CHAPTER THREE

Mutation of *HSD3B2* Gene and Fate of Dehydroepiandrosterone

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Abstract

3βHSD2 enzyme is crucial for adrenal and gonad steroid biosynthesis. In enzyme deficiency states, due to recessive loss-of-function *HSD3B2* mutations, steroid flux is altered and clinical manifestations result. Deficiency of 3βHSD2 activity in the adrenals precludes normal aldosterone and cortisol synthesis and the alternative backdoor and 11-oxygenated C19 steroid pathways and the flooding of cortisol precursors along the Δ5 pathway with a marked rise in DHEA and DHEAS production. In gonads, it precludes normal T and estrogen synthesis. Here, we review androgen-dependent male differentiation of the external genitalia in humans and link this to female development and steroidogenesis in the developing adrenal cortex. The molecular mechanisms governing postnatal adrenal cortex zonation and ZR development were also revised. This chapter will review relevant clinical, hormonal, and genetic aspects of 3βHSD2 deficiency with emphasis on the significance of alternate fates encountered by steroid hormone precursors in the adrenal gland and gonads. Our current knowledge of the

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process of steroidogenesis and steroid action is derived from pathological conditions. In humans the 3βHSD2 deficiency represents a model of nature that reinforces our knowledge about the role of the steroidogenic alternative pathway in sex differentiation in both sexes. However, the physiological role of the high serum DHEAS levels in fetal life as well as after adrenarche remains to be elucidated.

1. ROLE OF 3β-HYDROXYSTEROID DEHYDROGENASE IN STEROID FORMATION

Type 2 3β-hydroxysteroid dehydrogenase Δ^4–Δ^5 isomerase (3βHSD2) is a bifunctional microsomal and mitochondrial nicotinamide adenine dinucleotide (NAD)+-dependent membrane-bound enzyme that catalyzes the conversion of the three principal endogenous Δ^5-steroid precursors, pregnenolone, 17α-hydroxypregnenolone, and dehydroepiandrosterone (DHEA) into their respective Δ^4-ketosteroids, namely progesterone, 17α-hydroxyprogesterone (17OHP), and androstenedione (A4), all with similar efficiency (apparent K_m and apparent V_{max}) (Lee, Miller, & Auchus, 1999; Rhéaume et al., 1991). The first reaction is the oxidation of the 3β-hydroxyl group to the ketone by dehydrogenase activity; during this process, NAD + is reduced to NADH. The intermediate Δ^5,3-ketosteroid remains tightly bound to the enzyme with nascent NADH, and the presence of NADH in the cofactor-binding site activates the Δ^4–Δ^5-isomerase activity, residing in the same enzyme that adapts different conformation (Thomas, Boswell, Scaccia, Pletnev, & Umland, 2005; Thomas, Frieden, Nash, & Strickler, 1995). Therefore, 3βHSD2 is essential for the biosynthesis of all classes of active steroid hormones, including aldosterone, cortisol in the adrenal cortex, and sex steroids in the adrenals and gonads.

1.1 Human Steroidogenesis

Steroidogenesis entails processes by which cholesterol is converted to biologically active steroid hormones. These processes are repeated in each steroidogenic tissue with cell-type-specific patterns that are dictated by the cell-specific expression of specific steroidogenic enzymes.

The cleavage of cholesterol to pregnenolone is the first, rate-limiting, and hormonally regulated step (John, John, Boggaram, Simpson, & Waterman, 1986) in the biosynthesis of steroid hormones common to all steroidogenic cells. The cholesterol side-chain cleavage enzyme, P450scc (*CYP11A1*), supported by its electron transport system consisting of reduced

nicotinamide adenine dinucleotide phosphate, adrenodoxin reductase (*FDXR*), and adrenodoxin (*FDX1*) (Miller, 2005), catalyzes three sequential reactions: 20α-hydroxylation, 22-hydroxylation, and cleavage of the 20–22 carbon bond of cholesterol to yield pregnenolone. Because P450scc resides on the inner mitochondrial membrane (Black, Harikrishna, Szklarz, & Miller, 1994), steroid acute regulatory (StAR) protein, as part of a multiprotein complex, termed transduceosome (Rone, Fan, & Papadopoulos, 2009), facilitates the movement of cholesterol from the outer to the inner mitochondrial membrane, thus providing the substrate for steroid hormone biosynthesis (Lin et al., 1995). The specific repertoire of enzymes distal to CYP11A1 in a cell determines the fate of pregnenolone metabolism and defines the function of that cell.

A schematic overview of steroidogenesis, including the classic androgen synthesis pathway and the two alternative androgen synthesis pathways (11-oxygenated 19 carbon steroid and alternative pathway to DHT), is shown in Fig. 1.

1.2 Adrenal Steroidogenesis

The coexpression of 3β-hydroxysteroid dehydrogenase/Δ^4–Δ^5 isomerase, 3βHSD (*HSD3B1* (Doi et al., 2014) and *HSD3B2*), and aldosterone synthase, P450c11AS (*CYP11B2*) in the zona glomerulosa (ZG) leads to aldosterone production under regulation by the renin/angiotensin system. The adrenal zona fasciculata (ZF) does not express angiotensin II receptors or P450c11AS, but instead expresses the ACTH receptor, MC2R, and 11β-hydroxylase, P450c11 (*CYP11B1*). The coexpression of 3βHSD type 2 (*HSD3B2*) and cytochrome P450 17α-hydroxylase/17,20-lyase, P450c17 (*CYP17A1*) along with P450c11 in the ZF, results in the production of cortisol under the influence of ACTH. By contrast, the zona reticularis (ZR) expresses relatively very little 3βHSD2 and large amounts of P450c17, cytochrome *b*5, which selectively activates the 17,20-lyase activity of P450c17 (Auchus, Lee, & Miller, 1998), and DHEA-sulfotransferase (*SULT2A1*) (Suzuki et al., 2000). Consequently, the C19 steroid DHEA is produced, much of which is sulfated to DHEAS (Miller & Auchus, 2011; Turcu, Smith, Auchus, & Rainey, 2014). This Δ^5 pathway is the preferred route to C19 steroid production in humans as the 17,20-lyase activity of human *CYP17A1* does not efficiently convert 17α-hydroxyprogesterone (17OHP) to androstenedione (A4) (Auchus, Lee, & Miller, 1998; Fig. 1). Androgen production in human adrenal cortex

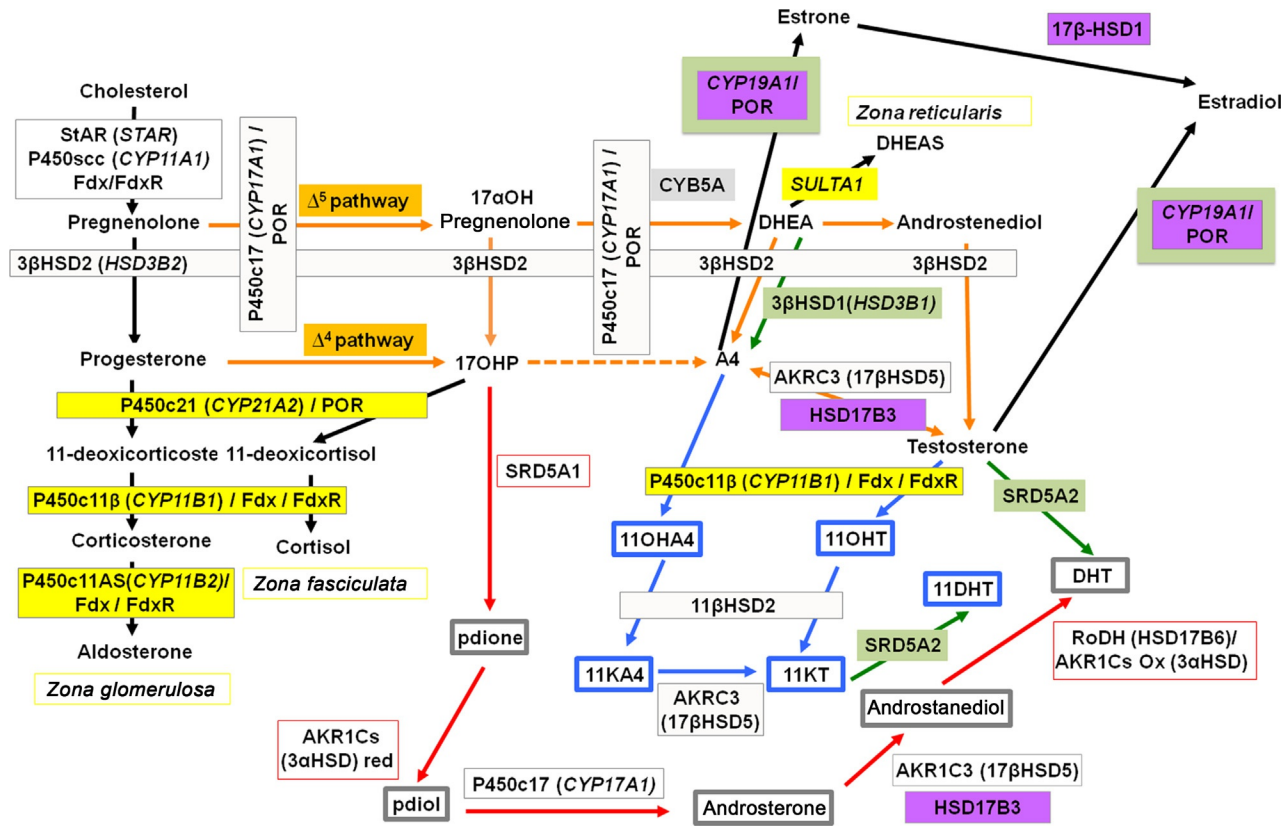

Fig. 1 See legend on opposite page.

is zonally and developmentally regulated. The 3βHSD2-deficient ZR is indistinct during infancy, but a continuous layer of reticularis cells starts to develop and thicken around 4–5 years (Remer, Boye, Hartmann, & Wudy, 2005). This process, known as adrenarche, is followed by a rise in circulating concentrations of DHEAS, with clinical signs physiologically observed between the ages of 6 and 10 (Auchus, 2011; Belgorosky, Baquedano, Guercio, & Rivarola, 2008). The human adrenal gland also makes little A4 and testosterone (T). It has been proposed that A4 is produced from DHEA in a layer of cells in the interface of the ZF and ZR which expresses CYP17A1, cytochrome *b*5, and 3βHSD2 (Nakamura et al., 2015, 2011). The expression of aldo-ketoreductase 1C3 (AKR1C3), also known as 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5), in the ZR would be responsible for the conversion of DHEA and A4 to androstenediol and T, respectively (Nakamura, Hornsby, et al., 2009; Fig. 1).

Furthermore, the advent of comprehensive analyses of steroid profiles by sensitive and specific liquid chromatography (LC) and gas chromatography/tandem mass spectrometry (GC-MS/MS) revealed adrenal alternative androgen synthesis pathways (Homma et al., 2006; Jones et al., 2017; Kamrath, Hochberg, Hartmann, Remer, & Wudy, 2012; O'Reilly et al., 2017; Rege et al., 2013; Turcu et al., 2016). 17OHP is also a substrate for an alternative, so-called backdoor pathway to androgen synthesis, which generates DHT without the intermediacy of DHEA, A4, or T (Auchus, 2004). This pathway depends on the 5α- and 3α-reduction of 17OHP to

Fig. 1 Pathways of human steroid hormone synthesis. The biosynthetic pathways shown are relevant to the gonads and the adrenal cortex. In *white boxes* are shown enzymes that are common to adrenals and gonads; in *purple*, those related to gonadal steroids, and in *yellow boxes* those related to adrenal-specific pathways. The classical androgen synthesis pathway is showed in *orange*. Δ^5 pathway involving pregnenolone through DHEA, and Δ^4 pathway involving progesterone through testosterone are indicated. Minor routes indicated by *dashed arrows*. The alternative "backdoor" androgen pathway is in *red*, and the 11oxC19-steroid pathway is showed in *blue*. In *green* are depicted enzymes involved in routes to androgens and estrogens in the periphery. A small amount of DHEA is converted in the adrenal by HSD3B2, and larger amounts derive from peripheral metabolism, mediated by HSD3B1. *11KA4*, 11-ketoandrostenedione; *11KT*, 11-ketotestosterone; *11OHA4*, 11β-hydroxyandrostenedione; *11OHT*, 11β-hydroxytestosterone; *17OHP*, 17α-hydroxyprogesterone; *A4*, androstenedione; *CYB5A*, cytochrome *b*5; *DHEA*, dehydroepiandrosterone; *DHEAS*, dehydroepiandrosterone sulfate; *DHT*, 5α-dihydrotestosterone; *pdiol*, 17-hydroxyallopregnenolone (5α-pregnane-3α,17α-diol-20-one); *pdione*, 17-hydroxydihydroprogesterone (5α-pregnane-17α-ol-3,20-dione); *T*, testosterone.

produce 17-hydroxyallopregnenolone (pdiol), followed by 17,20-lyase activity of P450c17 and 17βHSD dehydrogenase activity, yielding androstanediol, and finally a 3α-oxidation step to DHT (Fig. 1) (Auchus, 2004). In contrast to the classical pathway, human CYP17A1 has a very high affinity for backdoor pathway intermediate, pdiol, which is an excellent substrate for its 17,20-lyase activity, not dependent from cytochrome *b*5, leading to androsterone production (Auchus, 2004; Gupta, Guryev, & Auchus, 2003). Recent evidence showed that the human adrenal cortex would express the enzymes to complete all the steps in the backdoor pathway to DHT (Baquedano et al., 2014; Flück et al., 2011; Marti et al., 2017) and that this pathway would contribute to the androgen production in pathological states in which 17OHP accumulates, including 21α-hydroxylase and POR deficiencies (Homma et al., 2006; Jones et al., 2017; Kamrath, Hochberg, Hartmann, Remer, & Wudy, 2012). Moreover, recent studies have demonstrated that human adrenal also produces a unique set of 11-oxygenated C19 steroid (11oxC19) (Fig. 1) (O'Reilly et al., 2017;Rege et al., 2013; Turcu et al., 2016). The first step of the 11-oxygenated androgen pathway is dependent on the adrenal CYP11B1-catalyzed 11β-hydroxylation of A4 to 11β-hydroxyandrostenedione (11OHA4), which is a major product of adrenal steroidogenesis (Rege et al., 2013; Turcu et al., 2016). Low quantities of the 11-oxygenated C19 steroid 11-ketoandrostenedione (11KA4), 11β-hydroxytestosterone (11OHT), and 11-ketotestosterone (11KT) are also produced by the human adrenal (Rege et al., 2013; Turcu et al., 2016; Fig. 1). 11KT and its peripheral 5α-reduced metabolite, 11-keto-5α-dihydrotestosterone (11KDHT), are potent agonists of the human androgen receptor (AR) with affinities and potencies similar to that of T and 5α-dihydrotestosterone (DHT), respectively (Pretorius et al., 2016; Rege et al., 2013; Storbeck et al., 2013).

