Steroidogenesis of the testis – new genes and pathways

Christa E. Flück a,∗, Amit V. Pandey b

a Department of Pediatrics and Department of Clinical Research, Pediatric Endocrinology and Diabetology, University Children’s Hospital, University of Bern, Freiburgstrasse 15/C845, 3010 Bern, Switzerland
b Pediatric Endocrinology Steroid Laboratory, University Children’s Hospital, Freiburgstrasse 15/C837, 3010 Bern, Switzerland

Abstract

Defects of androgen biosynthesis cause 46,XY disorder of sexual development (DSD). All steroids are produced from cholesterol and the early steps of steroidogenesis are common to mineralocorticoid, glucocorticoid and sex steroid production. Genetic mutations in enzymes and proteins supporting the early biosynthesis pathways cause adrenal insufficiency (AI), DSD and gonadal insufficiency. The classic androgen biosynthesis defects with AI are lipid CAH, CYP11A1 and HSD3B2 deficiencies. Deficiency of CYP17A1 rarely causes AI, and HSD17B3 or SRD5A2 deficiencies only cause 46,XY DSD and gonadal insufficiency. All androgen biosynthesis depends on 17,20 lyase activity of CYP17A1 which is supported by P450 oxidoreductase (POR) and cytochrome b5 (CYB5). Therefore, 46,XY DSD with apparent 17,20 lyase deficiency may be due to mutations in CYP17A1, POR or CYB5. Illustrated by patients harboring mutations in SRD5A2, normal development of the male external genitalia depends largely on dihydrotestosterone (DHT) which is converted from circulating testicular testosterone (T) through SRD5A2 in the genital skin. In the classic androgen biosynthetic pathway, T is produced from DHEA and androstenedione/diol in the testis. However, recently found mutations in AKR1C2/4 genes in undervirilized 46,XY individuals have established a role for a novel, alternative, backdoor pathway for fetal testicular DHT synthesis. In this pathway, which has been first elucidated for the tammar wallaby pouch young, 17-hydroxyprogesterone is converted directly to DHT by 5α-3α reductive steps without going through the androgens of the classic pathway. Enzymes AKR1C2/4 catalyse the critical 3αHSD reductive reaction which feeds 17OH-DHP into the backdoor pathway. In conclusion, androgen production in the fetal testis seems to utilize two pathways but their exact interplay remains to be elucidated.

© 2014 Elsevier Masson SAS. All rights reserved.

Keywords: Disorder of sexual development; Lipoid congenital adrenal hyperplasia; CYP11A1; HSD3B2; CYP17A1; HSD17B3; SRD5A2; POR and CYB5 deficiencies; AKR1C2/4 genes

Résumé

troubles du développement sexuel ; hyperplasie congénitale lipoïde des surrénales ; déficits en production d’androgènes dans le testicule fœtal semble utiliser 2 voies de biosynthèse mais leurs inter-relations exactes demeurent à élucider.

1. Introduction

Le testicule est un organe complexe composé de deux unités distinctes, l’androgyne biosynthèse unité dans le Leydig cellules du compartiment interstitiel et le sperme unité nourrie par les cellules Sertoli de la tubule seminifère qui fait partir de 90% de la taille testiculaire [1].

Testostérone (T) production des testicules joue un rôle crucial dans le développement sexuel, fonction sexuelle et reproduction. Élevée pendant les 4-5 premières semaines de la grossesse, la nouvelle formation de testicule est déterminée par 46,XY chromosomes lorsque le testicule commence à se différencier (Fig. 1). De puis on T production ensemble avec Anti-Müllerian hormon and sex differentiating factors qui sont essentiels pour le développement sexuel du sexe masculin. La fonction ovariale est observée dans les premières 6 mois de la vie avec l’augmentation de la production de testostérone [2]. Cette fonction est d’origine hormonale que l’ovaire est une opportunité pour les tests avant la puberté qui devient hormoniquement quiescent till puberty. Stimulé par les hypothalamus pituitaire hormones, le testicule commence à produire de la testostérone à partir de la prostatique (DHEA) et de l’androsténone/diol dans le testiculaire. Cependant, des mutations récemment identifiées dans des gènes AKRIC2/4 chez des individus 46,XY peu virilisés, on montre le rôle d’une nouvelle voie de biosynthèse alternative de recours permettant la production de la testostérone testiculaire fœtale. Par cette voie, d’abord identifiée chez le jeune Wallaby Tammar lorsqu’il vit encore dans la poche de sa mère, la 17 OH progesterone est convertie directement en dihydrotestostérone par une étape de biosynthèse qui court-circuite la biosynthèse classique des androgènes. Les enzymes AKRIC2/4 catalysent l’étape de réduction critique passant par la 3a hydroxy-stéroïde-déshydrogénase (3aHSD) qui oriente la 17 hydroxy-dihydroxyprogestérone vers la voie de recours. En conclusion, la production d’androgènes dans le testicule fœtal semble utiliser 2 voies de biosynthèse mais leurs inter-relations exactes demeurent à élucider.

