Human 3 β -hydroxysteroid dehydrogenase deficiency seems to affect fertility but may not harbor a tumor risk: lesson from an experiment of nature

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Abstract

Context: 3 β -hydroxysteroid dehydrogenase deficiency (3 β HSD) is a rare disorder of sexual development and steroidogenesis. There are two isozymes of 3 β HSD, *HSD3B1* and *HSD3B2*. Human mutations are known for the *HSD3B2* gene which is expressed in the gonads and the adrenals. Little is known about testis histology, fertility and malignancy risk.

Objective: To describe the molecular genetics, the steroid biochemistry, the (immuno-)histochemistry and the clinical implications of a loss-of-function *HSD3B2* mutation.

Methods: Biochemical, genetic and immunohistochemical investigations on human biomaterials.

Results: A 46,XY boy presented at birth with severe undervirilization of the external genitalia. Steroid profiling showed low steroid production for mineralocorticoids, glucocorticoids and sex steroids with typical precursor metabolites for HSD3B2 deficiency. The genetic analysis of the *HSD3B2* gene revealed a homozygous c.687del27 deletion. At pubertal age, he showed some virilization of the external genitalia and some sex steroid metabolites appeared likely through conversion of precursors secreted by the testis and converted by unaffected *HSD3B1* in peripheral tissues. However, he also developed enlarged breasts through production of estrogens in the periphery. Testis histology in late puberty revealed primarily a Sertoli-cell-only pattern and only few tubules with arrested spermatogenesis, presence of few Leydig cells in stroma, but no neoplastic changes.

Conclusions: The testis with HSD3B2 deficiency due to the c.687del27 deletion does not express the defective protein.

This patient is unlikely to be fertile and his risk for gonadal malignancy is low. Further studies are needed to obtain firm knowledge on malignancy risk for gonads harboring defects of androgen biosynthesis.

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Introduction

Deficiency of 3 β -hydroxysteroid dehydrogenase type 2 (3 β HSD2, *HSD3B2*, OMIM 613890) is a rare disorder of sexual development (DSD) affecting both sexes and overall steroidogenesis. The *HSD3B2* gene is located on chromosome 1 (1). There are two isozymes of HSD3B, encoded by

separate genes *HSD3B1* and *HSD3B2*. HSD3B2 regulates the conversion of steroid precursors into the respective pathways producing aldosterone, cortisol and sex hormones in the cortex of the adrenal gland and for sex steroid production in the gonads (1, 2). Human mutations are only

known for the *HSD3B2* gene. For the *HSD3B1* gene, which is specifically expressed in the placenta and peripheral tissues, no human mutations are described (3).

At birth, 46,XY individuals with HSD3B2 deficiency usually present with hypospadias and ambiguous genitalia because the defect lowers testosterone and dihydrotestosterone production needed for a normal development of the external genitalia (4). Females show normal or mildly virilized external genitalia (mild clitoromegaly, labial fusion) due to DHEA accumulation and conversion to androgens by the normal HSD3B1 (4).

HSD3B2 is exclusively expressed in the gonads and the adrenal cortex. In testicular Leydig cells, cholesterol is converted to pregnenolone, 17-hydroxypregnenolone, DHEA and androstenedione ($\Delta 4$ A) by the same enzymes and cofactors as in the adrenal cortex (Fig. 1) (1). In healthy subjects, Leydig cells contain abundant *HSD3B2* to produce testosterone via $\Delta 4$ A or androstenediol. By contrast, HSD3B2 deficiency blocks the conversion of all $\Delta 5$ - $\Delta 4$ steroids causing adrenal insufficiency and disordered sex development at birth. Depending on the genetic defect, the severity of the HSD3B2 enzyme deficiency may vary leading to variable phenotypes in both DSD and adrenal insufficiency. Accordingly, HSD3B2 deficiency may manifest with or without salt loss and may manifest late, similar to 21-hydroxylase deficiency due to CYP21A2 mutations (1).

We found few longitudinal studies reporting on puberty and early adulthood of 46,XY patients with HSD3B2 deficiency (5, 6, 7, 8, 9, 10, 11, 12, 13). Moreover little is known about fertility (2, 5, 6, 9) and virtually nothing about gonadal malignancy risk in patients with HSD3B2 deficiency, although some data on testis histology are reported (8, 9, 12, 13, 14). An adrenal rest tumor has been visualized by ultrasound in an 18-year-old 46,XY DSD patient with a HSD3B2 mutation, but no biopsy was performed (5). However, information on tumor risk is needed for clinical decision-making, because several types of 46,XY DSD, especially those characterized by testicular dysgenesis and increased peripubertal virilization, carry an increased risk of germ cell neoplasia (15, 16).