1.3 Gonadal Steroidogenesis

Testicular synthesis of T follows a pathway that is similar to C19-steroid production in the adrenal ZR, but the stimulus for steroidogenesis is transduced by the LH receptor rather than MC2R and Leydig cells express abundant 3βHSD2 and 17βHSD3, but no SULT2A1 (Miller & Auchus, 2011). Thus, DHEA produced in the testis is not sulfated but is readily converted to A4 and/or androstenediol and then T via 3βHSD2 and 17βHSD3 (Fig. 1). As in the adrenal, the principal pathway to C19-steroids is via Δ^5 steroids to DHEA. Testicular T is converted to DHT in peripheral tissues, such as

prostate and genital skin, by steroid 5α-reductase type 2 (*SRDA2*) (Auchus & Miller, 2012). Gonadal T is not an important precursor for adrenal 11β-hydroxylated 11OHT and 11KT (Turcu et al., 2016).

In the ovary, enzymatic steps of steroidogenesis are partitioned between the granulosa and theca cells, which surround the oocyte and form a follicle. Furthermore, the patterns of steroidogenesis vary during the cycle: estradiol is the principal product in the follicular phase, and progesterone is produced in the luteal phase. Granulosa cells do not express P450c17 (Voutilainen, Tapanainen, Chung, Matteson, & Miller, 1986). Thus, steroidogenesis is initiated in granulosa cells under the influence of LH, which stimulates the P450scc expression (Voutilainen, Tapanainen, Chung, Matteson, & Miller, 1986). Pregnenolone and progesterone from granulosa cells diffuse into adjacent theca cells, where P450c17 and 3βHSD2 catalyze A4 synthesis. Small amounts of this A4 are secreted or converted to T by AKR1C3/17βHSD5, but most A4 returns to the granulosa cells where P450 aromatase (*CYP19A1*) and 17βHSD1 convert it to estrone and then to estradiol, respectively, under the influence of FSH.

Recent gene and protein expression studies revealed that human gonads express all genes necessary for DHT production via the backdoor pathway (Flück et al., 2011; Marti et al., 2017). Furthermore, the expression of the backdoor pathway in the fetal testis appears to play an important role during male sexual differentiation (Flück et al., 2011; see below).

1.4 Gonadal and Adrenocortical Organogenesis. Human Sexual Differentiation

The adrenal cortex and gonads derive from a common embryological adrenogonadal precursor lineage. In human embryos, these adrenogonadal progenitors, referred to as the adrenogonadal primordium, can be identified from about 3 weeks postconception (wpc) as a thickening on the inner surface of the mesonephros, the equivalent to the primitive kidney system (Else & Hammer, 2005; Parker et al., 2002). As this primordium grows, these cells subsequently differentiate and the gonadal primordium and adrenal primordium then separate. This occurs at 33 dpc (Goto et al., 2006).

In the 7th wpc, the bipotential gonad begins to differentiate into a testis or an ovary, depending on the genetic sex of the individual and, during 8–12 wpc, the sexual dimorphism of the external genitalia is established. The male gonad initiates the expression of the sex-determining region of the Y chromosome (*SRY*) gene in the cells destined to become Sertoli cells, which secrete anti-Müllerian hormone leading to regression of the

mesonephric ducts, which would otherwise give rise to the female internal genitalia (Wilhelm, Palmer, & Koopman, 2007). A few days later, a second wave of differentiation within the testis gives rise to a population of Leydig cells that secrete androgens, which virilize the male internal and external genitalia (Sobel, Zhu, & Imperato-McGinley, 2004). The evidence shows that both testicular T converted to DHT in genital skin by 5α-reductase type 2 and DHT from testicular backdoor pathway are needed for normal male sexual development (Flück et al., 2011). DHT signals to the bipotential external genitalia via the AR to elicit differentiation irrevocably along a male pathway: the urethral folds fuse in the midline to close the urogenital sinus, the genital swellings develop into the scrotum, and the phallus enlarges into a penis. Thus, during this narrow window of sexual differentiation (8–12 wpc), maintaining the appropriate intrauterine hormone environment is critical (Asby, Arlt, & Hanley, 2009; Hanley & Arlt, 2006).

Meanwhile, the cells of the adrenal primordium continue to proliferate, and at about 48 dpc, chromaffin cells originating from the neural crest migrate to the area where the adrenogonadal primordium is developing (Xing, Lerario, Rainey, & Hammer, 2015). At the same time, the adrenal cortex becomes encapsulated and by 50–52 dpc, two discrete zones are detectable in the adrenal cortex, the inner fetal zone (FZ), and the smaller outer zone, or definitive zone (DZ) (Goto et al., 2006; Xing, Lerario, Rainey, & Hammer, 2015). All key steroidogenic enzymes, including StAR, CYP11A, CYP17, and CYP21, are first detected robustly at 50–52 dpc within the nascent inner FZ and weaker within the outer DZ, except for CYP17 that is absent (Goto et al., 2006). Importantly, 3βHSD2 is detected in a wave of expression from 8 wpc until the end of the first trimester at the interface between the DZ and FZ. The presence of 3βHSD2 facilitates de novo cortisol biosynthesis, but it would also be relevant to androgen biosynthesis by either classical or alternative pathways. In the former, DHEA is converted to androstenedione by 3βHSD2; in the latter, 3βHSD2 would be a means of providing progesterone from pregnenolone (Auchus, 2004; Hanley & Arlt, 2006). Indeed, Goto and coworkers have demonstrated that the anterior pituitary–adrenal axis does appear mature as early as 50–52 dpc and that ACTH stimulates the secretion of A4 and T by the fetal adrenal cortex during the first trimester (Goto et al., 2006). This intimately ties the developing adrenal gland to the process of sexual differentiation and led to the hypothesis that the transient fetal cortisol production is capable of attenuating ACTH stimulation of the adrenal and subsequent upregulation of androgen production, thereby acting as a safeguard mechanism for normal female sexual development (Goto et al., 2006).

Regardless of the source of androgen production, the target tissue responds by male sexual differentiation of the external genitalia during a period spanning from approximately 8 weeks of fetal development until the end of the first trimester. From the second trimester onward, the potential for further virilization, even in the presence of androgen excess, is limited at least partly by diminishing expression of the AR in females (Shapiro, Huang, & Wu, 2000) and the onset of fetal P450 aromatase (*CYP19A1*) expression in both sexes (Grumbach & Auchus, 1999).

During the second and third trimesters, the adrenal glands continue to grow rapidly, largely due to an increase in the size of the FZ, which produce large amounts of DHEA and DHEAS (Ishimoto & Jaffe, 2011). A third cortical zone becomes evident by 14 weeks (Goto et al., 2006). This transitional zone (TZ) is located between the DZ and the FZ. After 23–24 weeks' gestation, 3βHSD2 expression is again detectable in the DZ and TZ (Ishimoto & Jaffe, 2011). Immediately before birth, there is a second peak in cortisol production that is required for fetal organ maturation.

After birth, a strong remodeling of the adrenal gland occurs; the fetal zone undergoes apoptosis, followed by encapsulation of the medulla and final zonation of the adult cortex. These morphologic changes are accompanied by a rapid drop in DHEA and DHEAS production due to the involution of the fetal zone. In preadrenarche children, the ZG and the ZF are clearly present, but only focal islands of ZR cells, insufficient to influence serum DHEAS levels, can be identified at the ages 3–5 years (Belgorosky, Baquedano, Guercio, & Rivarola, 2008; Dardis, Saraco, Rivarola, & Belgorosky, 1999; Gell et al., 1998; Nakamura, Gang, Suzuki, Sasano, & Rainey, 2009; Suzuki et al., 2000). After adrenarche, there is a development and thickening of a continuous ZR associated with detectable increases in circulating DHEA and DHEAS (de Peretti & Forest, 1976; Endoh, Kristiansen, Casson, Buster, & Hornsby, 1996; Havelock, Auchus, & Rainey, 2004; Hui et al., 2009).

2. HUMAN 3βHSD2 DEFICIENCY

In enzyme deficiency states, steroid flux is altered and clinical manifestations often result (Ghayee & Auchus, 2007). Deficiency of 3βHSD2 activity in the adrenals precludes normal aldosterone and cortisol synthesis and the flooding of cortisol precursors along the Δ5 pathway with a marked rise in pregnenolone, 17α-hydroxypregnenolone, DHEA, DHEAS, and androstenediol production (Fig. 2). Deficiency of 3βHSD2 activity in gonads precludes normal T and estrogen synthesis. The blockage caused

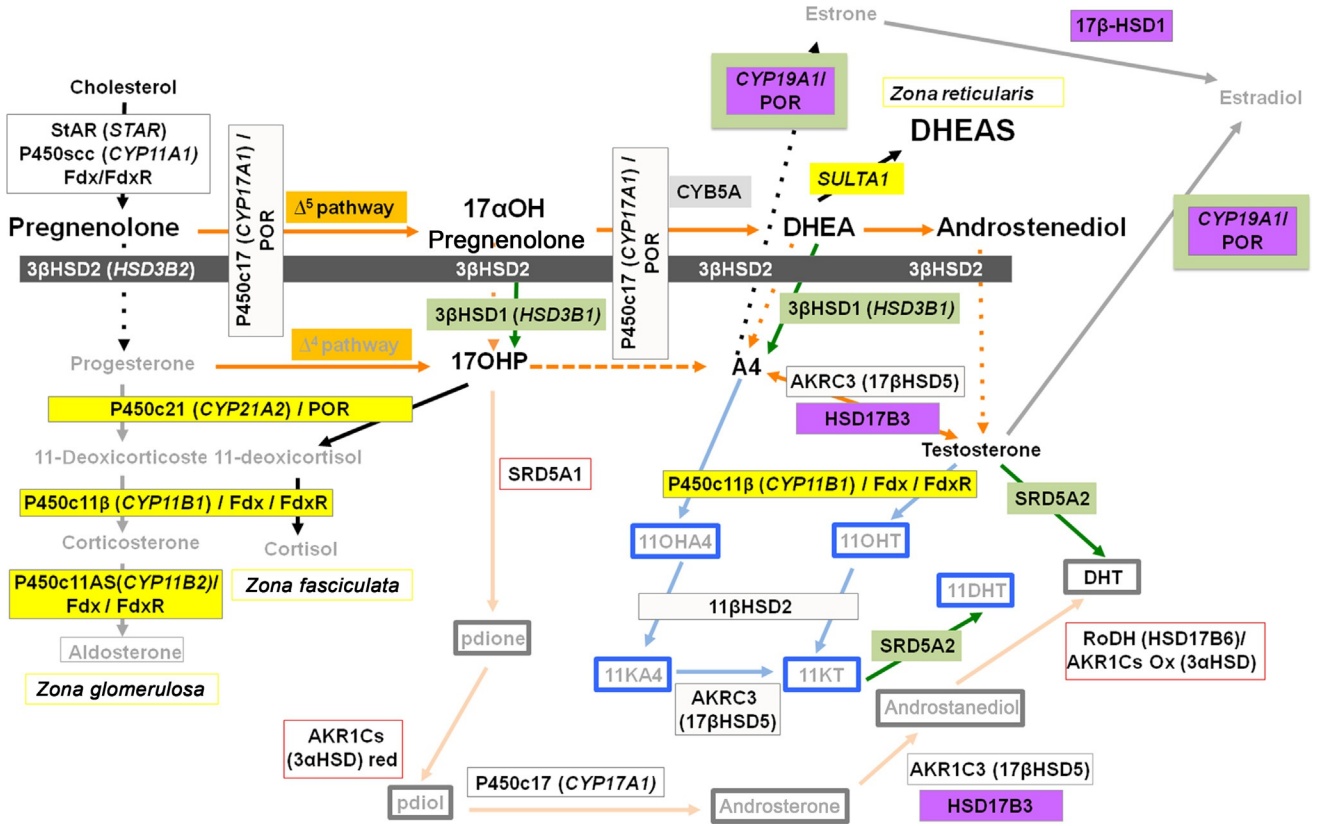

Fig. 2 See legend on opposite page.

by 3βHSD2 deficiency precludes flux to the backdoor pathway and 11-oxygenated C19 steroid synthesis due to very low intraadrenal 17OHP and A4 substrate levels, respectively (Fig. 2). However, peripheral 3βHSD type 1 (3βHSD1) conversion of Δ^5 steroids to Δ^4 steroids can result in high serum 17OHP and A4 (see below) (Fig. 2).

2.1 Molecular Genetics of Human 3βHSD2 Deficiency

In humans there are two closely linked 3βHSD genes and cognate enzymes. Type 1 (*HSD3B1*) and type 2 (*HSD3B2*) 3βHSD genes map to the short arm of chromosome 1 (1p13.1) with identical intron/exon organizations (Luu et al., 1989; Rhéaume et al., 1991). The structure of each of the *HSD3B1* and *HSD3B2* genes consists of four exons, which are included within a DNA fragment of 7.8 kb (Lorence, Corbin, Kamimura, Mahendroo, & Mason, 1990; Fig. 3A). *HSD3B1* gene encodes an enzyme of 372 amino acids predominantly expressed in placenta, breast, liver, brain, and some other peripheral tissues (Simard et al., 2005). *HSD3B2* gene, which encodes a protein of 371 amino acids (Fig. 3B), shares 93.5% identity with the type 1 and is almost exclusively expressed in the adrenals and gonads (Lorence et al., 1990; Rhéaume et al., 1991). The purified 3βHSD2 enzyme has a Michaelis constant (K_m) of 47 μM and a maximal velocity (V_{max}) of 82 nmol/min mg for 3βHSD substrate (DHEA) and a K_m of 88 μM and V_{max} of 970 nmol/min mg for the isomerase substrate (5-androstene-3,17-dione), while 3βHSD1 has a K_m of 3.7 μM and a V_{max} of 43 nmol/min mg for 3βHSD substrate (DHEA) and a K_m of 28 μM and V_{max} of 598 nmol/min mg for the isomerase substrate (5-androstene-3,17-dione) (Thomas, Mason, Blanco, & Veisaga, 2001). The higher affinity of 3βHSD1 could facilitate

Fig. 2 Altered steroid pathways in 3βHSD2 deficiency. The block of adrenal cortisol and aldosterone synthesis hunts precursors to 19-carbon steroids. Deficiency of 3βHSD2 activity in gonads precludes normal testosterone and estrogen synthesis. Peripheral 3βHSD1-catalyzed conversion of Δ^5 steroids to Δ^4 steroids results in high 17OHP and A4 in 46,XX patients. However, the alternative androgen synthesis pathways (11-oxygenated 19 carbon steroids and the "backdoor" pathway to DHT) are precluded due to very low intraadrenal Δ^4 steroids. Steroids that are produced in excess have larger font. Steroids that are deficient are graphically not emphasized, with minor pathways shown with *dotted arrows*. *11KA4*, 11-ketoandrostenedione; *11KT*, 11-ketotestosterone; *11OHA4*, 11β-hydroxyandrostenedione; *11OHT*, 11β-hydroxytestosterone; *17OHP*, 17α-hydroxyprogesterone; *A4*, androstenedione; *CYB5A*, cytochrome *b5*; *DHEA*, dehydroepiandrosterone; *DHEAS*, dehydroepiandrosterone sulfate; *DHT*, 5α-dihydrotestosterone; *pdiol*, 17-hydroxyallopregnenolone (5α-pregnane-3α,17α-diol-20-one); *pdione*,17-hydroxydihydroprogesterone (5α-pregnane-17α-ol-3,20-dione); *T*, testosterone.