Mots clés : Troubles du développement sexuel ; Hyperplasie congénitale lipoïde des surrénales ; Déficits en CYP11A1 ; HSD3B2 ; CYP17A1 ; HSD17B3 ; SRD5A2 ; POR ; CYB5 ; Gène AKR1C2/4

2. Normal steroid biosynthesis

All steroid hormones are produced from cholesterol through a cascade of enzymes which are encoded by genes that are common to all steroid producing organs [7]. In most cases the organ specific gene expression determines the steroid profile of each specialized organ. For instance, the testis is determined to produce androgens from cholesterol (Fig. 2). The cholesterol molecule is transported to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR) where cholesterol is the substrate for the first step of steroidogenesis. In the mitochondria cholesterol is converted to pregnenolone by the side chain cleavage system comprised of CYP11A1 (P450sccl), ferrodoxin (FDX1) and ferrodoxireductase (FDXR). In the classical pathway, pregnenolone is then converted through the delta 5 pathway by CYP17A1 (P450c17) to 17α-hydroxyprogrenenolone (17OHpred) and dehydroepiandrosterone (DHEA) with the first reaction catalysed by its 17α-hydroxylase activity supported by P450 oxidoreductase (POR) and the second reaction by the 17,20 lyase activity supported by POR and cytochrome b5 (CYB5). DHEA is then turned over to testosteron through androstenediol or androstenediol catalysed by 3β-hydroxysteroid dehydrogenase type II (HSD3B2/3βHSDII) and 17β-hydroxysteroid dehydrogenase 3 (HSD17B3/17βHSD3). Testosterone may be converted to DHT which has about 10-times more affinity for the androgen receptor. This conversion is catalysed by 5α-reductase type II (SRD5A2/5αRed2) which is expressed in genital skin and the prostate. In humans, only little conversion to androstenedione occurs through the delta 4 pathway coming from progesterone (Prog) and 17-hydroxyprogesterone (17OHProg) because 17,20 lyase activity is poor on the substrate 17OHProg compared to 17OHProg [8].