In this study, we describe the molecular genetics, the clinical course, the steroid biochemistry and the testis histology as well as immunohistochemistry (IHC) in a patient harboring a severe, homozygous HSD3B2 mutation followed from birth to 16 years of age. We provide data on testis tumor risk assessment and reviewed the literature for information regarding histology, fertility and the malignancy risk for gonads with genetic defects affecting steroid biosynthesis.

Patient and methods

Case report

The patient was the first child of consanguineous parents originating from Sri Lanka. He was born at term after an uneventful pregnancy. His birth weight was 3530 g. At birth, the boy was noted to have perinatal asphyxia and severe hypospadias, cryptorchidism and undervirilization. Over the next 3 days, he decompensated with respiratory distress and neonatal hypoglycemia. At day 8 of life, hyponatremia and hyperkalemia were noticed and the neonatal screening taken on the 5th day of life showed 17-hydroxyprogesterone (17OHP) levels above the detection limits with a value of 61 nmol/l ($n < 30$ nmol/l). The elevation was confirmed with a serum level of 967 nmol/l at day 11 of life (normal age range < 30 nmol/l). Biochemical work-up revealed elevated adrenocorticotrophin and renin with relatively low glucocorticoid production (Table 1). DHEA was markedly elevated with a ratio to $\Delta 4$ A and testosterone hinting HSD3B2 deficiency (Table 1). Therefore, the child was started on glucocorticoid and mineralocorticoid replacement for congenital adrenal hyperplasia (CAH). Karyotype was 46,XY. Although the patient improved, he was noted to have severe neurological impairments most likely due to

Figure 1

Steroid biosynthesis pathways with HSD3B2 deficiency. In the human adrenal cortex and in testicular Leydig cells, expression of 3β -hydroxysteroid dehydrogenase type 2 (HSD3B2) is needed to convert DHEA to androstenedione ($\Delta 4$ A). Lack of HSD3B2 will lead to DHEA accumulation and secretion. In peripheral tissues, the isoenzyme HSD3B1 is expressed and can convert circulating DHEA to $\Delta 4$ A. Further conversion to testosterone, estrone (E_1) and estradiol (E_2) is catalyzed by peripheral HSD17B5, CYP19A1 (aromatase) and HSD17B1 activities. Preg, pregnenolone; Prog, progesterone; 17OHPreg, 17-hydroxypregnenolone; 17OHP, 17-hydroxyprogesterone; DHEAS, DHEA sulphate.

Table 1 Clinical characteristics and biochemical data of the index patient over 16 years. ACTH, cortisol, FSH, LH and E₂ were measured by immunochemiluminometric assays in a routine laboratory setting. Free testosterone, DHEA, DHEAS and Δ4A were assessed by RIAs after extraction; E₁ was measured using a RIA. Values in bold are outside the normative range.

Age (years)	0	0.16	6.3	12.2	13.2	15.7	16.4
Clinics							
Tanner B	1	1	1	NA	NA	4	Post testosterone
Tanner P	1	1	3	3	3	3–4	3–4 (3.5 cm)
Testes volume (ml)	Not palpable	Not palpable	Not palpable	Not palpable	Not palpable	4	4
Treatment	None	GC and MC	GC and MC	GC and MC	GC and MC	4	4
Lab						GC and MC	GC and MC, testosterone
Adrenal							
ACTH (ng/l)	2638 (9.0–50)	55.5 (9.0–50.0)	79.9 (7.2–63.3)	19.3 (5–50)	26.9 (5–50)	188.4 (7.2–63.6)	255 (7.2–63.6)
Cortisol (nmol/l)	284 (140–470)		343	240	238		
DHEA (nmol/l)	> 105 (<3)	11 (1–13)	1.3 (0.7–9.0)			39 (7–30)	21 (7–30)
DHEAS (μmol/l)		1.3 (<3)	< 0.4 (<3)	1.5 (<3)	2.2 (<9)	6.68 (<9)	8.69 (<9)
Δ4A (nmol/l)	> 34.5	1.6	0.5 (0.3–1.7)			35 (2.2–7.3)	28.9 (2.2–7.3)
Testes							
LH (U/l)					<0.1	5.7 (1.3–9.8)	<0.1 (1.3–9.8)
FSH (U/l)					1.5	5.1 (1.5–12.9)	0.4 (1.5–12.9)
fTesto (pmol/l)	171 (6.4–37)					9.4 (0–76.9)	56.3 (19.2–97.1)
Periphery							
E ₁ (pmol/l)						233 (37–185)	204 (37–185)
E ₂ (pmol/l)						108 (28–156)	101 (28–156)