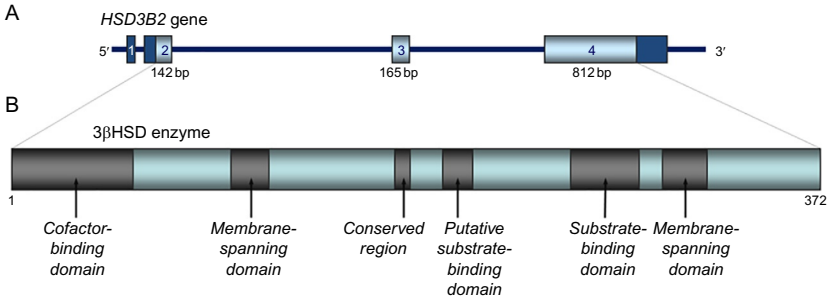

Fig. 3 (A) Genomic organization for *HSD3B2* gene. *Numbered boxes* represent exons. *Lines* represent introns. The HSD3B2 gene has four exons, whereof exon 1 and the 5′ part of exon 2 are not translated. (B) A cartoon of the different functional domains of the 3βHSD2 enzyme.

steroid formation from relatively low concentrations of substrates usually present in peripheral tissues.

The *HSD3B2* gene is mutated in 3βHSD deficiency, while *HSD3B1* gene mutations have never been described, possibly because placental 3βHSD1 deficiency would preclude progesterone production during pregnancy. Mutations throughout the *HSD3B2* gene have been reported. To date, a total of 50 mutations (including frameshift, nonsense, in-frame deletion, splicing, and missense mutations) have been identified in the *HSD3B2* gene in 82 individuals from 66 families suffering from classical 3βHSD deficiency as shown in Table 1. Gene conversions with the *HSD3B1* gene have not been described, and founder mutations account for only small cluster of cases in remote areas.

2.2 Clinical Features of Human 3βHSD2 Deficiency

Recessive loss-of-function *HSD3B2* mutations cause a rare form of congenital adrenal hyperplasia (CAH) that impairs both adrenal and gonadal steroidogenesis. It is one of the rarest forms of CAH with an incidence of only 1 in approximately 1,000,000 births and no ethnic pockets with higher prevalence have been described. Cortisol deficiency leads to an increase in corticotropin (ACTH) secretion through the feedback mechanism that results in increased production of Δ^5 steroid precursors, such as pregnenolone, 17α-hydroxypregnenolone, DHEA, and androstenediol from the adrenal cortex. Aldosterone deficiency causes the salt wasting, and combined cortisol and aldosterone deficiencies cause adrenal crisis if the affected subjects are not early identified and treated (Auchus & Chang, 2010; Auchus & Miller, 2012; Pang, 2001; Simard, Moisan, & Morel, 2002).

Table 1 *HSD3B2* Gene Mutations and Their Residual In Vitro Activity Reported in 3βHSD2-Deficient Patients

Mutation	Variant ID	Activity In Vitro (% of wt)	Domain of the protein	Evidence of Protein Instability	References
Missense					
p.Leu6Phe c.16C > T	CM000720	Δ5-P to P 47.8%, DHEA to Δ4-A 39.3%		Yes	Zhang et al. (2000)
p.Ala10Glu c.29C > A	CM001196 rs28934880	DHEA to Δ4-A 0%	Putative NAD-binding domain	Yes	Alos et al. (2000)
p.Ala10Val c.29C > T	CM993167	DHEA to Δ4-A 29.1%	Putative NAD-binding domain	No	Moisan et al. (1999)
p.Gly12Glu c.35G > A	rs756607591	ND		ND	Benkert et al. (2015)
p.Gly15Asp c.44G > A	CM952220	Δ5-P to P 0%	Putative NAD-binding domain	No	Rhéaume et al. (1995)
p.Ala82Thr c.244G > A	CM940954 rs757033996	DHEA to Δ4-A 7.6%		No	Mendonça et al. (1994)
p.Ala82Pro c.244G > C	CM124707	ND	Membrane-binding domain	Yes	Rabbani, Mahdieh, Haghi Ashtiani, Setoodeh, & Rabbani, 2012)
p.Ala82Asp c.245C > A		ND		ND	Nordenström, Forest, and Wedell (2007)
p.Asn100Ser c.299A > G	CM950653	Δ5-P to P 2.7%, DHEA to Δ4-A 11%		No	Mébarki et al. (1995)

Continued

Table 1 *HSD3B2* Gene Mutations and Their Residual In Vitro Activity Reported in 3βHSD2-Deficient Patients—cont'd

Mutation	Variant ID	Activity In Vitro (% of wt)	Domain of the protein	Evidence of Protein Instability	References
p.Leu108Trp c.323T>G	CM940955	Δ5-P to P 0%		Yes	Sanchez, Mébarki, et al. (1994), Sanchez, Rhéaume, et al. (1994)
p.Gly129Arg c.385G>A	CM940956	Δ5-P to P 2%, DHEA to Δ4-A 4.7%	Cofactor binding domain	No	Rhéaume et al. (1994)
p.Glu142Lys c.424G>A	CM930411 rs80358219	Δ5-P to P 0%		No	Simard et al. (1993)
p.Pro155Leu c.464C>T	CM993168 rs779418168	DHEA to Δ4-A 0%	YXXXK sequence located in the active site of short-chain alcohol dehydrogenases	No	Moisan et al. (1999)
p.Ala167Val c.500C>T	CM985528	ND		ND	Nayak, Lee, and Witchel (1998)
p.Leu173Arg c.518T>G	CM940957 rs762479018	DHEA to Δ4-A 52.8%		No	Moisan et al. (1999)
p.Thr181Ile c.542C>A		ND		ND	Johannsen et al. (2005)
p.Pro186Leu c.557C>T	CM940958	Δ5-P to P 0%		Yes	Sanchez, Mébarki, et al. (1994), Sanchez, Rhéaume, et al. (1994)

p.Tyr190Cys c.569A>G		Δ5-17P to 17-OHP <5%, Δ5-P to P <1%, DHEA to Δ4-A <1%	Between the two putative substrate-binding domains. 3D model: extremely close to the putative substrate-binding pocket	No	Takasawa et al. (2014)
p.Leu205Pro c.614T>C	CM950654	DHEA to Δ4-A 0%		No	Moisan et al. (1999)
p.Ser213Thr c.638G>C	CM040433 rs137867568	DHEA to Δ4-A 40%		ND	Codner et al. (2004)
p.Ser213Gly c.637A>G	CM995305 rs759422374	DHEA to Δ4-A 58.4 ± 0.6%		No	Moisan et al. (1999)
p.Lys216Glu c.646A>G	CM995306	DHEA to Δ4-A 58.9%		No	Moisan et al. (1999)
p.Ser218Pro c.652T>C		Δ5-17P to 17-OHP <10%, Δ5-P to P <1%, DHEA to Δ4-A <1%	Between the two putative substrate-binding domains. 3D model: extremely close to the putative substrate-binding pocket	No	Takasawa et al. (2014)
p.Pro222Gln c.665C>A	CM993169 rs765547422	DHEA to Δ4-A 0%		No	Moisan et al. (1999)
p.Pro222His		DHEA to Δ4-A 0%		No	Moisan et al. (1999)
p.Pro222Thr c.664C>A	CM023090 rs80358220	Δ5-P to P 0%, DHEA to Δ4-A 0%	Adjacent flanking substrate-binding domain	Yes	Pang et al. (2002)

Continued

Table 1 *HSD3B2* Gene Mutations and Their Residual In Vitro Activity Reported in 3βHSD2-Deficient Patients—cont'd

Mutation	Variant ID	Activity In Vitro (% of wt)	Domain of the protein	Evidence of Protein Instability	References
p.Leu236Ser c.707T > C	CM993170 rs35887327	DHEA to Δ4-A 100%		No	Moisan et al. (1999)
p.Ala245Pro c.733G > C	CM930412	Δ5-P to P 11.9%, DHEA to Δ4-A 13.1%		No	Simard et al. (1993)
p.Gly250Val c.749G > T	CM1210438	Δ5-P to P 20%, DHEA to Δ4-A 27%	Loop next to a beta-sheet structure in the cofactor domain	No	Baquedano et al. (2015)
p.Tyr253Asn c.757T > A	CM930413	Δ5-P to P 0%, DHEA to Δ4-A 0%		No	Simard et al. (1993)
p.Tyr254Asp c.760T > G	CM940959	Δ5-P to P 0%, DHEA to Δ4-A 0%	Substrate-binding domain	No	Sanchez, Mébarki, et al. (1994), Sanchez, Rhéaume, et al. (1994)
p.Thr259Arg c.776C > G	CM950656	DHEA to Δ4-A 0%	Substrate-binding domain	Yes	Moisan et al. (1999)
p.Thr259Met c.776C > T	CM993171 rs80358221	DHEA to Δ4-A 0%	Substrate-binding domain	Yes	Moisan et al. (1999)
p.Ser284Arg c.852C > G	CM040434	DHEA to Δ4-A 32%		ND	Codner et al. (2004)
p.Gly294Val c.881G > T	CM993172	DHEA to Δ4-A 20.5%	Membrane-spanning domain	No	Moisan et al. (1999)

p.Tyr339Cys c.1016A>G		ND		ND	Bahíllo-Curieses, Loidi Fernández de Trocóniz, del Cañizo López, and Martínez-Sopena (2016)
p.Pro341Leu c.1022C>T	CM081307	Δ5-P to P 2%, DHEA to Δ4-A 6%	C-Terminal part of the protein	Yes	Welzel et al. (2008)
p.Ter373Cys c.1119A>C	CM021276	Δ5-P to P 27%, DHEA to Δ4-A 27%	C-Terminal part of the protein	Yes	Pang et al. (2002)
Nonsense					
p.Glu25Ter c.73G>T		No predicted activity		ND	Huang et al. (2014)
p.Glu135Ter c.403G>T	CM970746	No predicted activity		ND	Marui et al. (1998)
p.Trp171Ter c.512G>A	CM920363 rs80358216	No predicted activity		ND	Rhéaume et al. (1992)
p.Trp230Ter c.690G>A		No predicted activity		ND	Nordenström, Forest, and Wedell (2007)
p.Arg249Ter c.745C>T	CM950655 rs80358217	No predicted activity		ND	Tajima et al. (1995)
p.Tyr308Ter c.924C>G	CM950657	No predicted activity		ND	Tajima et al. (1995)

Continued

Table 1 *HSD3B2* Gene Mutations and Their Residual In Vitro Activity Reported in 3βHSD2-Deficient Patients—cont'd

Mutation	Variant ID	Activity In Vitro (% of wt)	Domain of the protein	Evidence of Protein Instability	References
p.Gln334Ter c.1000C>T	CM125506	No predicted activity	C-Terminal part of the protein	ND	Jeandron and Sahakitrungruang (2012)
p.Arg335Ter c.1003C>T	CM081308 rs148200568	Δ5-P to P 2 %, DHEA to Δ4-A 2%	C-Terminal part of the protein	Yes	Welzel et al. (2008)
p.Trp355Ter c.1064G>A	CM081309	Δ5-P to P 1.5 %, DHEA to Δ4-A 2%	C-Terminal part of the protein	Yes	Welzel et al. (2008)
Deletions					
p.Ala168Valfs c.503delC		No predicted activity		ND	Probst-Scheidegger, Udhane, l'Allemand, Flück, and Camats (2016)
p.Trp230_Ala238del c.687_713del	CG994940	DHEA to Δ4-A 0%		Yes	Moisan et al. (1999)
p.Asn266ThrfsTer6 c.797delA	CD000265	No predicted activity		ND	McCartin et al. (2000)
p.Lys273ArgfsTer7 c.818_819delAA	CD941706 rs754609778	No predicted activity		ND	Simard et al. (1994)
p. Met290CysfsTer10 c.867delG	CD994557 rs767167623	No predicted activity		ND	Moisan et al. (1999)

p.Thr318LysfsTer50 c.953delC	CD962037	No predicted activity		ND	Zhang, Sakkal-Alkaddour, Chang, Yang, and Pang (1996)
p.Val319AlafsTer49 c.956delT		No predicted activity	3D modeling predicts a conformational change in the substrate-binding pocket	ND	Bizzarri et al. (2016)
p.Lys368SerfsTer129 c.1103delA		No predicted activity		ND	Johannsen et al. (2005)
Insertions					
p.Thr187HisfsTer17 c.558insC	CI920941 rs770815049	No predicted activity		ND	Rhéaume et al. (1992)
Indels					
p.Val248AsnfsTer2 c.742_746delinsaact	CX931241	No predicted activity		ND	Chang, Kappy, et al. (1993)
Splicing					
c.308-6G>A	CS942333 rs755048400	No predicted activity		ND	Rhéaume et al. (1994)

All mutations were designated following the recommendations of the Human Genome Variation Society and HUGO Gene Nomenclature Committee (den Dunnen et al., 2016). Nucleotides change considering reference sequences: NM_001166120.1, NP_001159592.1, and CCDS902.1. *DHEA*, dehydroepiandrosterone; *ND*, not determined; *P*, progesterone; *Δ4-A*, androstenedione; *Δ5-P*, pregnenolone; *Δ5-17P*, 17OH pregnenolone; *17-OHP*, 17 OH progesterone.

The clinical phenotype in the affected patients is significantly heterogeneous with different degrees of genital ambiguity and adrenal failure in both sexes (Table 2 and 3).