© 2014 Elsevier Masson SAS. Tous droits réservés.
Table 1
DSD due to androgen biosynthetic defects with and without adrenal insufficiency.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Gene</th>
<th>OMIM</th>
<th>46,XY DSD Phenotype</th>
<th>46,XX Phenotype</th>
<th>Adrenal Insufficiency</th>
<th>Other Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoid congenital adrenal hyperplasia (LCAH)</td>
<td>StAR</td>
<td>201710</td>
<td>Classic form: 46,XY DSD, gonadal insufficiency</td>
<td>Non-classic form: None</td>
<td>YES</td>
<td>Maybe – lack of pubertal development or premature ovarian failure</td>
</tr>
<tr>
<td>P450 side chain cleavage syndrome (CAH)</td>
<td>CYP11A1</td>
<td>118485</td>
<td>Classic form: 46,XY DSD, gonadal insufficiency</td>
<td>Non-classic form: None</td>
<td>YES</td>
<td>Maybe – lack of pubertal development or premature ovarian failure</td>
</tr>
<tr>
<td>3β-hydroxysteroid dehydrogenase II deficiency (CAH)</td>
<td>HSD3B2</td>
<td>201810</td>
<td>46,XY DSD, gonadal insufficiency</td>
<td>Non-classic form: No DSD, but premature adrenarche</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td>Combined 17-hydroxylase, 17,20 lyase deficiency (CAH)</td>
<td>CYP17A1</td>
<td>202110</td>
<td>46,XY DSD, gonadal insufficiency</td>
<td>Lack of pubertal development, gonadal insufficiency</td>
<td>Variable</td>
<td>Hypertension and hypokalemic alkalosis (not seen with isolated lyase deficiency)</td>
</tr>
<tr>
<td>P450 oxidoreductase deficiency (CAH)</td>
<td>POR</td>
<td>124015</td>
<td>46,XY DSD, gonadal insufficiency</td>
<td>46,XX DSD, gonadal insufficiency; PCOS-like</td>
<td>Variable</td>
<td>Maternal virilization during pregnancy; Antley-Bixler skeletal malformation syndrome; changes in drug metabolism</td>
</tr>
<tr>
<td>Cytochrome b5 deficiency</td>
<td>CYB5A</td>
<td>613218</td>
<td>46,XY DSD</td>
<td>?</td>
<td>NO</td>
<td>Methemoglobinemia</td>
</tr>
<tr>
<td>17β-hydroxysteroid dehydrogenase III deficiency / 17-ketosteroid reductase deficiency</td>
<td>HSD17B3</td>
<td>264300</td>
<td>46,XY DSD; progressive virilisation and gynecomastia at puberty</td>
<td>None</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>5α-reductase II deficiency</td>
<td>SRD5A2</td>
<td>607306</td>
<td>46,XY DSD; progressive virilisation and gynecomastia at puberty</td>
<td>None</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>3α-hydroxysteroid dehydrogenase deficiency</td>
<td>AKR1C2/4</td>
<td>600450</td>
<td>46,XY DSD; gonadal insufficiency</td>
<td>None</td>
<td>NO</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Androgen biosynthesis in the testis and sexual differentiation. Around 6–8 weeks gestation the neutral gonad develops into a testis in case of 46,XY determination and starts to produce androgens from cholesterol in the Leydig cells after 8–10 weeks gestation. In the classic pathway, androgen production in the testis leads to testosterone (T) which then enters the circulation and is turned over to dihydrotestosterone (DHT) by 5α-reductase II in the genital skin. Through means of the novel, alternative backdoor pathway (Fig. 2), DHT may be formed directly in the testis. DHT is required for normal differentiation of the male external genitalia. By contrast, the female gonad is hormonally inactive during fetal life and the female external genitalia must be protected from influence of androgens to remain female.

3. Classic androgen biosynthesis defects (StAR, CYP11A1, HSD3B2, CYP17A1, HSD17B3, SRD5A2)

3.1. StAR and CYP11A1

Patients with severe defects in the genes for StAR or CYP11A1 present clinically indistinguishable with adrenal insufficiency including mineralocorticoid (MC) and glucocorticoid (GC) deficiency, as well as 46,XY DSD and gonadal insufficiency due to a lack of androgen production (Table 1) [9]. StAR triggers the import of cholesterol from the outer to the inner mitochondrial membrane making it available as essential substrate to the side chain cleavage system (CYP11A1/FDX1/FDXR) for all steroid biosynthesis [7]. In 1955, Prader described a 46,XY DSD patient who died in an adrenal crisis very early in life and was found to have grossly enlarged, fatty transformed adrenal glands [10]. This prompted him to name this disorder lipoid CAH (LCAH). However, the StAR gene as the underlying genetic defect of LCAH and the mechanism of disease were only described years later [11]. Infants with severe StAR mutations (= classic lipoid CAH) manifest with adrenal insufficiency soon after birth or within the first year of life and affected 46,XY babies have female-appearing external genitalia [9]. Less severe StAR mutations which retain partial activity cause non-classic LCAH and can present with late-onset primary adrenal insufficiency only from 4 years of age to adulthood, without affecting male sex development [9,12]. The mechanism of disease in StAR deficiency is described in a “two hit model” [11]. The first hit consists of the actual loss of StAR activity for cholesterol import into the
mitochondrium of the steroid producing cell. However, as there is about 10% of StAR-independent cholesterol import, this may not fully explain a severe phenotype. Thus the second hit consists of the destruction of the steroidogenic cells through accumulation of cholesterol and cholesterol esters. In keeping with this model, the male gonad which is fully active in androgen production early in fetal life, will be damaged early resulting in 46,XY DSD. By contrast, the female gonad which is basically inactive until puberty may only be affected by StAR deficiency later on allowing even normal pubertal development and menses in affected females for a certain time [9].

Human CYP11A1 mutations are known since 2001 [13] and their clinical manifestation is identical to StAR mutations with a classic form with severe mutations and a non-classic form with partial enzyme activity loss [9]. However, in contrast to StAR, adrenal enlargement which characterizes CAH is not observed in CYP11A1 deficiency making adrenal imaging an option for possibly differentiating StAR from CYP11A1 mutations. For both classic StAR and CYP11A1 deficiency, diagnostic steroid profile (LCMSMS, GCMS) shows overall (very) low steroid production (MC, GC and sex steroids).