ACTH, adrenocorticotrophin; fTesto, free testosterone; DHEAS, DHEA sulphate; Δ4A, androstenedione; FSH, follicle-stimulating hormone; LH, luteinising hormone; E₁, estrone; E₂, estradiol; NA, not assessed; GC, glucocorticoid (hydrocortisone); MC, mineralocorticoid (fludrocortisone).

perinatal asphyxia. Over the following years, he developed severe cerebral palsy with psychomotor retardation and a dyskinetic movement disorder. Magnetic resonance imaging of his brain at age 2 and 13 years revealed atrophic basal ganglia as seen after stroke-like damages. He needed intensive neurologic and developmental care but remained wheelchair bound and unable to communicate. He was fed over a percutaneous gastric tube and was not able to control urine or stool.

From an endocrine point of view, the follow-up over the first decade on physiologic replacement therapy with fludrocortisone and hydrocortisone was unremarkable (Table 1). During childhood, testes remained unpalpable and it was discussed critically if laparotomy in search for the testicles and hypospadias repair should be performed. Together with the parents, our team declined such a surgical intervention based on the fact that the patient suffered from severe neurological handicaps.

At the age of puberty the patient was noted to show clear signs of virilization (pubic hair, penile enlargement). His gonads became palpable in the inguinal area and he presented with a remarkable gynecomastia (Tanner B4). Biochemical studies revealed elevated serum androgen precursors and estrone but rather low testosterone. Luteinising hormone (LH) and follicle-stimulating hormone were in the range for early puberty (Table 1). Serum inhibin B (240 pg/ml, normal range 60–300) and anti-Müllerian hormone (AMH, 10.6 ng/ml, normal range 10–130) were both normal. Given this natural evolution, orchidopexy was performed at age 15.5 years and both testes were biopsied to assess the malignancy risk. Post-operatively, the patient was started on low dose testosterone replacement therapy. Clinically, the gynecomastia improved soon on treatment but biochemically estrogens remained elevated suggesting too low testosterone dosage.

Genetic analysis

Parental informed consent was obtained for the genetic analysis of the DNAs extracted from blood of the patient and both parents. The study was approved by the Ethics Committee of the Canton Bern, Switzerland. Genetic analysis of the *HSD3B2* gene was performed by PCR and direct sequencing on blood leukocyte DNA using specific primers designed according to NCBI entries NG_013349.1 and NM_000198.3. Sequencing of the amplified PCR products was performed by Microsynth AG (Balgach, Switzerland). The primer sequences are communicated on request.

HSD3B expression studies

Total RNA was extracted from testis biopsy material of the patient using TRIzol reagent (Invitrogen AG). Extracted RNA was reverse transcribed and then analyzed by amplification with specific primers for HSD3B expression. GAPDH served as control for semi-quantitative RT-PCR analysis. PCR products were visualized on an agarose gel. Control (normal) testis cDNA was purchased from Amsbio (AMS Biotechnology (Europe) Limited, Abingdon, UK).

For protein expression analysis, testis tissues were incubated in lysis buffer and sonicated to obtain a homogenate. Western blot analysis was then performed on this homogenate using an anti-human HSD3B antibody as described (17).