Clinical symptoms of adrenal failure could be present early after birth. Mineralocorticoid deficiency with salt wasting, hypotension, and hyperkalemia is observed in 60%–70% of the reported cases (Simard, Moisan, & Morel, 2002). No functional 3βHSD2 isoenzyme is expressed in the adrenals and gonads of these patients. The nonsalt-wasting form of 3βHSD2 deficiency results from variations in the *HSD3B2* gene causing an incomplete loss of enzymatic activity (Baquedano et al., 2015; Moisan et al., 1999).

Testicular steroidogenic defect leads to different degrees of under virilization in 46,XY affected subjects. The phenotypic spectrum includes patients with severe under virilization and female appearance. Most frequently affected individuals present with genital ambiguity, hypospadias, microphallus, and cryptorchidism (Auchus & Miller, 2012; Pang, 2001).

Mild genital virilization with clitoromegaly with or without partial labial fusion, and even normal female genitalia has been reported in the 46,XX affected subjects (Auchus & Chang, 2010; Benkert et al., 2015; Pang, 2001). This lesser genital virilization, compared to 21-hydroxylase and 11-hydroxylase deficiencies, is related to peripheral conversion of the DHEA excess through the homologous enzyme 3βHSD1 into more potent androgens. The absence of adrenal 3βHSD activity precludes elevation of intra-adrenal 17OHP and the production of backdoor-derived DHT and 11-oxygenated androgens, which would be the major source of virilizing androgens in other forms of CAH (Jones et al., 2017; Kamrath, Hochberg, Hartmann, Remer, & Wudy, 2012; Turcu et al., 2016). The underrepresentation of 46,XX patients in this autosomal recessive disorder may be explained by the milder genital phenotype that could delay diagnosis and/or lead to unrecognized cause of infant death (Alos et al., 2000; Moisan et al., 1999).

Peripheral conversion does not rescue cortisol and aldosterone deficiency, because the 21-carbon steroid precursors do not efficiently return to the adrenal for subsequent hydroxylations. Peripheral cytochromes P450 cannot substitute for the adrenal 21α- and 11α-hydroxylases to make significant amounts of cortisol, but progesterone 21α-hydroxylation through CYP3A4 and CYP2C9 in the liver can partially compensate for the mineralocorticoid deficiency by making 11-deoxycorticosterone in older children and adults (Casey & MacDonald, 1982).

As mentioned earlier, patients with less severe enzymatic defects can maintain a normal hydroelectrolyte balance. If not treated, they will develop symptoms of androgen excess later in life (body odor, pubarche, axilarche,

Table 2 Ethnic Origin, Genotype, and Clinical Findings of 46,XY Described Patients

			At Diagnosis					
Families/ Patients	**Population**	**Genotype**	**Dead Relatives**	**Newborn Screening (17OHP4)**	**Chronological Age**	**Clinical Phenotype**	**Salt Wasting**	**Long-Term Follow-up**	**References**
I.1	Japanese	p.[Arg249Ter]; [Arg249Ter]			Birth	Skin pigmentation. Hypospadias with micropenis, bifid scrotum, palpable gonads	Yes	Gynecomastia at 7.6 years. Spontaneous pubertal development	Yoshimoto et al. (1997)
II.1	American	p.[Trp171Ter]; [Thr187HisfsTer17]	Yes		1 month	Mild hypospadias	Yes	Spontaneous puberty. Gynecomastia. Fathered two children	Parks, Bermudez, Anast, Bongiovanni, and New (1971), Rhéaume et al. (1992)
III.1	Mexican Hispanic	p.[Val248AsnfsTer2]; [Val248AsnfsTer2]			Birth	Peineal hypospadias, micropenis, bifid scrotum, scrotal testes	Yes	Gynecomastia at 12 years	Chang, Kappy, et al. (1993)
IV.1	Dutch	p.[Tyr253Asn]; [Thr187HisfsTer17]			Birth	Urethral diverticula, hypospadias	Yes		Simard et al. (1993)
V.1	American	p.[Glu142Lys]; [Trp171Ter]			1 week	Perineal hypospadias, bifid scrotum	Yes		Simard et al. (1993)
VI.1	Turkish	p.[Ala245Pro]; [Ala245Pro]	Yes		4 years	Scrotal hypospadias, bifid scrotum	No		Simard et al. (1993)

Continued

Table 2 Ethnic Origin, Genotype, and Clinical Findings of 46,XY Described Patients—cont'd

					At Diagnosis				
Families/ Patients	**Population**	**Genotype**	**Dead Relatives**	**Newborn Screening (17OHP4)**	**Chronological Age**	**Clinical Phenotype**	**Salt Wasting**	**Long-Term Follow-up**	**References**
VII.1	Pakistani	p.[Lys273ArgfsTer7]; [Lys273ArgfsTer7]			Birth	Hypospadias	Yes		Simard et al. (1994)
VIII.1	Afghan	p.[Lys273ArgfsTer7]; [Lys273ArgfsTer7]			Birth	Sever hypospadias, bifid scrotum, palpable gonads	Yes		Simard et al. (1994)
IX.1	Afghan	p.[Lys273ArgfsTer7]; [Lys273ArgfsTer7]	Yes		Birth	Severe hypospadias, bifid scrotum	Yes		Simard et al. (1994)
X.1	Brazilian	p.[Ala82Thr]; [Ala82Thr]			Birth	Ambiguous genitalia	Compensated salt loss (elevated renin activity)	Assigned female. Gonadectomized	Mendonça et al. (1994), Moisan et al. (1999)
X.2	Brazilian	p.[Ala82Thr]; [Ala82Thr]			Birth	Perineal hypospadias, bifid scrotum	Compensated salt loss (elevated renin activity)	Assigned female. Spontaneous typically male puberty at 14 years. No gynecomastia. Reassigned male at 17 years	Mendonça et al. (1994), Moisan et al. (1999)

XI.1	Spanish/ Portuguese	p.[Leu108Trp]; [Pro186Leu]	Birth	Perineal hypospadias, bifid scrotum	Yes		De Peretti, Forest, Feit, and David (1980), Sanchez, Mébarki, et al. (1994), Sanchez, Rhéaume, et al. (1994), Moisan et al. (1999)
XII.1	American	c.[385G>A]; [308-6G>A] p.[Gly129Arg]	6 years	Premature pubarche. History of multiple surgeries for perineal hypospadias and bilateral cryptorchidism	No	Spontaneous puberty at 12 years	Pang et al. (1983), Chang, Kulin, et al. (1993), Rhéaume et al. (1994), Moisan et al. (1999)
XIII.1	Scottish	p.[Leu173Arg]; [Leu173Arg]	5 years	Ambiguous genitalia	No	Assigned female at birth. Gonadectomized	Russell et al. (1994), Moisan et al. (1999)
XIV.1	Algerian	p.[Gly15Asp]; [Gly15Asp]	Birth	Perineoscrotal hypospadias, bifid scrotum	Yes		Gendrel, Chaussain, Roger, and Job (1979), Rhéaume et al. (1995), Moisan et al. (1999)
XV.1	French, English	p.[Asn100Ser]; [Asn100Ser]	20 months	Perineal hypospadias, palpable testes	Compensated salt loss (elevated renin activity)	Assigned female. Gonadectomized	De Peretti et al. (1980), Mébarki et al. (1995), Moisan et al. (1999)

Continued

Table 2 Ethnic Origin, Genotype, and Clinical Findings of 46,XY Described Patients—cont'd

Families/ Patients	Population	Genotype	Dead Relatives	Newborn Screening (17OHP4)	At Diagnosis: Chronological Age	Clinical Phenotype	Salt Wasting	Long-Term Follow-up	References
XVI.1	Japanese	p.[Leu205Pro]; [Leu205Pro]			3 months	Hyperpigmentation, severe hypospadias, bifid scrotum	Yes		Katsumata et al. (1995), Moisan et al. (1999)
XVII.1	Japanese	p.[Thr259Arg]; [Thr259Arg]			N/A	Pigmentation, hypospadias, palpable testes in bifid scrotum	Yes		Tajima et al. (1995), Moisan et al. (1999)
XVIII.1	Japanese	p.[Arg249Ter]; [Arg249Ter]			N/A	Pigmentation, hypospadias, bifid scrotum	Yes		Tajima et al. (1995)
XIX.1	Japanese	p.[Arg249Ter]; [Arg249Ter]			N/A	Pigmentation, severe undervirilization	Yes		Tajima et al. (1995)
XX.1	Japanese	p.[Tyr308Ter]; [Tyr308Ter]	Yes		N/A	Pigmentation, perineal hypospadias, bifid scrotum	Yes		Tajima et al. (1995)
XXI.1	French	p.[Pro155Leu]; [Gly294Val]			Birth	Perineal hypospadias, palpable testes in scrotum	No		Moisan et al. (1999), Gendrel et al. (1979)
XXI.2	French	p.[Pro155Leu]; [Gly294Val]			Birth	Perineal hypospadias, scrotal testes	No		Moisan et al. (1999), Gendrel et al. (1979)

XXII.1	Egyptian	p.[Ala10Val];[Ala10Val]	6 months	Perineoscrotal hypospadias, scrotal testes	No		Moisan et al. (1999)
XXII.2	Egyptian	p.[Ala10Val];[Ala10Val]	4 months	Perineoscrotal hypospadias, micropenis, scrotal testes	No		Moisan et al. (1999)
XXIII.1	Algerian	p.[Pro222Gln]; [Pro222Gln]	Birth	Perineal hypospadias, micropenis, scrotal testes	Yes		Moisan et al. (1999)
XXIV.1	French, American	p.[Leu236Ser]; [Met290CysfsTer10]	Birth	Perineal hypospadias with micropenis, no palpable testis	No		Moisan et al. (1999)
XXV.1	French	p.[Tyr259Met]; [Met290CysfsTer10]	Birth	Perineal hypospadias, scrotal testes	Yes		Moisan et al. (1999)
XXVI.1	Sri-Lankan	p.[Trp230_Ala238del]; [Trp230_Ala238del]	Birth	Perineal hypospadias with micropenis, scrotal testes	Yes		Moisan et al. (1999)
XXVII.1	Sri-Lankan	p.[Trp230_Ala238del]; [Trp230_Ala238del]	Birth	Perineal hypospadias	Yes		Moisan et al. (1999)
XXVIII.1	English	p.[Asn266ThrfsTer6]; [Asn100Ser]	11 years	Perineal hypospadias, micropenis with normal testes	No	Premature pubarche (7 years)	McCartin et al. (2000)
XXVIII.2	English	p.[Asn266ThrfsTer6]; [Asn100Ser]	9 years	Perineal hypospadias with normal testes	Compensated salt loss (elevated renin activity)		McCartin et al. (2000)

Continued

Table 2 Ethnic Origin, Genotype, and Clinical Findings of 46,XY Described Patients—cont'd

			At Diagnosis					
Families/ Patients	**Population**	**Genotype**	**Dead Relatives**	**Newborn Screening (17OHP4)**	**Chronological Age**	**Clinical Phenotype**	**Salt Wasting**	**Long-Term Follow-up**	**References**
XXIX.1	ND	p.[Ala82Thr]; [Ala82Thr]	Yes		10 months	Perineal hypospadias, inguinal testes	Compensated salt loss (elevated renin activity)		McCartin et al. (2000)
XXX.1	Pakistani	p.[Leu6Phe];[Leu6Phe]			Birth	Hyperpigmented scrotum, severe hypospadias, scrotal gonads	No		Zhang et al. (2000)
XXXI.1	Taiwanese	p.[Thr259Met]; [Thr259Met]			Birth	Perineal hypospadias, chordee	Yes	Assigned female. Gonadectomized	Zhang et al. (2000), Moisan et al. (1999)
XXXII.1	French-Canadian	p.[Ala10Glu]; [Ala10Glu]			13 days	Perineal hypospadias with micropenis, palpable testes in bifid scrotum	Yes	Spontaneous puberty at 10.5 years. No gynecomastia. Testicular adrenal rest. Azoospermia at 18.5 years	Alos et al. (2000)
XXXIII.1	ND	p.[Ser284Arg];ND[a]			7 years	Midshaft hypospadias	No		Codner et al. (2004)
XXXIV.1	ND	p.[Ser213Thr];ND[a]			12.9 years	Scrotal hypospadias, bilateral cryptorchidism, chordee, precocious pubarche	No		Codner et al. (2004)

XXXV.1	Brazilian	p.[Pro222Gln]; [Pro222Gln]		Birth	Perineal hypospadias, palpable testes in bifid scrotum	Yes	Mermejo et al. (2005)
XXXVI.1	Lebanese	p.[Pro341Leu]; [Pro341Leu]		Birth	Micropenis, broad urogenital sinus, palpable gonads	Yes	Welzel et al. (2008)
XXXVII.1	Turkish	p.[Arg335Ter]; [Arg335Ter]	Elevated	Birth	Micropenis, scrotal hypospadias, a broad urogenital sinus, palpable testes	Yes	Welzel et al. (2008)
XXXVII.2	Turkish	p.[Arg335Ter]; [Arg335Ter]	Elevated	Birth	Micropenis, penoscrotal hypospadias, maldescended testes. Incomplete cleft lip	Yes	Welzel et al. (2008)
XXXVIII.1	Bangladeshi	p.[Trp355Ter]; [Trp355Ter]		Birth	Micropenis, perineal hypospadias, bifid scrotum, palpable testes	Yes	Welzel et al. (2008)
XXXIX.1	Caucasian	p.[Pro222Gln]; [Pro222Gln]		2 months	Normal male, unilateral cryptorchidism	Yes	Lusa et al. (2010)
XL.1	Iranian	p.[Ala82Pro]; [Ala82Pro]		20 days	Mild hyperpigmentation, micropenis with chordee, bifid scrotum, scrotal testis, perineal hypospadias	Yes	Rabbani, Mahdieh, Haghi Ashtiani, Setoodeh, and Rabbani (2012)

Continued

Table 2 Ethnic Origin, Genotype, and Clinical Findings of 46,XY Described Patients—cont'd

				At Diagnosis					
Families/ Patients	**Population**	**Genotype**	**Dead Relatives**	**Newborn Screening (17OHP4)**	**Chronological Age**	**Clinical Phenotype**	**Salt Wasting**	**Long-Term Follow-up**	**References**
XLI.1	Brazilian	p.[Pro222Gln]; [Pro222Gln]		Elevated	3 months	2.5 cm phallus, penoscrotal proximal hypospadias, incompletely fused labioscrotal folds, bilaterally palpable gonads	Yes		de Araújo, de Oliveira, Gameleira, Cruz, and Lofrano-Porto (2014)
XLII.1	Sri-Lankan	p.[Trp230_Ala238del]; [Trp230_Ala238del]			Birth	Severe hypospadias, cryptorchidism, undervirilization. Perinatal asphyxia	Yes	Severe cerebral palsy. pH 3 at 6.3 years. Spontaneous puberty. Gynecomastia. Testicular biopsy at 15.5 years. Few germ cells with spermatogenic arrest, no evidence of malignancy	Burckhardt et al. (2015)

XLIII.1-6	Old Order Amish of North America	p.[Gly12Glu]; [Gly12Glu]	Variable	Variable	Hypospadias	Yes	Postnatal virilization (premature pubarche). Gynecomastia. Testicular adrenal rests in 2	Benkert et al. (2015)
XLIV.1	Italian	p.[Val319AlafsTer49]; [Val319AlafsTer49]		Birth	Perineal hypospadias, palpable testes within the labial scrotal folds and a micropenis.	Yes		Bizzarri et al. (2016)
XLV.1	Spanish	p.[Ala82Thr]; [Tyr339Cys]	Elevated	15 days	Female assigned at birth. Mild clitoromegaly, urogenital sinus	No		Bahíllo-Curieses, Loidi Fernández de Trocóniz, del Cañizo López, and Martínez-Sopena (2016)
XLVI.1	Caucasian	UPD Crh1 p.[Glu142Lys]; [Glu142Lys]	Bordeline elevation	7 days	Perineal hypospadias, bifid scrotum, penile chordee, scrotal testes. Hypotonia, nonspecific dysmorphic facial findings	Yes		Panzer, Ekhaguere, Darbro, Cook, and Shchelochkov (2017)

[a]Heterozygous reported case, see text for details.
Families are designated with roman numbers and siblings are designated as II.1, II.2.