3.2. HSD3B2

CAH due to HSD3B2 has been described biochemically more than 50 years ago [14]. Severe HSD3B2 deficiency causes MC and GC deficiency as well as (partial) androgen deficiency resulting in 46,XY undervirilization and 46,XX virilization [9]. This is explained by the fact that there are two functional HSD3B genes in humans. HSD3B2 is exclusively expressed in the gonads and the adrenals. HSD3B1 is expressed in the placenta and in peripheral tissues including the liver and the skin. Human mutations in the HSD3B1 gene are not known. But in case of HSD3B2 deficiency, active HSD3B1 may convert circulating androgen precursors secreted from the adrenals or gonads into more active androgens in the periphery explaining the virilization of females. Similarly, it is because of the HSD3B1 activity that babies with severe HSD3B2 deficiency might be picked up on 21-hydroxylase deficiency newborn screening for elevated 17OHP levels resulting from peripheral conversion of massively increased 17OHP levels. Generally, 3β-hydroxysteroid dehydrogenases (3βHSDs) convert delta 5 steroids (Preg, 17OHPreg, DHEA, androstenediol) to delta 4 steroids (Prog, 17OHPreg, DHEA, androstenediol) to delta 4 steroids (Prog, 17OHP, androstenedione, T) (Fig. 2). Thus high ratios of the delta 5 over delta 4 steroids are the diagnostic fingerprint of HSD3B2 deficiency.

3.3. CYP17A1

CYP17 deficiency comes in two forms, both affect androgen biosynthesis and thus cause 46,XY DSD and gonadal insufficiency. The first, more frequent form consists of combined loss of both 17α-hydroxylase and 17,20 lyase activities, while the second form consists of an isolated loss of 17,20 lyase activity which is described only in few patients so far and affects androgen biosynthesis exclusively (Table 1, Fig. 2) [9,15]. Clinical presentation of 46,XY due to CYP17 deficiency varies from apparently female to undervirilized male with absence of Müllerian structures, hypoplastic Wolff structures and intra-abdominal or maldescended testes. Pubertal development is missing including pubic or axillary hair, and gynecomastia is only seen in partial insufficiency. With severe 17-hydroxylase deficiency, only the mineralocorticoid synthesis pathway of steroidogenesis is functional and the CYP17 enzyme block will lead to
elevated levels of corticosterone and 11-deoxycorticosterone (DOC) which will suppress aldosterone and renin and result in hypertension and hypokalemic alkalosis in the patient. As corticosterone has glucocorticoid activity, patients do not generally suffer from adrenal insufficiency although ACTH is elevated. Thus the typical steroid profile of combined CYP17 deficiency consists of high DOC but low cortisol and androgens.

In total, 17,20 lyase activity is required for androgen production. It is supported by POR and CYB5 for its full functionality. Mutations at locations E305, R347 and R358 of the CYP17 protein have been shown to cause isolated lyase deficiency [7,15].

3.4. HSD17B3

There are at least 14 isoforms of human 17β-hydroxysteroid dehydrogenases (17βHSDs), some are preferentially reductases, some oxidases, and they fulfill variable physiological functions. However, human mutations are only known for the HSD17B3 gene causing 46,XY DSD due to 17-ketosteroid reductase/17βHSD3 deficiency. Type 3 17βHSD (HSD17B3) is exclusively expressed in the testes where it reduces androstenedione to T, DHEA to androstenediol, androstanediol to DHT and androsterone to androstanediol (Fig. 2). Thus HSD17B3 deficiency is a male sex-limited disorder causing 46,XY DSD with severe to complete undervirilization of the external genitalia with a blind vaginal pouch. Müllerian structures are absent while Wolff structures are present, and testes are often located inguinal. Patients raised female virilize at puberty as redundant genitalia as the virilization of the external genitalia seems to depend largely on DHT, but have otherwise undergone a rather wide variety of mutations in chromosome 2p23 and predominantly found in male reproductive tissues [9]. The syndrome of 5α-reductase deficiency is due to a wide variety of mutations in SRD5A2. Typically, affected 46,XY individuals manifest at birth with a female-appearing external genitalia as the virilization of the external genitalia seems to depend largely on DHT, but have otherwise undergone a rather normal male sex determination and differentiation during fetal development. At puberty, progressive virilization and gynecomastia occur brought by the intact activity of 5α-Red1. This may prompt a change in gender role to male in individuals raised as female. Diagnosis of 5α-reductase deficiency may be suggested when a high serum T/DHT ratio (basal and hCG stimulated) is observed. However, assessment of a whole steroid profile (GCMS, LCMSMS) is even more informative as 5α-Reods are important for the reduction of a variety of steroids (e.g. C21 steroids) in their metabolism which can be seen on the profile [7].