Histology and IHC

Testis biopsy material for histology was fixed in Bouin's fluid (for good morphology) or formalin (for IHC) and paraffin-embedded. The tissue sections (4 µm) were stained in hematoxylin and eosin for morphological description, which was done independently by two observers experienced in testis pathology (C Tapia and E Rajpert-De Meyts). The IHC staining was performed using a panel of antibodies (Table 2), including markers of germ cell neoplasia *in situ* (GCNIS), testicular adrenal rest tumours (TART) and markers of maturation of Leydig and Sertoli cells. Tissue sections from adult orchiectomy specimens containing GCNIS and normal testis parenchyma, and biopsies with TART were used as positive controls. A standard three-layered indirect immunoperoxidase protocol was applied, using Histostain Kit (Zymed, San Francisco, CA, USA) followed by colour development with aminoethyl-carbazole substrate (DAKO, Glostrup, Denmark), as described previously (18). To control for specificity of each antibody, a serial section was stained with the full protocol but with the primary antibody replaced by dilution buffer. The sections were subsequently scanned on a NanoZoomer 2.0 HT Scanner (Hamamatsu Photonics, Herrsching am Ammersee, Germany) and evaluated by J E Nielsen and E Rajpert-De Meyts.

Results

Bypass steroid pathways employed with HSD3B2 deficiency

Our 46,XY patient presented at birth with a severely undervirilized external genitalia, non-palpable gonads and a steroid profile suggesting HSD3B2 deficiency

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Table 2 Summary of the findings in the immunohistochemical evaluation of testis tissue of the reported patient with HSD3B2 deficiency. Details of primary antibodies used are also given.

Antibody/antigen	Antibody ID and source	Recognised cell types	Staining pattern in the patient
Placental-like alkaline phosphatase (PLAP)	M7191, DAKO (Glostrup, Denmark)	Gonocytes, malignant germ cells	Negative
D2-40/podoplanin (PDPN)	M3619, DAKO	Gonocytes, malignant germ cells and lymphatic vessels	Negative
MAGE-A4	Clone 57B, gift from G C Spagnoli, Basel University (Basel, Switzerland)	Subset of GCNIS cells, fetal pre-spermatogonia, spermatogonia and early primary spermatocytes	Positive
Smooth muscle actin (SMA)	AB7817, Abcam (Cambridge, UK)	Peritubular cells	Positive
CYP11A1	HPA016436, Atlas Antibodies AB (Stockholm, Sweden)	Leydig cells	Positive
Insulin-like factor 3 (INSL3)	HPA028615, Atlas Antibodies AB	Leydig cells	Positive
Delta-like homolog 1 (DLK1)	Gift from C H Jensen, University of Southern Denmark (Odense, Denmark)	Immature Leydig cells	Largely negative, except in spindle-shaped Leydig precursors
Anti-Müllerian hormone (AMH)	Gift from R L Cate (previously Biogen, Cambridge, MA, USA)	Immature Sertoli cells	Largely negative, except very weak staining in a few tubules
Androgen receptor (AR)	AR441, Neo Markers (Lab Vision, Freemont, CA, USA)	Sertoli cells, peritubular cells and Leydig cells	Positive
Vimentin	sc-373717, Santa Cruz Biotechnology	Sertoli cells and mature Leydig cells	Positive
GATA4	sc-1237, Santa Cruz Biotechnology	Sertoli cells, Leydig cells and subset of gonocytes	Positive (in Sertoli cells and a subset of Leydig cells)
HSD3B2	ab154385, Abcam	Adrenocortical cells, TART and Leydig cells	Negative

(Table 1 and Fig. 1). Under physiologic replacement therapy with fludrocortisone and hydrocortisone he was well controlled during infancy and childhood. At pubertal age, he was able to virilize to some degree (phallic growth and pubic hair P3) and testes became palpable in the inguinal region. However, he also developed markedly enlarged breasts (Tanner stage 4) due to excessive estrogen production through peripheral conversion of high amounts of androgen precursors (DHEA, androstenediol excreted by the stimulated gonads (Table 1)). Circulating androgen precursors can be converted to $\Delta 4$ A or testosterone by the unaffected HSD3B1 isoenzyme followed by catalytic reactions enabled by normal HSD17B1/5 and CYP19A1 enzyme activities yielding estrogens (Fig. 1). Accordingly, gynecomastia in the patient improved with low dose testosterone replacement therapy (50–100 mg monthly), which suppressed gonadotrophins and serum DHEA (Table 1). But higher dosage seems necessary to inhibit the bypass pathways leading to estrogens.