Table 3 Ethnic Origin, Genotype, and Clinical Findings of 46,XX Described Patients

Families/ Patients	Population	Genotype	Dead Relatives	Newborn Screening (17OHP4)	Chronological Age	Clinical Phenotype	Salt Wasting	Long-Term Follow-up	References
XLVII.1	Swiss	p.[Trp171Ter]; [Trp171Ter]	Yes		Birth	No signs of virilization. Skin pigmentation	Yes	No spontaneous breast development at 14.7 years	Zachmann, Vollmin, Murset, Curtius, and Prader (1970), Zachmann, Forest, and De Peretti (1979), Rhéaume et al. (1992)
XLVIII.1	Swiss	p.[Trp171Ter]; [Trp171Ter]			Unknown	No signs of virilization	Yes		Rhéaume et al. (1992)
X.3	Brazilian	p.[Ala82Thr]; [Ala82Thr]			31 years	Clinically normal	No	Spontaneous puberty and menarche, regular menses	Mendonça et al. (1994), Moisan et al. (1999)
XII.2	American	c[385G > A]; [308-6G > A] p.[Gly129Arg]			8 years	Normal genitalia at birth. Postnatal virilization: Premature pubarche (4 years), acne, mild clitoromegaly (8 years)	No	Spontaneous puberty and menarche, Irregular menses	Pang et al. (1983), Chang, Kulin, et al. (1993), Rhéaume et al. (1994)

XLIX.1	Brazilian	p.[Ala82Thr]; [Ala82Thr]	5 years	Premature pubarche	No		Mendonça et al. (1994), Moisan et al. (1999)
L.1	Brazilian	p.[Thr259Met]; [Thr259Met]	41 years	Incidentally discovered bilateral accessory adrenal tissue in the ovaries and in the paraaortic region. Clitoromegaly, severe virilization	No		Paula et al. (1994), Moisan et al. (1999)
XIII.2	Scottish	p.[Leu173Arg]; [Leu173Arg]	2 years	Clinically normal	No		Russell et al. (1994), Moisan et al. (1999)
LI.1	American	p.[Tyr254Asp];ND[a]	17 years	Primary amenorrhea, slight clitoromegaly, moderate hirsutism, enlarged ovaries. Hyperpigmented skin	Compensated salt loss (elevated renin activity)	Spontaneous puberty at 11 years	Rosenfield et al. (1980), Sanchez, Mébarki, et al. (1994a), Sanchez, Rhéaume, et al. (1994b), Moisan et al. (1999)
XVI.2	Japanese	p.[Leu205Pro]; [Leu205Pro]	11 months	Hyperpigmentation, mild clitoromegaly	Yes		Katsumata et al. (1995), Moisan et al. (1999)
XVII.2	Japanese	p.[Thr259Arg]; [Thr259Arg]	2 weeks	Normal genitalia with severe pigmentation	Yes		Tajima et al. (1995), Moisan et al. (1999)

Continued

Table 3 Ethnic Origin, Genotype, and Clinical Findings of 46,XX Described Patients—cont'd

Families/ Patients	Population	Genotype	Dead Relatives	Newborn Screening (17OHP4)	Chronological Age	Clinical Phenotype	Salt Wasting	Long-Term Follow-up	References
LII.1	Pakistani	p.[Lys273ArgfsTer7]; [Thr318LysfsTer50]			Birth	Pigmentation, mildly enlarged clitoris	Yes	Spontaneous puberty, menarche (13 years), secondary amenorrhea, acne and hirsutism	Zhang, Sakkal-Alkaddour, Chang, Yang, and Pang (1996) Pang et al. (2002)
LIII.1	Chilean	p.[Glu135Ter]; [Glu135Ter]			20 months	Normal, hyper pigmented external genitalia	Yes		Marui et al. (1998)
XXV.2	French	p.[Tyr259Met]; [Met290CysfsTer10]			Birth	Normal genitalia	Yes		Moisan et al. (1999)
LIV.1	American	p.[Ser213Gly]; [Lys216Glu]			7 years	Premature pubarche at 4 years, growth acceleration	No		Moisan et al. (1999)
LV.1	Brazilian	p.[Pro222His]; [Gly129Arg]			7 years	Normal genitalia, premature pubarche	No		Marui et al. (2000), Moisan et al. (1999)
LV.2	Brazilian	p.[Pro222His]; [Gly129Arg]			6.7 years	Normal genitalia, premature pubarche	No		Marui et al. (2000), Moisan et al. (1999)
LVI.1	Brazilian	p.[Thr259Met]; [Thr259Met]			7.8 years	Clitoromegaly, precocious pubarche	No		Marui et al. (2000), Moisan et al. (1999)

XXIII.2	Algerian	p.[Pro222Gln]; [Pro222Gln]		1 month	Mild clitoromegaly	Yes		Moisan et al. (1999)
LVII.1	French Canadian	p.[Ala10Glu]; [Ala10Glu]		3 weeks	Normal external genitalia. Enlarged adrenals	Yes	Premature pubarche at 4 years. Spontaneous puberty (8 years) and menarche (10.5 years). Multicystic enlarged ovaries	Alos et al. (2000)
LVIII.1	Eastern European	p.[Pro222Thr]; [Pro222Thr]		4 weeks	Normal external genitalia	Yes		Pang et al. (2002)
LIX.1	Caucasian	p.[Glu142Lys]; [Ter373Cys]		7 years	Normal external genitalia	No	Premature pubarche (5.5 years). Increased somatic growth and acne (7.3 years). Telarche (9 years)	Pang et al. (2002)
LX.1	Caucasian	p.[Lys368SerfsTer129]; [Thr181Ile]	Elevated	7.6 years	Precocious pubarche	Yes	Spontaneous puberty (10.1 years)	Johannsen et al. (2005)
LX.2	Caucasian	p.[Lys368SerfsTer129]; [Thr181Ile]	Elevated	3.7 years	Sister	Yes		Johannsen et al. (2005)

Continued

Table 3 Ethnic Origin, Genotype, and Clinical Findings of 46,XX Described Patients—cont'd

Families/ Patients	Population	Genotype	Dead Relatives	Newborn Screening (17OHP4)	Chronological Age	Clinical Phenotype	Salt Wasting	Long-Term Follow-up	References
LXI.1	Russian	p.[Ala82Asp]; [Trp230Ter]		Elevated	Birth	Neonatal screening	Yes		Nordenström, Forest, and Wedell (2007)
LXII.1	American/ Salvadoran	p.[Gln334Ter]; [Gln334Ter]		Elevated	8 days	Normal external genitalia. Skin pigmentation	Yes		Jeandron and Sahakitrungruang (2012)
LXII.2	Salvadoran	p.[Gln334Ter]; [Gln334Ter]			28 days	Normal external genitalia	Yes		Jeandron and Sahakitrungruang (2012)
LXIII.1	Japanese	p.[Tyr190Cys]; [Ser218Pro]			Birth	Mild virilization with clitoromegaly. Skin pigmentation	Compensated salt loss (elevated renin activity)		Takasawa et al. (2014)
LXIV.1	Chinese	p.[Glu25Ter]; [Glu25Ter]			1 month	Skin pigmentation. Clitoromegaly	Yes	Spontaneous puberty (9 years) and menarche (12 years). Oligomenorrhea. Postmenarcheal recurrent ovarian cysts	Huang et al. (2014)

LXV.1	ND (adopted)	p.[Gly250Val]; [Gly250Val]		7 months	Precocious pubarche PH II–III and postnatal clitoromegaly. Two orifices, and nonpalpable gonads	Compensated salt loss (elevated renin activity)		Baquedano et al. (2015)
XLIII.7–16	Old Order Amish of North America	p.[Gly12Glu]; [Gly12Glu]	Variable	Variable	Normal female external genitalia. No ambiguous genitalia	Yes	Premature pubarche. Two adult females developed polycystic ovaries	Benkert et al. (2015)
LXVI.1	Switzerland	p.[Trp171Ter]; [Ala168Valfs]	Elevated	8 days	Normal female external genitalia without virilization or palpable gonads	Yes		Probst-Scheidegger, Udhane, l'Allemand, Flück, and Camats (2016)

[a]Heterozygous reported case, see text for details.
Families are designated with roman numbers and siblings are designated as II.1, II.2.

rapid somatic growth, and advance skeletal maturation) (Baquedano et al., 2015; Codner et al., 2004; Mendonça et al., 1994; Pang et al., 2002; Rhéaume et al., 1994). Signs of androgen excess worsen with age, probably due to an increase in DHEA production with the development of the ZR in the adrenal cortex.

Puberty in affected boys showed a broad clinical heterogeneity, but spontaneous pubertal development, with or without enlarged testes and gynecomastia, has been reported even in the presence of a severe salt-wasting form (Alos et al., 2000; Mendonça et al., 1994; Parks, Bermudez, Anast, Bongiovanni, & New, 1971; Rhéaume et al., 1992). Indeed, an affected male with proven severe 3βHSD2 deficiency has fathered children (Rhéaume et al., 1992). Testicular adrenal rest tumors (TART) were also reported in male affected individuals (Benkert et al., 2015).

In affected girls, ovarian estrogen synthesis is compromised and the development of secondary sexual characteristics is impaired (Auchus & Chang, 2010). However, spontaneous pubertal development and menarche have been reported in a few of the affected females with severe mutation in the *HSD3B2* gene without any residual enzyme activity (Alos et al., 2000; Huang et al., 2014; Moisan et al., 1999). Hirsutism and menstrual disorders were described in older affected females (Rosenfield et al., 1980).

Taking together the findings observed in affected boys and girls suggest that Δ^4 steroids could originate from gonadal 3βHSD1 activity, because even though only very low levels of 3βHSD1 mRNA could be detected in normal gonads, this enzyme possesses a 5- to 10-fold higher activity than the type 2 isoenzyme and could be stimulated by the increased gonadotropin secretion that results from low sex steroid levels.

A nonclassic or late-onset clinical form was described in girls presenting with precocious pubarche and a PCO-like syndrome (Marui et al., 2000; Pang et al., 1985). The genetic basis for this clinical form has not been properly described, and use of this terminology and diagnosis is discouraged, and should be reserved only for patients with markedly elevated ratios of Δ^5 steroid/Δ^4 steroids and confirmatory molecular studies.

The biochemical marker of this steroidogenic disorder is the increased serum level of Δ^5 steroids, especially 17-hydroxypregnenolone, DHEA, and DHEAS. Peripheral conversion of these Δ^5 precursors by 3βHSD1 isoenzyme leads to an increase in serum levels of 17OHP and A4. Even though androgen excess is less severe than in other forms of CAH, especially than 21α-hydroxylase deficiency, the increase in 17OHP might lead to misinterpretations of neonatal screening results. An increase in precursor/product ratio

(Δ^5/Δ^4) more than 10 SDS above the normal reference value is very useful in the diagnosis of this enzymatic disorder (17-hydroxypregnenolone/17-hydroxyprogesterone, DHEA/androstenedione, pregnenolone/progesterone) (Auchus & Chang, 2010; Auchus & Miller, 2012; Lutfallah et al., 2002). However, confirmatory molecular studies are needed.

Initial management and long-term follow-up of the affected patients, as in other DSD conditions, require the participation of a multidisciplinary team with experience in the field.

Initial treatment is directed to restore fluid and electrolyte balance. Affected infants should receive glucocorticoid, mineralocorticoid, and salt supplementation as in 21α-hydroxylase deficiency. Usually lower hydrocortisone doses are needed in this period, as androgen excess is easier to control. With the development of the ZR, androgen excess becomes more problematic and glucocorticoid and mineralocorticoid treatment should continue. T and DHEAS are used to monitor treatment.

In 3βHSD2 deficiency the variable impairment in gonadal steroidogenesis could lead to primary hypogonadism that would need sex steroid replacement therapies to develop and sustain secondary sexual characteristics.

Testicular ultrasound should be considered to detect the presence of TART (Benkert et al., 2015).

2.3 Genotype–Phenotype Relationships. Structure–Function Relationships

Mutations in the *HSD3B2* gene result in a wide spectrum of molecular repercussions, which are associated with the different phenotypic manifestations of classical 3βHSD2 deficiency. In almost all the reported cases, the severity of salt wasting usually showed good correlation with the functional consequences of *HSD3B2* mutations as assayed in in vitro cell systems (Baquedano et al., 2015; Moisan et al., 1999; Simard, Moisan, & Morel, 2002; Simard et al., 2005; Tables 1–3). Evidences showed that it is more appropriate to assess the enzymatic activity of transiently expressed mutant proteins using intact cells rather than homogenates from cells, because addition of exogenous cofactor can drive a reaction that may not occur in vivo (Moisan et al., 1999). However, the relationship between the genotype and the gonadal phenotype in severe 3βHSD2 deficiency is more complex and fertility is difficult to predict (Mendonca et al., 1987; Mendonça et al., 1994; Rhéaume et al., 1992; Zachmann, Forest, & De Peretti, 1979).

