Steroidogenesis of the testis – new genes and pathways

Stéroïdogenèse testiculaire – nouveaux gènes et nouvelles voies

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 lipoid 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 leads 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


Corresponding author.

E-mail addresses: christa.flueck@dkf.unibe.ch (C.E. Flück), amit@pandeylab.org (A.V. Pandey).

http://dx.doi.org/10.1016/j.ando.2014.03.002

0003-4266/© 2014 Elsevier Masson SAS. All rights reserved.
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

The human testis is a complex organ comprised of two distinct units, the androgen biosynthesis unit in the Leydig cells of the interstitial compartment and the sperm producing unit nourished by the Sertoli cells of the seminiferous tubule compartment which makes around 90% of the testis volume [1]. Testosterone (T) production of the testis plays a critical role in sex development, sexual function and reproduction. Early in fetal life (4–5 weeks gestation), the neutral anlage is determined by 46,XY chromosomes to become a testis (Fig. 1). From then on T production together with Anti-Müllerian hormone and sex differentiating factors will lead to normal male sex differentiation. In this process T and more so dihydrotestosterone (DHT) are particularly crucial for the formation of the normal male external genitalia. Soon after birth, micropuberty is observed in the first 6 months of life with elevated testosterone production [2]. This phenomenon is of unknown function but offers a window of opportunity for functional testing of the testis before it becomes hormonally quiescent till puberty. Stimulated by the hypothalamic-pituitary hormones, the testis resumes androgen biosynthesis at puberty to prompt the development of secondary male sex characteristics and initiate sexual function and spermatogenesis for reproduction in adult life. Thus it is easy to understand that abnormalities in androgen biosynthesis cause disorders of sex development (DSD) and function.

The biochemical pathway for T synthesis is long known. But recently, an alternative, so-called “backdoor” pathway for the production of DHT, not using DHEA, androstenedione and T as precursors (Fig. 2), has been described first in the tammar wallaby pouch young [3,4]. We have found mutations in genes of this backdoor pathway (AKR1C2/4;3αHSDs) in subjects manifesting with moderate to severe forms of 46,XY DSD [5,6]. However, the role of this novel pathway in human androgen biosynthesis is largely unknown.

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 (P450sc(c)), ferrodoxin (FDX1) and ferrodoxin-reductase (FDXR). In the classic pathway, pregnenolone is then converted through the delta 5 pathway by CYP17A1 (P450c17) to 17α-hydroxyprogrenenolone (17OHpreg) and dehydropiandrosterone (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 testosterone through androstenedione or androstenediol catalyzed by 3β-hydroxysteroid dehydrogenase type II (HSD3B2/3βHSDII) and 17β-hydroxysteroid dehydrogenase 3 (HSD17B3/17HSD3). Testosterone can 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 (17OH) because 17,20 lyase activity is poor on the substrate 17OH compared to 17OHpreg [8].
<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>Maybe – lack of pubertal development or premature ovarian failure</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>Maybe – lack of pubertal development or premature ovarian failure</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>Classic form: 46,XY DSD, gonadal insufficiency</td>
<td>46,XX DSD, gonadal insufficiency</td>
<td>YES</td>
<td>Non-classic form: Premature adrenarche, PCOS-like</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>Rare (only with OHase deficiency)</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</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>SRS5A2</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>
C.E. Flück, A.V. Pandey / Annales d’Endocrinologie 75 (2014) 40–47

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.

androstenedione. Therefore, genetic defects in genes involved in the initial steps of steroidogenesis shared by the adrenal cortex and the gonads affect steroid production of both organs and may lead to disorders of sexual development and function as well as adrenal insufficiency, also known as congenital adrenal hyperplasias (CAH) in medical literature (Table 1) [7,9].