Human mutations for SRD5A1 are not described. Overall, the type 1 and 2 genes show a complex pattern of developmental regulation of expression which is thought to also play a role for fetal androgen biosynthesis through the classic versus the alternative, backdoor pathway in the testis (Figs. 1 and 2) [6,7].

4. Cofactor defects (POR, CYB5)

Basic studies of isolated lyase deficiency have elucidated the important role of cofactors P450 oxidoreductase (POR) and cytochrome b5 (CYB5) for the enzymatic reaction of CYP17 [15]. In fact, the same clinical phenotype seen with isolated 17,20 lyase deficiency (see above) due to specific CYP17A1 mutations may be mimicked by certain POR or CYB5 mutations [15,17,18].

4.1. POR

Symptoms of POR deficiency (PORD) were first described in 1985 in a 46,XY DSD patient with a steroid profile for combined 21- and 17-hydroxylase deficiency [19]. We found the first human POR mutations in patients with a broad phenotype ranging from 46,XY and 46,XX DSD, adrenal insufficiency and bony malformations (known as Antley-Bixler syndrome by geneticists) on one side of the spectrum to mild polycystic ovary syndrome-like phenotype on the other side [20]. POR is the obligate electron donor to all microsomal type 2 P450s which comprise many proteins involved in steroidogenesis and xenobiotic metabolism, heme catabolism and bile acid synthesis (comprehensively reviewed in [21]). For adrenal and gonadal steroidogenesis, reactions catalyzed by enzymes CYP21A2 (21-hydroxylase), CYP17A1 and CYP19A1 (aromatase) depend on POR for electron transfer from NADPH. Typical for PORD is the broad phenotype which may be explained by two facts: one, different mutations in the POR protein affect the electron transfer to its partners (e.g. CYP17A1) to different degrees; two, the same POR mutation affects the activity of different P450 partners to different degrees as their interaction might differ [21]. For example, a severe POR mutation which destroys electron donation to all P450 (e.g. R457H) may be found in patients with either 46,XY or 46,XX DSD, adrenal insufficiency and skeletal malformations, and virilization of the mother during pregnancy may occur [20]. By contrast, the POR mutation G539R which has been shown to affect predominantly 17,20 lyase activity will resemble isolated 17,20 lyase deficiency [22]. Clinical diagnosis of PORD is difficult but the steroid profile from urine or plasma (GCMS or LCMSMS) can be diagnostic. Final proof requires genetic testing.

4.2. CYB5

Human CYB5 mutations have been found in 46,XY patients with low androgens and gonadal insufficiency but normal MC and GC production due to true isolated 17,20 lyase deficiency where the genes for CYP17A1 and POR were normal [18]. CYB5 enhances the activity of 17,20 lyase by facilitating the allosteric interaction between POR and CYP17A1 [9,15]. So far, only two
5. Alternative androgen production through the backdoor pathway and AKR1C deficiency

5.1. The backdoor pathway

Studies of sex development using the tammar wallaby as model revealed predominance of an alternative pathway for androgen production in the testis of the pouch young rather than the classic pathway (Fig. 2) [3]. In this pathway, 17OH is 5α-reduced to 17OH-dihydroprogesterone (17OH-DHP) and 3α-reduced to 17OH-allopregnanolone (17OH-Allo, P’diol). P’diol is then an excellent substrate for CYP17-lyase to form androsterone which is further converted to androstenedione (A’diol) and finally oxidized to DHT. In this backdoor pathway the steroid flux bypasses conventional intermediates (DHEA, androstenedione, T) and uses different enzymes (SRD5A1, AKR1C2/4, HSD17B6) for DHT production. As this pathway has been found in rodents as well, its existence in human fetuses was hypothesized. First evidence for it in men came from urine steroid analysis of patients suffering from PORD showing a disproportionate increase of 17OH-Allo (P’diol) and androsterone which mainly originate from the backdoor pathway [23]. Similarly, urine steroid profiling in untreated patients with 21OH-ase deficiency revealed an elevated P’diol and ratio for androsterone to etiocholanolone in the neonatal period indicating that there is increased steroid flux through the backdoor pathway in this disease state [24]. Thus it has been suggested that in utero virilization of girls (46,XX DSD) with PORD (and in part 21OH-ase deficiency) might be caused by increased 17OH being processed to androgens through the backdoor pathway [3,23]. On the other hand, these studies have also shown that the functionality of the backdoor pathway changes from fetal to postnatal to adult life and changes with disease states. In addition, performing gene expression studies on fetal versus adult testis and adrenal tissues, we found that genes of the backdoor pathway are tissue and developmentally specific expressed suggesting a differential regulation in the gonads (ovary, testis) and the adrenals [6]. But to date, detailed functional studies of the backdoor pathway performed on human tissues are missing and many questions concerning the interplay between the classic versus the alternative, backdoor pathway remain unsolved.