As previously mentioned, *HSD3B2* gene defects appeared to follow an autosomal recessive mode of inheritance, as with other steroidogenic

enzyme defects. However, heterozygous *HSD3B2* gene mutations in a female patient with nonsalt-losing 3βHSD deficiency diagnose at puberty (Sanchez, Rhéaume, et al., 1994) and in two male patients with apparent idiopathic hypospadias without clear steroidogenic pattern of 3βHSD deficiency (Codner et al., 2004) have been reported. In vitro experiments argued for its functional impact (Codner et al., 2004; Sanchez, Rhéaume, et al., 1994) and dominant-negative properties (Codner et al., 2004). Nevertheless, the absence of symptoms in the heterozygous patients' relatives argues against such a dominant effect. Noteworthy, a putative second mutation located in the promoter or within intronic regions potentially affecting the normal expression of this gene or generating an alternative aberrant splicing site cannot be refuted. Associated genetic or environmental/hormonal influences that may contribute to the clinical phenotype should be ruled out in those patients.

The amino acid residues that are the sites of missense mutations are generally in highly conserved regions in members of the vertebrate 3βHSD isoenzymes, suggesting the crucial role of these residues for the activity and/stability of the enzyme. Because the crystal structure of 3βHSD has not been determined, several 3D model structures of the 3βHSD2 were generated by the comparative modeling technique to study the potential impact of mutations on the 3βHSD2 structure (Baquedano et al., 2015; Rabbani, Mahdieh, Haghi Ashtiani, Setoodeh, & Rabbani, 2012). Mutations that impair steroid binding, nicotinamide cofactor binding, and enzyme stability have all been described (Fig. 3; Table 1), and in many instances, the enzymatic defect is complex and multifactorial.

3. CONCLUDING REMARKS

3βHSD2 is essential for the biosynthesis of all classes of active steroid hormones, including aldosterone, cortisol in the adrenal cortex, and sex steroids in the adrenals and gonads. In the classical steroidogenic pathway, known as front door steroidogenic pathway, the Δ^5 steroids represent the preferred route for C19 steroid production in humans. In the last decade, an alternative, so-called backdoor pathway to androgen synthesis has been described. 17OHP is the substrate from which can be generated DHT without the intermediacy of DHEA, A4, or T. It has been proposed that this pathway would contribute to the androgen production in pathological states in which 17OHP accumulates, including 21α-hydroxylase and POR deficiencies. Furthermore, the expression of the backdoor pathway in the fetal testis appears to play an important role during male sexual differentiation.

Recent studies have demonstrated that human steroidogenic tissues also produce a unique set of 11-oxygenated C19 steroids. 11-Oxygenated C19 steroid and its peripheral 5α-reduced metabolites are potent agonists of the human AR with affinities and potencies similar to that of T and DHT. Androgen production in the adrenal cortex is zonally and developmentally regulated. The 3βHSD2-deficient ZR is indistinct during infancy, but a continuous layer of reticularis cells starts to develop and thicken around 4–5 years of age. This process, known as adrenarche, is followed by a rise in circulating concentrations of DHEAS, with clinical signs physiologically observed between the ages of 6 and 10. In the testes the synthesis of T by Leydig cells follows a pathway that is similar to C19-steroid production in the adrenal ZR. Leydig cells express abundant 3βHSD2 and 17βHSD3, but no SULT2A1 is required to produce sulfo conjugate compounds. Thus, DHEA produced in the testis is not sulfurylated but is readily converted to A4 and/or androstenediol and then T via 3βHSD2 and 17βHSD3. In the ovary, small amounts of A4 are secreted or converted to T in Teca cells, but most A4 is converted to estradiol by granulosa cells, under the influence of FSH.

Recessive loss-of-function HSD3B2 mutations cause a rare form of CAH that impairs both adrenal and gonadal steroidogenesis. Deficiency of 3βHSD2 activity in the adrenals precludes normal aldosterone and cortisol synthesis and the flooding of cortisol precursors along the Δ^5 pathway with a marked rise in pregnenolone, 17-hydroxypregnenolone, DHEA, DHEAS, and androstenediol production which represent serum biomarkers for suspecting the diagnosis of 3βHSD2 deficiency. In gonads, deficiency of 3βHSD2 activity precludes normal T and estrogen synthesis. The blockage caused by 3βHSD2 deficiency precludes flux to the backdoor pathway and 11-oxygenated C19 steroid synthesis due to very low intraadrenal 17OHP and A4 substrate levels, respectively. Peripheral 3βHSD1 conversion of Δ5 steroids to Δ4 steroids can result in high serum 17OHP and A4.

The clinical phenotype in the affected patients is significantly heterogeneous with different degrees of genital ambiguity and adrenal failure in both sexes. Clinical symptoms of adrenal failure could be present early after birth. Mineralocorticoid deficiency with salt wasting, hypotension, and hyperkalemia is observed in 60%–70% of the reported cases. No functional 3βHSD2 isoenzyme is expressed in the adrenals and gonads of these patients. The nonsalt-wasting form of 3βHSD2 deficiency results from variations in the HSD3B2 gene causing an incomplete loss of enzymatic activity. Testicular steroidogenic defect leads to different degrees of under virilization.

In 46,XX affected patients, mild genital virilization with clitoromegaly with or without partial labial fusion and even normal female genitalia has been reported. The absence of adrenal 3βHSD activity precludes elevation of intraadrenal 17OHP and the production of backdoor-derived DHT and 11-oxygenated androgens, which would be the major source of virilizing androgens in other forms of CAH.

Finally, in humans the 3βHSD2 deficiency represents a model of nature that reinforces our knowledge about the role of the steroidogenic alternative pathway in sex differentiation in both sexes. In addition, the physiological role of the high serum DHEAS levels in fetal life as well as after adrenarche remains to be elucidated.

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All authors have nothing to declare.

REFERENCES

Alos, N., Moisan, A. M., Ward, L., Desrochers, M., Legault, L., Leboeuf, G., et al. (2000). A novel A10E homozygous mutation in the HSD3B2 gene causing severe salt-wasting 3beta-hydroxysteroid dehydrogenase deficiency in 46,XX and 46,XY French-Canadians: Evaluation of gonadal function after puberty. *The Journal of Clinical Endocrinology and Metabolism*, *85*(5), 1968–1974. https://doi.org/10.1210/jcem.85.5.6581.

Asby, D. J., Arlt, W., & Hanley, N. A. (2009). The adrenal cortex and sexual differentiation during early human development. *Reviews in Endocrine & Metabolic Disorders*, *10*(1 spec. issue), 43–49. https://doi.org/10.1007/s11154-008-9098-9.

Auchus, R. J. (2004). The backdoor pathway to dihydrotestosterone. *Trends in Endocrinology and Metabolism: TEM*, *15*(9), 432–438. https://doi.org/10.1016/j.tem.2004.09.004.

Auchus, R. J. (2011). The physiology and biochemistry of adrenarche. *Endocrine Development*, *20*, 20–27. https://doi.org/10.1159/000321209.

Auchus, R. J., & Chang, A. Y. (2010). 46,XX DSD: The masculinised female. *Best Practice & Research Clinical Endocrinology & Metabolism*, *24*(2), 219–242. https://doi.org/10.1016/j.beem.2009.11.001.

Auchus, R. J., Lee, T. C., & Miller, W. L. (1998). Cytochrome b5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *The Journal of Biological Chemistry*, *273*(6), 3158–3165. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9452426.

Auchus, R., & Miller, W. (2012). Defects in androgen biosynthesis causing 46,XY disorders of sexual development. *Seminars in Reproductive Medicine*, *30*(5), 417–426. https://doi.org/10.1055/s-0032-1324726.

Bahíllo-Curieses, M. P., Loidi Fernández de Trocóniz, L., del Cañizo López, A., & Martínez-Sopena, M. J. (2016). Partial 3ß-hydroxysteroid dehydrogenase type 2 deficiency:

Diagnosis of a novel mutation after positive newborn screening for 21-hydroxylase deficiency. *Medicina Clínica*, *146*(2), 92–93. https://doi.org/10.1016/j.medcli.2015.04.004.

Baquedano, M. S., Ciaccio, M., Marino, R., Perez Garrido, N., Ramirez, P., Maceiras, M., et al. (2015). A novel missense mutation in the *HSD3B2* gene, underlying nonsalt-wasting congenital adrenal hyperplasia. New insight into the structure-function relationships of 3β-hydroxysteroid dehydrogenase type II. *The Journal of Clinical Endocrinology & Metabolism*, *100*(1), E191–E196. https://doi.org/10.1210/jc.2014-2676.

Baquedano, M. S., Madjinca, S., Aliberti, P., Saraco, N. I., Warman, M., Rivarola, M. A., et al. (2014). Expression of androgen receptor (AR) and the alternative "Backdoor" pathway to dihydrotestosterone (DHT) in normal and 21-hydroxylase deficient human adrenal cortex. Abstract SAT-0753. *Endocrine Reviews*, *35*(3 Suppl), E320.

Belgorosky, A., Baquedano, M. S., Guercio, G., & Rivarola, M. A. (2008). Adrenarche: Postnatal adrenal zonation and hormonal and metabolic regulation. *Hormone Research*, *70*(5), 257–267.

Benkert, A. R., Young, M., Robinson, D., Hendrickson, C., Lee, P. A., & Strauss, K. A. (2015). Severe salt-losing 3β-hydroxysteroid dehydrogenase deficiency: Treatment and outcomes of *HSD3B2* c.35G > A homozygotes. *The Journal of Clinical Endocrinology & Metabolism*, *100*(8), E1105–E1115. https://doi.org/10.1210/jc.2015-2098.

Bizzarri, C., Massimi, A., Federici, L., Cualbu, A., Loche, S., Bellincampi, L., et al. (2016). A new homozygous frameshift mutation in the HSD3B2 gene in an apparently non-consanguineous Italian family. *Hormone Research in Pædiatrics*, *86*(1), 53–61. https://doi.org/10.1159/000444712.

Black, S. M., Harikrishna, J. A., Szklarz, G. D., & Miller, W. L. (1994). The mitochondrial environment is required for activity of the cholesterol side-chain cleavage enzyme, cytochrome P450scc. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(15), 7247–7251. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8041774.

Burckhardt, M.-A., Udhane, S. S., Marti, N., Schnyder, I., Tapia, C., Nielsen, J. E., et al. (2015). Human 3β-hydroxysteroid dehydrogenase deficiency seems to affect fertility but may not harbor a tumor risk: Lesson from an experiment of nature. *European Journal of Endocrinology*, *173*(5), K1–K12. https://doi.org/10.1530/EJE-15-0599.

Casey, M. L., & MacDonald, P. C. (1982). Extraadrenal formation of a mineralocorticosteroid: Deoxycorticosterone and deoxycorticosterone sulfate biosynthesis and metabolism. *Endocrine Reviews*, *3*(4), 396–403. https://doi.org/10.1210/edrv-3-4-396.

Chang, Y. T., Kappy, M. S., Iwamoto, K., Wang, J., Yang, X., & Pang, S. (1993). Mutations in the type II 3 beta-hydroxysteroid dehydrogenase gene in a patient with classic salt-wasting 3 beta-hydroxysteroid dehydrogenase deficiency congenital adrenal hyperplasia. *Pediatric Research*, *34*(5), 698–700. https://doi.org/10.1203/00006450-199311000-00026.

Chang, Y. T., Kulin, H. E., Garibaldi, L., Suriano, M. J., Bracki, K., & Pang, S. (1993). Hypothalamic-pituitary-gonadal axis function in pubertal male and female siblings with glucocorticoid-treated nonsalt-wasting 3 beta-hydroxysteroid dehydrogenase deficiency congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology and Metabolism*, *77*(5), 1251–1257. https://doi.org/10.1210/jcem.77.5.8077318.

Codner, E., Okuma, C., Iñiguez, G., Boric, M. A., Avila, A., Johnson, M. C., et al. (2004). Molecular study of the 3β-hydroxysteroid dehydrogenase gene type II in patients with hypospadias. *The Journal of Clinical Endocrinology & Metabolism*, *89*(2), 957–964. https://doi.org/10.1210/jc.2002-020873.

Dardis, A., Saraco, N., Rivarola, M. A., & Belgorosky, A. (1999). Decrease in the expression of the 3beta-hydroxysteroid dehydrogenase gene in human adrenal tissue during prepuberty and early puberty: Implications for the mechanism of adrenarche. *Pediatric Research*, *45*(3), 384–388. https://doi.org/10.1203/00006450-199903000-00016.

de Araújo, V. G. B., de Oliveira, R. S., Gameleira, K. P. D., Cruz, C. B., & Lofrano-Porto, A. (2014). 3β-Hydroxysteroid dehydrogenase type II deficiency on newborn screening test. *Arquivos Brasileiros de Endocrinologia e Metabologia*, *58*(6), 650–655. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25211449.

de Peretti, E., & Forest, M. G. (1976). Unconjugated dehydroepiandrosterone plasma levels in normal subjects from birth to adolescence in human: The use of a sensitive radioimmunoassay 1. *The Journal of Clinical Endocrinology & Metabolism*, *43*(5), 982–991. https://doi.org/10.1210/jcem-43-5-982.

De Peretti, E., Forest, M. G., Feit, J. P., & David, M. (1980). Endocrine studies in two children with male pseudohermapbroditism due to to S/3-hydroxysteroid dehydrogenase defect. In A. R. Genazzani, J. H. H. Thijssen, & P. K. Siiteri (Eds.), *Adrenal androgens* (pp. 141–145). New York: Raven Press.

den Dunnen, J. T., Dalgleish, R., Maglott, D. R., Hart, R. K., Greenblatt, M. S., McGowan-Jordan, J., et al. (2016). HGVS recommendations for the description of sequence variants: 2016 update. *Human Mutation*, *37*(6), 564–569.

Doi, M., Satoh, F., Maekawa, T., Nakamura, Y., Fustin, J.-M., Tainaka, M., et al. (2014). Isoform-specific monoclonal antibodies against 3β-hydroxysteroid dehydrogenase/isomerase family provide markers for subclassification of human primary aldosteronism. *The Journal of Clinical Endocrinology & Metabolism*, *99*(2), E257–E262. https://doi.org/10.1210/jc.2013-3279.

Else, T., & Hammer, G. D. (2005). Genetic analysis of adrenal absence: Agenesis and aplasia. *Trends in Endocrinology and Metabolism*, *16*(10), 458–468. https://doi.org/10.1016/j.tem.2005.10.004.

Endoh, A., Kristiansen, S. B., Casson, P. R., Buster, J. E., & Hornsby, P. J. (1996). The zona reticularis is the site of biosynthesis of dehydroepiandrosterone and dehydroepiandrosterone sulfate in the adult human adrenal cortex resulting from its low expression of 3 beta-hydroxysteroid dehydrogenase. *The Journal of Clinical Endocrinology & Metabolism*, *81*(10), 3558–3565. https://doi.org/10.1210/jcem.81.10.8855801.

Flück, C. E., Meyer-Böni, M., Pandey, A. V., Kempná, P., Miller, W. L., Schoenle, E. J., et al. (2011). Why boys will be boys: Two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *The American Journal of Human Genetics*, *89*(2), 201–218. https://doi.org/10.1016/j.ajhg.2011.06.009.