Genetic characterization of the *HSD3B2* mutation

In our patient, a homozygous c.687del27 deletion was identified (Fig. 2A, B and C). Both parents were

heterozygote carriers of this deletion. The deletion of 27 bp led to a loss of the amino acid sequence Trp–Ala–His–Ile–Leu–Ala–Leu–Arg–Ala (p.W230_A238del), but did not alter the normal reading frame. This mutation has been described previously in four unrelated Sri Lankan families presenting with a similar phenotype (4, 19). Sequence alignment revealed that the amino acids contained in this deletion were highly conserved between isoenzymes and species (Supplementary Figure 1A, see section on supplementary data given at the end of this article). Finally, also structure analysis revealed that the deleted protein sequence from W230 to A238 was essential for HSD3B2 activity because it is part of an important alpha helix forming the protein (Supplementary Figure 1B). In line with this finding, previously performed, *in vitro* studies revealed complete loss of function for this mutation (19).

p.W230_A238del is not expressed in testis

We performed expression studies on testis biopsy material obtained from our patient at age 15.5 years and compared it to normal, adult testis tissue. RT-PCR studies on total RNA extracted from testis tissues revealed a PCR product

Figure 2

Characterization of the novel HSD3B2 mutation from bio-materials (blood, testis tissue). (A) Family pedigree. The patient (P) is homozygote for the deletion c.687_713del whereas both parents are heterozygotes. Two healthy brothers did not consent for genetic analysis. (B) HSD3B2 DNA sequencing of the patient and parents; the mutation site in HSD3B2 is marked with an arrow. (C) Scheme of the WT and mutated *HSD3B2* gene and protein sequences (NM_000198 and NP_000189.1). Coding exons are shown as black boxes, non-coding exons are shown in gray. The deleted sequences at DNA and protein level are highlighted in grey. (D and E) Expression of HSD3B in biopsy material of the testis obtained from the patient at age 16 years before testosterone therapy. Control testis tissue was from a healthy adult subject. For mRNA expression analysis (D), total RNA was extracted and semi-quantitative RT-PCR for HSD3B performed. A shorter HSD3B product (476 bp) was obtained from the patient's testis tissue compared to control testis (503 bp). GAPDH was used as internal control. Western blot was performed on the same material with an antibody recognizing HSD3B (E). No HSD3B protein expression was found in the testis material of the patient compared to a healthy control. β -actin staining served as loading control.

for the mutated HSD3B2 which was shorter than the WT product and corresponded to the deletion (Fig. 2D). By contrast, Western blot analysis for HSD3B2 revealed no protein expression for the testis of the patient while the control testis showed abundant HSD3B2 protein expression. Thus the mutated protein most likely got

degraded. As the HSD3B antibody is not specific for the human type 2 enzyme which differs only by one amino acid from the human type 1 enzyme, the missing protein band also excludes (compensatory) expression of HSD3B1 in the testis with mutated HSD3B2.

Histology and IHC of the testis tissue deficient in HSD3B2

At age 15.5 years, the patient underwent orchidopexy before testosterone replacement treatment was started. Both testicles were found in the inguinal area and biopsies were taken (Fig. 3A). General histology in all biopsies showed testis parenchyma with usually well-developed seminiferous tubules but predominantly Sertoli-cell-only pattern (Fig. 3B). The interstitial compartment appeared somewhat fibrotic but morphologically normal INSL3-positive Leydig cells were present (Table 2). Occasionally, clusters of less developed and slightly distorted tubules were seen. Only few germ cells were present in both testes (in ~5–10% tubules in a cross-section), as visualized by MAGE-A4 staining (Fig. 3C). Germ cells displayed spermatogenic arrest at the level of spermatogonia and no spermiogenesis was seen (Fig. 3C). There was no evidence of malignancy risk, because neither persisting immature fetal gonocytes nor premalignant germ cells (GCNIS) were detected. This was confirmed by negative staining with markers for these cells; PLAP, D2-40/PDPN (Table 2 and Fig. 3D). In general, Sertoli cells had an immature appearance, with some heterogeneity (more mature Sertoli cells in tubules with germ cells, less differentiated Sertoli cells in less developed tubules; Fig. 3E and F). Accordingly, the immunohistochemical expression of AR was variable (Fig. 3E) and weaker than in Sertoli cells in the control adult testis. However, there was only a very weak trace of AMH staining in a few tubules. Peritubular cells were present (visualized by SMA in Fig. 3G) and seemed morphologically normally developed, except around some clusters of poorly developed tubules, where layers of spindle-shaped cells recognized as immature Leydig cells due to the presence of DLK1 were seen (Fig. 3H). No Leydig cell hyperplasia or large Leydig cell nodules were present, no adrenal rests were seen (Table 2).