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
Fig. 2. Androgen biosynthesis from cholesterol through the classic and alternative backdoor pathway. The classic pathway proceeds from cholesterol via pregnenolone, 17OHPreg and DHEA to androstenedione or androstenediol and then to testosterone in testicular Leydig cells (shown in black). Circulating testosterone is then converted to DHT in genital skin. The backdoor pathway proceeds mainly from 17OHPreg to 17OH-Prog, 17OH-DHP, 17OH-Allo, androstanediol (A’diol) (all shown in red) and then to DHT (highlighted in yellow) in the testis. The involved enzymes and proteins shown in the classic pathway are: CYP11A1 (P450ccc, cholesterol side chain cleavage enzyme), StAR (steroidogenic acute regulatory protein), CYP17A1 (P450c17, 17α-hydroxylase/17,20 lyase), HSD3B2 (3βHSD2, 3β-hydroxysteroid dehydrogenase, type 2), cytochrome b5, P450 oxidoreductase (POR), HSD17B3 (17βHSD3, 17β-hydroxysteroid dehydrogenase, type 3), and 5αRed2 (5α-reductase, type 2). The alternative pathway comprises three different enzymes: 5αRed1 (5α-reductase, type 1); AKR1C2 and AKR1C4 for reductive 3αHSD activity; and 17βHSD6 and/or AKR1C2/4 for oxidative 3αHSD activity. Steroid names include: 17OHPreg, 17-hydroxyprogrenolone; 17OHPreg, 17-hydroxyprogesterone; 17OH-DHP, 17-hydroxydihydroprogesterone (5α-pregn-3α,17α-ol-20-one); 17OH-Allo, 17-hydroxyallopregnanolone (5α-pregn-3α,17α-diol-20-one; P’diol); androstanediol (A’diol).

mitochondrion 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 LCAS 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 17OHprog levels resulting from peripheral conversion of massively increased 17OHPreg levels. Generally, 3β-hydroxysteroid dehydrogenases (3βHSDs) convert delta 5 steroids (Preg, 17OHProg, DHEA, androstenediol) to delta 4 steroids (Prog, 17OHProg, androstenediol, 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, androstanedione to DHT and androsterone to androstaneol (Fig. 2). Thus HSD17B3 deficiency is a male sex-limited disorder causing 46,XY DSD with severe to complete undervirilization of the external genitalia as the virilization of the external genitalia seems to resemble 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. 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
CYB5 mutations (W28X, H44L) have been described in 46,XY DSD individuals of two families [18].

5. Alternative androgen production through the backdoor pathway and AKRIC 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, 17OHP 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, AKRIC2/4, 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 androstenedione 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 17OHP 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 AKRIC2/4 mutations

The backdoor pathway depends on reductive and oxidative 3α-hydroxysteroid dehydrogenase (3αHSD) activities for androgen production [7]. The four major human 3αHSDs are aldo-ketoreductases of the AKRIC family and have usually reductive activity. AKRIC1–4 lie on chromosome 10p14-15 and each enzyme has specific tissue distribution and specific catalytic characteristics. AKRIC3 is also known as 17βHSD5 and catalyzes the conversion of androstenedione to T in steroidogenic and non-steroidogenic tissues. AKRIC3 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, AKRIC2 and AKRIC4 are able to convert 17OHDHP to P’diol. They are both expressed in testis and adrenals but the fetal testis expresses more AKRIC2 than AKRIC4 [6]. By contrast, AKRIC2/4 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 AKRIC2/4 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 paternal 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 AKRIC2 mutations in the affected individuals and suggested autosomal recessive, male sex-limited inheritance. However, intrafamilial phenotypical variability and in vitro functional studies showing only moderate activity loss of 20–80% of the identified AKRIC2 mutant enzymes prompted a search for a second hit. Linkage analysis pointed to the AKRIC locus which contains five closely related AKRIC genes which were further investigated. Finally, the second hit was found in the AKRIC4 gene. Affected patients were all found to harbor a splicing mutation in AKRIC4 together with AKRIC2 (I79V).

In a second kindred with severe 46,XY DSD (female external genitalia, intra-abdominal testes) a complex chromosomal rearrangement in the AKRIC locus was found. This included an unequal crossing over between the AKRIC2 and the AKRIC1 gene, and an additional missense mutation (H222Q) in the AKRIC2 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 CEF) and 310031-134926 (to AVP) from the Swiss National Science Foundation.

References