Gell, J. S., Carr, B. R., Sasano, H., Atkins, B., Margraf, L., Mason, J. I., et al. (1998). Adrenarche results from development of a 3beta-hydroxysteroid dehydrogenase-deficient adrenal reticularis. *The Journal of Clinical Endocrinology and Metabolism*, *83*(10), 3695–3701. https://doi.org/10.1210/jcem.83.10.5070.

Gendrel, D., Chaussain, J. L., Roger, M., & Job, J. C. (1979). Congenital adrenal hyperplasia due to blockade of 3-beta-hydroxysteroid dehydrogenase. *Archives Françaises de Pédiatrie*, *36*(7), 647–655. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/161161.

Ghayee, H. K., & Auchus, R. J. (2007). Basic concepts and recent developments in human steroid hormone biosynthesis. *Reviews in Endocrine & Metabolic Disorders*, *8*(4), 289–300. https://doi.org/10.1007/s11154-007-9052-2.

Goto, M., Piper Hanley, K., Marcos, J., Wood, P. J., Wright, S., Postle, A. D., et al. (2006). In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. *The Journal of Clinical Investigation*, *116*(4), 953–960. https://doi.org/10.1172/JCI25091.

Grumbach, M. M., & Auchus, R. J. (1999). Estrogen: Consequences and implications of human mutations in synthesis and action. *The Journal of Clinical Endocrinology and Metabolism*, *84*(12), 4677–4694. https://doi.org/10.1210/jcem.84.12.6290.

Gupta, M. K., Guryev, O. L., & Auchus, R. J. (2003). 5alpha-reduced C21 steroids are substrates for human cytochrome P450c17. *Archives of Biochemistry and Biophysics*, *418*(2), 151–160. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14522586.

Hanley, N. A., & Arlt, W. (2006). The human fetal adrenal cortex and the window of sexual differentiation. *Trends in Endocrinology and Metabolism: TEM*, *17*(10), 391–397. https://doi.org/10.1016/j.tem.2006.10.001.

Havelock, J. C., Auchus, R. J., & Rainey, W. E. (2004). The rise in adrenal androgen biosynthesis: Adrenarche. *Seminars in Reproductive Medicine*, *22*(4), 337–347. https://doi.org/10.1055/s-2004-861550.

Homma, K., Hasegawa, T., Nagai, T., Adachi, M., Horikawa, R., Fujiwara, I., et al. (2006). Urine steroid hormone profile analysis in cytochrome P450 oxidoreductase deficiency: Implication for the backdoor pathway to dihydrotestosterone. *The Journal of Clinical Endocrinology & Metabolism*, *91*(7), 2643–2649. https://doi.org/10.1210/jc.2005-2460.

Huang, Y., Zheng, J., Xie, T., Xiao, Q., Lu, S., Li, X., et al. (2014). A novel homozygous mutation p.E25X in the HSD3B2 gene causing salt wasting 3β-hydroxysteroid dehydrogenases deficiency in a Chinese pubertal girl: A delayed diagnosis until recurrent ovary cysts. *Zhonghua Er Ke Za Zhi = Chinese Journal of Pediatrics*, *52*(12), 948–951. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25619355.

Hui, X. G., Akahira, J. I., Suzuki, T., Nio, M., Nakamura, Y., Suzuki, H., et al. (2009). Development of the human adrenal zona reticularis: Morphometric and immunohistochemical studies from birth to adolescence. *Journal of Endocrinology*, *203*(2), 241–252. https://doi.org/10.1677/JOE-09-0127.

Ishimoto, H., & Jaffe, R. B. (2011). Development and function of the human fetal adrenal cortex: A key component in the feto-placental unit. *Endocrine Reviews*, *32*(3), 317–355. https://doi.org/10.1210/er.2010-0001.

Jeandron, D. D., & Sahakitrungruang, T. (2012). A novel homozygous Q334X mutation in the HSD3B2 gene causing classic 3β-hydroxysteroid dehydrogenase deficiency: An unexpected diagnosis after a positive newborn screen for 21-hydroxylase deficiency. *Hormone Research in Pædiatrics*, *77*(5), 334–338. https://doi.org/10.1159/000336004.

Johannsen, T. H., Mallet, D., Dige-Petersen, H., Müller, J., Main, K. M., Morel, Y., et al. (2005). Delayed diagnosis of congenital adrenal hyperplasia with salt wasting due to type II 3beta-hydroxysteroid dehydrogenase deficiency. *The Journal of Clinical Endocrinology and Metabolism*, *90*(4), 2076–2080. https://doi.org/10.1210/jc.2004-1374.

John, M. E., John, M. C., Boggaram, V., Simpson, E. R., & Waterman, M. R. (1986). Transcriptional regulation of steroid hydroxylase genes by corticotropin. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(13), 4715–4719. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3014507.

Jones, C. M., Mallappa, A., Reisch, N., Nikolaou, N., Krone, N., Hughes, B. A., et al. (2017). Modified release and conventional glucocorticoids and diurnal androgen excretion in congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology and Metabolism*, *102*, 1797–1806. https://doi.org/10.1210/jc.2016-2855.

Kamrath, C., Hochberg, Z., Hartmann, M. F., Remer, T., & Wudy, S. A. (2012). Increased activation of the alternative "backdoor" pathway in patients with 21-hydroxylase deficiency: Evidence from urinary steroid hormone analysis. *The Journal of Clinical Endocrinology & Metabolism*, *97*(3), E367–E375. https://doi.org/10.1210/jc.2011-1997.

Katsumata, N., Tanae, A., Yasunaga, T., Horikawa, R., Tanaka, T., & Hibi, I. (1995). A novel missense mutation in the type II 3 beta-hydroxysteroid dehydrogenase gene in a family with classical salt-wasting congenital adrenal hyperplasia due to 3 beta-hydroxysteroid dehydrogenase deficiency. *Human Molecular Genetics*, *4*(4), 745–746. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7633426.

Lee, T. C., Miller, W. L., & Auchus, R. J. (1999). Medroxyprogesterone acetate and dexamethasone are competitive inhibitors of different human steroidogenic enzymes. *The Journal of Clinical Endocrinology and Metabolism*, *84*(6), 2104–2110. https://doi.org/10.1210/jcem.84.6.5646.

Lin, D., Sugawara, T., Strauss, J. F., Clark, B. J., Stocco, D. M., Saenger, P., et al. (1995). Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science (New York, N.Y.)*, *267*(5205), 1828–1831. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7892608.

Lorence, M. C., Corbin, C. J., Kamimura, N., Mahendroo, M. S., & Mason, J. I. (1990). Structural analysis of the gene encoding human 3 beta-hydroxysteroid dehydrogenase/delta 5----4-isomerase. *Molecular Endocrinology*, *4*(12), 1850–1855. https://doi.org/10.1210/mend.15.1.0585.

Lusa, L. G., de Lemos-Marini, S. H. V., Soardi, F. C., Ferraz, L. F. C., Guerra-Júnior, G., & de Mello, M. P. (2010). Structural aspects of the p.P222Q homozygous mutation of HSD3B2 gene in a patient with congenital adrenal hyperplasia. *Arquivos Brasileiros de Endocrinologia e Metabologia*, *54*(8), 768–774. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21340167.

Lutfallah, C., Wang, W., Mason, J. I., Chang, Y. T., Haider, A., Rich, B., et al. (2002). Newly proposed hormonal criteria via genotypic proof for type II 3beta-hydroxysteroid dehydrogenase deficiency. *The Journal of Clinical Endocrinology and Metabolism*, *87*(6), 2611–2622. https://doi.org/10.1210/jcem.87.6.8615.

Luu, T. V., Lachance, Y., Labrie, C., Leblanc, G., Thomas, J. L., Strickler, R. C., et al. (1989). Full length cDNA structure and deduced amino acid sequence of human 3 beta-hydroxy-5-ene steroid dehydrogenase. *Molecular Endocrinology (Baltimore, Md.)*, *3*(8), 1310–1312. https://doi.org/10.1210/mend-3-8-1310.

Marti, N., Galván, J. A., Pandey, A. V., Trippel, M., Tapia, C., Müller, M., et al. (2017). Genes and proteins of the alternative steroid backdoor pathway for dihydrotestosterone synthesis are expressed in the human ovary and seem enhanced in the polycystic ovary syndrome. *Molecular and Cellular Endocrinology*, *441*, 116–123. https://doi.org/10.1016/j.mce.2016.07.029.

Marui, S., Castro, M., Latronico, A. C., Elias, L. L., Arnhold, I. J., Moreira, A. C., et al. (2000). Mutations in the type II 3beta-hydroxysteroid dehydrogenase (HSD3B2) gene can cause premature pubarche in girls. *Clinical Endocrinology*, *52*(1), 67–75. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10651755.

Marui, S., Torrealba, I. M., Russell, A. J., Latronico, A. C., Sutcliffe, R. G., & Mendonca, B. B. (1998). A novel homozygous nonsense mutations E135* in the type II 3beta-hydroxysteroid dehydrogenase gene in a girl with salt-losing congenital adrenal hyperplasia. Mutations in brief no. 168. Online. *Human Mutation*, *12*(2), 139. https://doi.org/10.1002/(SICI)1098-1004(1998)12:2<139::AID-HUMU19>3.0.CO;2-4.

McCartin, S., Russell, A. J., Fisher, R. A., Wallace, A. M., Arnhold, I. J., Mason, J. I., et al. (2000). Phenotypic variability and origins of mutations in the gene encoding 3beta-hydroxysteroid dehydrogenase type II. *Journal of Molecular Endocrinology*, *24*(1), 75–82. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10656999.

Mébarki, F., Sanchez, R., Rhéaume, E., Laflamme, N., Simard, J., Forest, M. G., et al. (1995). Nonsalt-losing male pseudohermaphroditism due to the novel homozygous N100S mutation in the type II 3 beta-hydroxysteroid dehydrogenase gene. *The Journal of Clinical Endocrinology & Metabolism*, *80*(7), 2127–2134. https://doi.org/10.1210/jcem.80.7.7608265.

Mendonca, B. B., Bloise, W., Arnhold, I. J., Batista, M. C., Toledo, S. P., Drummond, M. C., et al. (1987). Male pseudohermaphroditism due to nonsalt-losing 3 beta-hydroxysteroid dehydrogenase deficiency: Gender role change and absence of gynecomastia at puberty. *Journal of Steroid Biochemistry*, *28*(6), 669–675. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2826919.

Mendonça, B. B., Russell, A. J., Vasconcelos-Leite, M., Arnhold, I. J., Bloise, W., Wajchenberg, B. L., et al. (1994). Mutation in 3 beta-hydroxysteroid dehydrogenase type II associated with pseudohermaphroditism in males and premature pubarche or

cryptic expression in females. *Journal of Molecular Endocrinology*, *12*(1), 119–122. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8185809.

Mermejo, L. M., Elias, L. L. K., Marui, S., Moreira, A. C., Mendonca, B. B., & de Castro, M. (2005). Refining hormonal diagnosis of type II 3beta-hydroxysteroid dehydrogenase deficiency in patients with premature pubarche and hirsutism based on HSD3B2 genotyping. *The Journal of Clinical Endocrinology and Metabolism*, *90*(3), 1287–1293. https://doi.org/10.1210/jc.2004-1552.

Miller, W. L. (2005). Minireview: Regulation of steroidogenesis by electron transfer. *Endocrinology*, *146*(6), 2544–2550. https://doi.org/10.1210/en.2005-0096.

Miller, W. L., & Auchus, R. J. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine Reviews*, *32*(1), 81–151. https://doi.org/10.1210/er.2010-0013.

Moisan, A. M., Ricketts, M. L., Tardy, V., Desrochers, M., Mébarki, F., Chaussain, J.-L., et al. (1999). New insight into the molecular basis of 3β-hydroxysteroid dehydrogenase deficiency: Identification of eight mutations in the *HSD3B2* gene in eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. *The Journal of Clinical Endocrinology & Metabolism*, *84*(12), 4410–4425. https://doi.org/10.1210/jcem.84.12.6288.

Nakamura, Y., Fujishima, F., Hui, X., Felizola, S. J. A., Shibahara, Y., Akahira, J., et al. (2015). 3βHSD and CYB5A double positive adrenocortical cells during adrenal development/aging. *Endocrine Research*, *40*(1), 8–13. https://doi.org/10.3109/07435800.2014.895377.

Nakamura, Y., Gang, H. X., Suzuki, T., Sasano, H., & Rainey, W. E. (2009). Adrenal changes associated with adrenarche. *Reviews in Endocrine & Metabolic Disorders*, *10*(1 spec. issue), 19–26. https://doi.org/10.1007/s11154-008-9092-2.

Nakamura, Y., Hornsby, P. J., Casson, P., Morimoto, R., Satoh, F., Xing, Y., et al. (2009). Type 5 17β-hydroxysteroid dehydrogenase (AKR1C3) contributes to testosterone production in the adrenal reticularis. *The Journal of Clinical Endocrinology & Metabolism*, *94*(6), 2192–2198. https://doi.org/10.1210/jc.2008-2374.

Nakamura, Y., Xing, Y., Hui, X.-G., Kurotaki, Y., Ono, K., Cohen, T., et al. (2011). Human adrenal cells that express both 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) and cytochrome b5 (CYB5A) contribute to adrenal androstenedione production. *The Journal of Steroid Biochemistry and Molecular Biology*, *123*(3–5), 122–126. https://doi.org/10.1016/j.jsbmb.2010.12.001.

Nayak, S., Lee, P. A., & Witchel, S. F. (1998). Variants of the type II 3beta-hydroxysteroid dehydrogenase gene in children with premature pubic hair and hyperandrogenic adolescents. *Molecular Genetics and Metabolism*, *64*(3), 184–192. https://doi.org/10.1006/mgme.1998.2715.

Nordenström, A., Forest, M. G., & Wedell, A. (2007). A case of 3beta-hydroxysteroid dehydrogenase type II (HSD3B2) deficiency picked up by neonatal screening for 21-hydroxylase deficiency: Difficulties and delay in etiologic diagnosis. *Hormone Research*, *68*(4), 204–208. https://doi.org/10.1159/000102593.

O'Reilly, M. W., Kempegowda, P., Jenkinson, C., Taylor, A. E., Quanson, J. L., Storbeck, K.-H., et al. (2017). 11-Oxygenated C19 steroids are the predominant androgens in polycystic ovary syndrome. *The Journal of Clinical Endocrinology and Metabolism*, *102*(3), 840–848. https://doi.org/10.1210/jc.2016-3285.