5.2. Human AKR1C2/4 mutations

The backdoor pathway depends on reductive and oxidative 3α-hydroxysteroid dehydrogenase (3αHSD) activities for androgen production [7]. The four major human 3αHSDs are aldoketoreductases of the AKR1C family and have usually reductive activity. AKR1C1-4 lie on chromosome 10p14-15 and each enzyme has specific tissue distribution and specific catalytic characteristics. AKR1C3 is also known as 17βHSD5 and catalyzes the conversion of androstenedione to T in steroiodogenic and non-steroidogenic tissues. AKR1C3 is higher expressed in the human fetal adrenal and testis and may participate (together with 17βHSD3) in the conversion of androsterone to A’diol in the backdoor pathway. Both, AKR1C2 and AKR1C4 are able to convert 17OH-DHP to P’diol. They are both expressed in testis and adrenals but the fetal testis expresses more AKR1C2 than AKR1C4 [6]. By contrast, AKR1C2/4 have both minimal oxidative activity. Therefore the conversion of A’diol to DHT in the fetal testis is not likely to be supported by those enzymes but rather by the oxidative HSD17B6/RODH (retinol dehydrogenase) which is also abundantly expressed in the human prostate.

Recently, we have identified first mutations in AKR1C2/4 in patients with a phenotype consistent with isolated 17,20 lyase deficiency when mutations in the CYP17A1, POR and CYB5 genes were not found [6,25].

The index family came to medical attention with two 46,XY DSD patients, one with cryptorchidism and genital undervirilization (raised male), the other with an apparent female phenotype and no uterus (raised female) [25]. Extended family history revealed a maternal aunt with severe 46,XY DSD (female gender, tall stature, primary amenorrhoe) and a maternal cousin with 46,XY DSD with moderate undervirilization (raised male). GC and MC production was normal in all. Genetic analysis of the genes of the backdoor pathway for androgen production revealed AKR1C2 mutations in the affected individuals and suggested autosomal recessive, male sex-limited inheritance. However, intrafamiliar phenotypical variability and in vitro functional studies showing only moderate activity loss of 20–80% of the identified AKR1C2 mutant enzymes prompted a search for a second hit. Linkage analysis pointed to the AKR1C locus which contains five closely related AKR1C genes which were further investigated. Finally, the second hit was found in the AKR1C4 gene. Affected patients were all found to harbor a splicing mutation in AKR1C4 together with AKR1C2 (I79V).

In a second kindred with severe 46,XY DSD (female external genitalia, intra-abdominal testes) a complex chromosomal rearrangement in the AKR1C locus was found. This included an unequal crossing over between the AKR1C2 and the AKR1C1 gene, and an additional missense mutation (H222Q) in the AKR1C2 gene which was tested functionally inactive [6].

Thus from these experiments of nature affecting androgen production of the backdoor pathway in the testis, we learned that the backdoor pathway plays an important role for fetal male sex development. In addition, we learned that not only T but also DHT is directly produced in the fetal testis (Fig. 2).

6. Perspective and conclusions

Normal male sex development and function requires normal androgen biosynthesis. At least in fetal life, this includes a fully functional classic as well as alternative, backdoor steroid pathway for the production of T and DHT in the testis. The role of the backdoor pathway beyond fetal life as well as the interplay between both pathways in health and disease are largely unsolved.
Disclosure of interest

This work was supported by grants 320030-146127 (to CEFL) and 310031-134926 (to AVP) from the Swiss National Science Foundation.

References