Review of HSD3B2 cases with reported pubertal development

To compare the development of our HSD3B2 deficient patient, we performed a literature search. We found 11 46,XY DSD cases owing to HSD3B2 mutations with

Figure 3

Histologic and immunohistochemical characterization of the testis deficient in HSD3B2. (A) At age 15.5 years, both testes found in the inguinal region were biopsied and surgically fixed within the scrotum before starting testosterone replacement therapy. (B) General histology of testis tissue showing well-developed seminiferous tubules but a predominantly Sertoli-cell-only pattern, with immature appearance of Sertoli cells. The interstitial compartment appears somewhat fibrotic but small groups of normal Leydig cells are present. (C) MAGE-A4

staining shows only few germ cells; few spermatogonia and no spermiogenesis are present. (D) Negative D2-40 (PDPN) staining confirming the absence of gonocytes and malignancy (CIS/GCNIS). (E and F) Androgen receptor (E) and GATA4 (F) staining identifying mostly Sertoli cells and some Leydig cells. (G) SMA staining revealing normal peritubular cells. (H) DLK1 staining showing spindle-shaped immature Leydig cells forming layers directly adjacent to peritubular cells of less well developed tubules.

described pubertal development (Table 3) (2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 19, 20). Remarkably, the adrenal phenotype was mostly more severe than the DSD phenotype, and all subjects spontaneously virilized at puberty (when not gonadectomized). In addition, gynecomastia was seen in 4/11 at puberty. Little information was found on testis histology and fertility (Table 3).

Discussion

In this study, we describe the longitudinal course of a 46,XY DSD patient with a severe HSD3B2 deficiency from birth to young adolescence. We performed detailed studies on testis biopsy material suggesting that this patient deficient in HSD3B2 most likely is not at significantly increased risk for gonadal malignancy.

At birth our patient presented with a typical steroid profile for HSD3B2 deficiency including high levels of 17OHP because accumulating $\Delta 5$ steroids from the adrenals will be converted by the intact peripheral or placental HSD3B1 in this defect (Fig. 1) (1). This may mislead initially to false diagnosis of 21-hydroxylase deficiency in patients with *HSD3B2* mutations (3). During infancy and childhood this bypass steroid production can be controlled by mineralocorticoid and glucocorticoid replacement therapy suppressing the hypothalamus-pituitary-adrenal (HPA) axis. However, at puberty with the stimulation of gonadotrophins on the testis, androgen precursors (DHEA and androstenediol) are secreted from the testis into the periphery and serve as substrates for testosterone and estrogen production enabling partial virilization (Fig. 1 and (9)). In addition, aromatization of

Table 3 Pubertal development and testis histology of reported 46,XY cases owing to HSD3B2 deficiency.

Case	Gender	Genetics	Phenotype		Pubertal development		Testis Histology	Spermatogenesis	References
			Adrenal	DSD	Virilization	Gynecomastia			
1	F	<i>W171X/P186insC-fs</i>	SW	Severe	Yes	Yes	NB	ND (fathered two children?)	(2, 10)
2	M	ND	SW	Mild	Yes	Yes	Normal	ND	(8)
3	M	ND	SW	Moderate	Yes	Yes	Immature testis, predominantly Sertoli cells	ND	(12)
4	M	<i>R249X/R249X</i>	SW	Moderate	Yes	Yes	No mature spermatogenesis, otherwise normal	ND	(13)
5	M	ND	SW	Moderate	Yes	No	Normal	ND	(11, 14)
6	M	ND	SW	Moderate	Yes	No	NB	ND	(11, 14)
7	M	<i>A10E/A10E</i>	SW	Moderate	Yes	No	TART	Azoospermia	(5)
8	M	ND	SW	Moderate	Yes	No	NB	Normal	(6)
9	F > M	<i>A82T/A82T</i>	SV	Moderate	Yes	No	Normal	Normal	(9)
10	F	<i>A82T/A82T</i>	SV	Moderate	No (gonadectomy at age 7)	No (gonadectomy)	NB	ND	(9)
11	M	<i>G129R/c.6651G>A</i>	LO (premature pubarche)	Moderate	Yes	No	NB	ND	(7, 19)

F, female; M, male; ND, not described; SW, classic, salt-wasting; SV, classic, non-salt wasting, simple virilizing; LO, late onset, non-classic; NB, no biopsy.

