Biol*. 2011;352:14–26.
32. **Griswold SL, Behringer RR.** Fetal Leydig cell origin and development. *Sex Dev*. 2009;3:1–15.
33. **Hu GX, Lian QQ, Ge RS, Hardy DO, Li XK.** Phthalate-induced testicular dysgenesis syndrome: Leydig cell influence. *Trends Endocrinol Metab*. 2009;20:139–145.
34. **Habert R, Lejeune H, Saez JM.** Origin, differentiation and regulation of fetal and adult Leydig cells. *Mol Cell Endocrinol*. 2001;179: 47–74.
35. **O'Shaughnessy PJ, Baker P, Sohnius U, Haavisto AM, Charlton HM, Huhtaniemi I.** Fetal development of Leydig cell activity in the mouse is independent of pituitary gonadotroph function. *Endocrinology*. 1998;139:1141–1146.
36. **O'Shaughnessy PJ, Fleming LM, Jackson G, Hochgeschwender U, Reed P, Baker PJ.** Adrenocorticotropic hormone directly stimulates testosterone production by the fetal and neonatal mouse testis. *Endocrinology*. 2003;144:3279–3284.
37. **Shima Y, Miyabayashi K, Haraguchi S, et al.** Contribution of Leydig and Sertoli cells to testosterone production in mouse fetal testes. *Mol Endocrinol*. 2013;27:63–73.
38. **Weisser J, Landreh L, Söder O, Svechnikov K.** Steroidogenesis and steroidogenic gene expression in postnatal fetal rat Leydig cells. *Mol Cell Endocrinol*. 2011;341:18–24.
39. **Aguilar F, Lo M, Claustrat B, Saez JM, Sassard J, Li JY.** Hypersensitivity of the adrenal cortex to trophic and secretory effects of angiotensin II in Lyon genetically-hypertensive rats. *Hypertension*. 2004;43:87–93.
40. **Finkielstain GP, Kim MS, Sinaii N, et al.** Clinical characteristics of a cohort of 244 patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2012;97:4429–4438.

Downloaded from https://academic.oup.com/jcem/article/100/3/E524/2840009 by Reprints Desk user on 14 November 2025