peripheral metabolites to estrogens leads to gynecomastia. To stop the testicular, false precursor supply to the periphery, testosterone supplementation therapy is used to inhibit gonadotrophins by negative feedback. In our case, initial low dose testosterone therapy was not extremely effective, raising the question whether (additional) aromatase inhibitor treatment would be beneficial. However, as aromatase inhibitors are not established drugs for the treatment of gynecomastia (21), we did not dare to use them. Instead, we planned to increase testosterone supplementation gradually to physiologic levels. Use of an aromatase inhibitor (testolactone) has been reported in an 11-year-old patient with gynecomastia due to HSD3B2 deficiency (13). Treatment resulted in testicular growth and pubertal advancement, but effect on gynecomastia was not further described.

In HSD3B2 deficiency, development of secondary sexual characteristics and marked gynecomastia at puberty has been described in 46,XY DSD individuals and seems not to correlate with the severity of enzyme deficiency (Table 3) (2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 19, 20, 22). Gynecomastia may rather correspond to peripheral precursor conversion to estrogens which depends on accuracy of replacement therapy and peripheral HSD3B1 activity.

So far there is little information on testis histology, fertility and tumor risk for testis tissue harboring a HSD3B2 defect in the literature (Table 3). Detailed immunohistochemical studies performed on testis biopsy material of our patient suggest low likelihood for fertility, because only very few spermatogonia were present and there was no spermiogenesis. Importantly, our patient seems at low risk for malignancy, because no delayed gonocytes or pre-malignant GCNIS changes were detected. Based on our findings and the previously published reports, it appears that HSD3B2 deficiency does affect development of the testis to a variable degree, but does not prevent maturation of fetal gonocytes to spermatogonia. However, from the limited literature available no firm conclusion about tumor risk may be drawn. Leydig cell hyperplasia (as sometimes seen in other disorders of testosterone synthesis, see Table 4) was not observed in our and the reported cases with HSD3B2 deficiency with obvious testosterone deficiency (Table 3) which could be expected to lead to an increase in LH and thus Leydig cell stimulation. This might be explained by feedback inhibition of other metabolites (e.g. estrogens) on gonadotrophins.

Overall, few studies are found on testis histology, fertility and malignancy risk in 46,XY DSD patients with androgen biosynthetic defects. We have summarized

Table 4 Summary of reported information on testis histology, possible fertility and malignancy risk in genetic steroid biosynthetic defects.

Disorder	Gene	Testis histology	Spermatogenesis	Malignancy risk	References
Lipoid congenital adrenal hyperplasia	StAR	Preserved seminiferous tubules with spermatogonia and Sertoli cells but without spermatocytes or spermatids. Hyalinized tubular walls. Fatty changes in Leydig cells (age 8 years, testes were located inguinal) (29) Reduced seminiferous tubule volume (26) Immature testes with uncanalated seminiferous tubules (24) Germ cell degeneration with aging (29, 30) Normal, but fewer germ cells (age 7 years; testes located intraabdominal); at 2 months normal number of germ cells (33) Normal testicular tissue for age; normal seminiferous tubules and Sertoli cells (25) This report Immature testis, predominantly Sertoli cells (12) No mature spermatogenesis, otherwise normal (13) Normal histology (8, 9, 14) Immature testicular tissue with extensive calcification Unknown Unknown Seminiferous tubules with immature Sertoli cells. Few germ cells. No Leydig cells or precursors in the interstitium (age 10 years; testes were located inguinal) (35) Variable presence and maturation of Sertoli and Leydig cells, hyperplastic Leydig cells (32)	Absent	Testicular carcinoma <i>in situ</i> (30)	(24, 26, 29, 30)
P450 side chain cleavage syndrome	CYP11A1		Unknown	Probably low (16)	(16, 25, 33)
3 β -hydroxysteroid dehydrogenase 2 deficiency	HSD3B2		Absent	Probably low	This report (8, 9, 12, 13, 14)
Combined 17-hydroxylase, 17,20 lyase deficiency	CYP17A1		Unknown	Unknown	(16)
P450 oxidoreductase deficiency	POR		Unknown	Unknown	
Cytochrome b5 deficiency	CYB5A		Unknown	Unknown	
17 β -hydroxysteroid dehydrogenase 3 deficiency/17-ketosteroid reductase deficiency	HSD17B3		Mostly decreased or absent	Probably low (16)	(15, 32, 35)
5 α -reductase 2 deficiency	SRD5A2		Impaired	Unclear for 'oncoytic' transformed Sertoli cells	(36)
3 α -hydroxysteroid dehydrogenase SF1 mutations	AKR1C2/4 NR5A1	Wide majority of seminiferous tubules (ST) containing Sertoli cells only, few germ cells; areas of Leydig cell hyperplasia (17 years, testes inguinal). Unknown Hypoplastic seminiferous tubules (27) Leydig cells with vacuoles (34) Very few germ cells (31) Poorly differentiated testis containing immature tubules and connective tissue (23)	Unknown Incomplete (few germ cells) (28)	Unknown Bilateral precursor lesions of testicular carcinoma <i>in situ</i> (28)	(23, 27, 28, 31, 34)

them in Table 4 (8, 9, 12, 13, 14, 16, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36). For spermatogenesis and thus fertility, the few data available suggest that it varies between different androgen biosynthetic defects as well as between individuals carrying defects in the same gene. In general, at least subfertility is a matter of fact. For tumor risk, we found only sparse data for *Star*, *CYP11A1*, *HSD17B3*, 5 α -reductase 2 deficiency (*SRD5A2*) and *NR5A1* mutations (Table 4). In *SRD5A2*, one study found oncocytic transformation in the cytoplasm of Sertoli cells in a patient aged 17 years (36), but the significance of this finding for potential tumor risk of the testis remains unclear. Precursor lesions of testicular CIS/GCNIS were described in one adolescent with a *NR5A1/SF1* mutation (28) and one individual with a *Star* mutation (22). In four subjects with *CYP11A1* and *HSD17B3* mutations, no signs of tumor risk were found (16).

Regarding 46,XY patients with steroid biosynthesis disorders without DSD (e.g. *CYP21A2*, *CYP11B1* and *NROB1/DAX1* mutations), the malignancy risk for the testis is estimated as being low (37, 38, 39). 46,XY patients with 21-hydroxylase deficiency and 11 β -hydroxylase deficiency may develop TARTs, which can compromise fertility and sometimes be mistaken for Leydig cell malignancy; but TARTs are benign (37, 40) and can be controlled by proper treatment (41). Importantly, testosterone overproduction as seen in some of these defects seems not to be associated with increased germ cell cancer risk (15, 37). In addition, patients with X-linked CAH due to *NROB1/DAX1* mutations may present in the testis a Sertoli-cell-only pattern with rare spermatogonia and no apparent spermatogenesis, but also pronounced Leydig cell hyperplasia (38, 39, 42). Contrary to 21-hydroxylase deficiency, they also have a hypogonadotrophic hypogonadism (43). Malignancy risk in these patients is probably also low (39). Generally, hypothalamic or hypogonadotrophic hypogonadism seems not to be associated with an increased germ cell cancer risk (15, 44).

In summary, through our studies of a severe c.687del27 *HSD3B2* mutation, we learned that the human testis does not express this defective protein and shows histologically a Sertoli-cell-only pattern without signs of spermatogenesis or signs of malignancy risk. Thus, it is suggested that routine prophylactic gonadectomy is not advisable; even less as the *HSD3B2* gonad will enable partial virilization at puberty through bypass steroid pathways (15). However, estrogen production and gynecomastia can be a problem requiring hormonal replacement, with aromatase inhibitors remaining a theoretical option for future trials. Furthermore, our literature search

revealed little information on fertility and the malignancy risk of gonads of 46,XY DSD patients with defects in the androgen biosynthesis. Therefore, we believe that detailed clinical studies on patients (and their biomaterials) harboring such rare disorders are important to generate the knowledge for answering clinically unsolved questions. Meanwhile, in view of the limited data available and the large phenotypic variability observed, it is recommended to place a non-scrotal gonad in a stable scrotal or at least inguinal position to allow for clinical follow-up and optimal preservation of residual testicular function (15).

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-15-0599>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Idea and design: M-A Burckhardt, E Rajpert-De Meyts and C E Flück; clinical work-up: M-A Burckhardt, P E Mullis, I Schnyder and C E Flück; experimental design and experiments: N Marti, S S Udhane and C E Flück; histological work-up: C Tapia, J E Nielsen and E Rajpert-De Meyts; manuscript writing: M-A Burckhardt, E Rajpert-De Meyts and C E Flück.

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