Pang, S. (2001). Congenital adrenal hyperplasia owing to 3 beta-hydroxysteroid dehydrogenase deficiency. *Endocrinology and Metabolism Clinics of North America*, *30*(1), 81–99. vi–vii. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11344940.

Pang, S. Y., Lerner, A. J., Stoner, E., Levine, L. S., Oberfield, S. E., Engel, I., et al. (1985). Late-onset adrenal steroid 3 beta-hydroxysteroid dehydrogenase deficiency. I. A cause of hirsutism in pubertal and postpubertal women. *The Journal of Clinical Endocrinology and Metabolism*, *60*(3), 428–439. https://doi.org/10.1210/jcem-60-3-428.

Pang, S., Levine, L. S., Stoner, E., Opitz, J. M., Pollack, M. S., Dupont, B., et al. (1983). Nonsalt-losing congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency with normal glomerulosa function. *The Journal of Clinical Endocrinology & Metabolism*, *56*(4), 808–818. https://doi.org/10.1210/jcem-56-4-808.

Pang, S., Wang, W., Rich, B., David, R., Chang, Y. T., Carbunaru, G., et al. (2002). A novel nonstop mutation in the stop codon and a novel missense mutation in the type II 3β-hydroxysteroid dehydrogenase (3β-HSD) gene causing, respectively, nonclassic and classic 3β-HSD deficiency congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology & Metabolism*, *87*(6), 2556–2563. https://doi.org/10.1210/jcem.87.6.8559.

Panzer, K., Ekhaguere, O. A., Darbro, B., Cook, J., & Shchelochkov, O. A. (2017). Uniparental isodisomy of chromosome 1 unmasking an autosomal recessive 3-beta hydroxysteroid dehydrogenase type II-related congenital adrenal hyperplasia. *Journal of Clinical Research in Pediatric Endocrinology*, *9*(1), 70–73. https://doi.org/10.4274/jcrpe.3680.

Parker, K. L., Rice, D. A., Lala, D. S., Ikeda, Y., Luo, X., Wong, M., et al. (2002). Steroidogenic factor 1: An essential mediator of endocrine development. *Recent Progress in Hormone Research*, *57*, 19–36.

Parks, G. A., Bermudez, J. A., Anast, C. S., Bongiovanni, A. M., & New, M. I. (1971). Pubertal boy with the 3β-hydroxy steroid dehydrogenase defect. *The Journal of Clinical Endocrinology & Metabolism*, *33*(2), 269–278. https://doi.org/10.1210/jcem-33-2-269.

Paula, F. J., Dick-de-Paula, I., Pontes, A., Schmitt, F. C., Mendonça, B. B., & Foss, M. C. (1994). Hyperandrogenism due to 3 beta-hydroxysteroid dehydrogenase deficiency with accessory adrenocortical tissue: A hormonal and metabolic evaluation. *Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Médicas e Biológicas*, *27*(5), 1149–1158. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8000336.

Pretorius, E., Africander, D. J., Vlok, M., Perkins, M. S., Quanson, J., & Storbeck, K.-H. (2016). 11-Ketotestosterone and 11-ketodihydrotestosterone in castration resistant prostate cancer: Potent androgens which can no longer be ignored. *PLoS One*, *11*(7). e0159867. https://doi.org/10.1371/journal.pone.0159867.

Probst-Scheidegger, U., Udhane, S. S., l'Allemand, D., Flück, C. E., & Camats, N. (2016). Non-virilizing congenital adrenal hyperplasia in a female patient with a novel HSD3B2 mutation. *Sexual Development: Genetics, Molecular Biology, Evolution, Endocrinology, Embryology, and Pathology of Sex Determination and Differentiation*, *10*(4), 200–204. https://doi.org/10.1159/000448724.

Rabbani, B., Mahdieh, N., Haghi Ashtiani, M. T., Setoodeh, A., & Rabbani, A. (2012). In silico structural, functional and pathogenicity evaluation of a novel mutation: An overview of HSD3B2 gene mutations. *Gene*, *503*(2), 215–221. https://doi.org/10.1016/j.gene.2012.04.080.

Rege, J., Nakamura, Y., Satoh, F., Morimoto, R., Kennedy, M. R., Layman, L. C., et al. (2013). Liquid chromatography–tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. *The Journal of Clinical Endocrinology & Metabolism*, *98*(3), 1182–1188. https://doi.org/10.1210/jc.2012-2912.

Remer, T., Boye, K. R., Hartmann, M. F., & Wudy, S. A. (2005). Urinary markers of adrenarche: Reference values in healthy subjects, aged 3-18 years. *The Journal of Clinical Endocrinology and Metabolism*, *90*(4), 2015–2021. https://doi.org/10.1210/jc.2004-1571.

Rhéaume, E., Lachance, Y., Zhao, H. F., Breton, N., Dumont, M., de Launoit, Y., et al. (1991). Structure and expression of a new complementary DNA encoding the almost exclusive 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase in human adrenals and gonads. *Molecular Endocrinology (Baltimore, Md.)*, *5*(8), 1147–1157. https://doi.org/10.1210/mend-5-8-1147.

Rhéaume, E., Sanchez, R., Mébarki, F., Gagnon, E., Carel, J. C., Chaussain, J. L., et al. (1995). Identification and characterization of the G15D mutation found in a male patient with 3 beta-hydroxysteroid dehydrogenase (3 beta-HSD) deficiency: Alteration of the putative NAD-binding domain of type II 3 beta-HSD. *Biochemistry*, *34*(9), 2893–2900. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7893703.

Rhéaume, E., Sanchez, R., Simard, J., Chang, Y. T., Wang, J., Pang, S., et al. (1994). Molecular basis of congenital adrenal hyperplasia in two siblings with classical nonsalt-losing 3 beta-hydroxysteroid dehydrogenase deficiency. *The Journal of Clinical Endocrinology and Metabolism*, *79*(4), 1012–1018. https://doi.org/10.1210/jcem.79.4.7962268.

Rhéaume, E., Simard, J., Morel, Y., Mebarki, F., Zachmann, M., Forest, M. G., et al. (1992). Congenital adrenal hyperplasia due to point mutations in the type II 3β-hydroxysteroid dehydrogenase gene. *Nature Genetics*, *1*(4), 239–245. https://doi.org/10.1038/ng0792-239.

Rone, M. B., Fan, J., & Papadopoulos, V. (2009). Cholesterol transport in steroid biosynthesis: Role of protein–protein interactions and implications in disease states. *Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids*, *1791*(7), 646–658. https://doi.org/10.1016/j.bbalip.2009.03.001.

Rosenfield, R. L., Rich, B. H., Wolfsdorf, J. I., Cassorla, F., Parks, J. S., Bongiovanni, A. M., et al. (1980). Pubertal presentation of congenital delta 5-3 beta-hydroxysteroid dehydrogenase deficiency. *The Journal of Clinical Endocrinology and Metabolism*, *51*(2), 345–353. https://doi.org/10.1210/jcem-51-2-345.

Russell, A. J., Wallace, A. M., Forest, M. G., Donaldson, M. D., Edwards, C. R., & Sutcliffe, R. G. (1994). Mutation in the human gene for 3 beta-hydroxysteroid dehydrogenase type II leading to male pseudohermaphroditism without salt loss. *Journal of Molecular Endocrinology*, *12*(2), 225–237. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8060486.

Sanchez, R., Mébarki, F., Rhéaume, E., Laflamme, N., Forest, M. G., Bey-Omard, F., et al. (1994). Functional characterization of the novel L108W and P186L mutations detected in the type II 3 beta-hydroxysteroid dehydrogenase gene of a male pseudohermaphrodite with congenital adrenal hyperplasia. *Human Molecular Genetics*, *3*(9), 1639–1645. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7833923.

Sanchez, R., Rhéaume, E., Laflamme, N., Rosenfield, R. L., Labrie, F., & Simard, J. (1994). Detection and functional characterization of the novel missense mutation Y254D in type II 3 beta-hydroxysteroid dehydrogenase (3 beta HSD) gene of a female patient with nonsalt-losing 3 beta HSD deficiency. *The Journal of Clinical Endocrinology & Metabolism*, *78*(3), 561–567. https://doi.org/10.1210/jcem.78.3.8126127.

Shapiro, E., Huang, H. Y., & Wu, X. R. (2000). Uroplakin and androgen receptor expression in the human fetal genital tract: Insights into the development of the vagina. *The Journal of Urology*, *164*(3 Pt. 2), 1048–1051. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10958738.

Simard, J., Moisan, A. M., & Morel, Y. (2002). Congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase deficiency. *Seminars in Reproductive Medicine*, *20*(3), 255–276. https://doi.org/10.1055/s-2002-35373.

Simard, J., Rhéaume, E., Leblanc, J. F., Wallis, S. C., Joplin, G. F., Gilbey, S., et al. (1994). Congenital adrenal hyperplasia caused by a novel homozygous frameshift mutation 273 delta AA in type II 3 beta-hydroxysteroid dehydrogenase gene (HSD3B2) in three male patients of Afghan/Pakistani origin. *Human Molecular Genetics*, *3*(2), 327–330. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8004103.

Simard, J., Rhéaume, E., Sanchez, R., Laflamme, N., de Launoit, Y., Luu-The, V., et al. (1993). Molecular basis of congenital adrenal hyperplasia due to 3 beta-hydroxysteroid dehydrogenase deficiency. *Molecular Endocrinology*, 7(5), 716–728. https://doi.org/10.1210/mend.7.5.8316254.

Simard, J., Ricketts, M.-L., Gingras, S., Soucy, P., Feltus, F. A., & Melner, M. H. (2005). Molecular biology of the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene family. *Endocrine Reviews*, *26*(4), 525–582. https://doi.org/10.1210/er.2002-0050.

Sobel, V., Zhu, Y.-S., & Imperato-McGinley, J. (2004). Fetal hormones and sexual differentiation. *Obstetrics and Gynecology Clinics of North America*, *31*(4), 837–856. x–xi. https://doi.org/10.1016/j.ogc.2004.08.005.

Storbeck, K.-H., Bloem, L. M., Africander, D., Schloms, L., Swart, P., & Swart, A. C. (2013). 11β-Hydroxydihydrotestosterone and 11-ketodihydrotestosterone, novel C19 steroids with androgenic activity: A putative role in castration resistant prostate cancer? *Molecular and Cellular Endocrinology*, *377*(1–2), 135–146. https://doi.org/10.1016/j.mce.2013.07.006.

Suzuki, T., Sasano, H., Takeyama, J., Kaneko, C., Freije, W. A., Carr, B. R., et al. (2000). Developmental changes in steroidogenic enzymes in human postnatal adrenal cortex: Immunohistochemical studies. *Clinical Endocrinology*, *53*(6), 739–747. https://doi.org/10.1046/j.1365-2265.2000.01144.x.

Tajima, T., Fujieda, K., Nakae, J., Shinohara, N., Yoshimoto, M., Baba, T., et al. (1995). Molecular analysis of type II 3 beta-hydroxysteroid dehydrogenase gene in Japanese patients with classical 3 beta-hydroxysteroid dehydrogenase deficiency. *Human Molecular Genetics*, *4*(5), 969–971. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7633460.

Takasawa, K., Ono, M., Hijikata, A., Matsubara, Y., Katsumata, N., Takagi, M., et al. (2014). Two novel *HSD3B2* missense mutations with diverse residual enzymatic activities for Δ5-steroids. *Clinical Endocrinology*, *80*(6), 782–789. https://doi.org/10.1111/cen.12394.

Thomas, J. L., Boswell, E. L., Scaccia, L. A., Pletnev, V., & Umland, T. C. (2005). Identification of key amino acids responsible for the substantially higher affinities of human type 1 3beta-hydroxysteroid dehydrogenase/isomerase (3beta-HSD1) for substrates, coenzymes, and inhibitors relative to human 3beta-HSD2. *The Journal of Biological Chemistry*, *280*(22), 21321–21328. https://doi.org/10.1074/jbc.M501269200.

Thomas, J. L., Frieden, C., Nash, W. E., & Strickler, R. C. (1995). An NADH-induced conformational change that mediates the sequential 3 beta-hydroxysteroid dehydrogenase/isomerase activities is supported by affinity labeling and the time-dependent activation of isomerase. *The Journal of Biological Chemistry*, *270*(36), 21003–21008. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7673125.

Thomas, J. L., Mason, J. I., Blanco, G., & Veisaga, ML. (2001). The engineered, cytosolic form of human type I 3beta-hydroxysteroid dehydrogenase/isomerase: Purification, characterization and crystallization. *Journal of Molecular Endocrinology*, *27*(1), 77–83.

Turcu, A. F., Nanba, A. T., Chomic, R., Upadhyay, S. K., Giordano, T. J., Shields, J. J., et al. (2016). Adrenal-derived 11-oxygenated 19-carbon steroids are the dominant androgens in classic 21-hydroxylase deficiency. *European Journal of Endocrinology*, *174*(5), 601–609. https://doi.org/10.1530/EJE-15-1181.

Turcu, A., Smith, J. M., Auchus, R., & Rainey, W. E. (2014). Adrenal androgens and androgen precursors-definition, synthesis, regulation and physiologic actions. *Comprehensive Physiology*, *4*(4), 1369–1381. https:/doi.org/10.1002/cphy.c140006.

Voutilainen, R., Tapanainen, J., Chung, B.-C., Matteson, K. J., & Miller, W. L. (1986). Hormonal regulation of P450scc (20,22-desmolase) and P450cl7 (17α-hydroxylase/17,20-lyase) in cultured human granulosa cells. *The Journal of Clinical Endocrinology & Metabolism*, *63*(1), 202–207. https://doi.org/10.1210/jcem-63-1-202.

Welzel, M., Wüstemann, N., Simic-Schleicher, G., Dörr, H. G., Schulze, E., Shaikh, G., et al. (2008). Carboxyl-terminal mutations in 3beta-hydroxysteroid dehydrogenase type II cause severe salt-wasting congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology and Metabolism*, *93*(4), 1418–1425. https://doi.org/10.1210/jc.2007-1874.

Wilhelm, D., Palmer, S., & Koopman, P. (2007). Sex determination and gonadal development in mammals. *Physiological Reviews*, *87*(1), 1–28. https://doi.org/10.1152/physrev.00009.2006.

Xing, Y., Lerario, A. M., Rainey, W., & Hammer, G. D. (2015). Development of adrenal cortex zonation. *Endocrinology and Metabolism Clinics of North America*, *44*(2), 243–274. https://doi.org/10.1016/j.ecl.2015.02.001.

Yoshimoto, M., Kawaguchi, T., Mori, R., Kinoshita, E., Baba, T., Tajima, T., et al. (1997). Pubertal changes in testicular 3 beta-hydroxysteroid dehydrogenase activity in a male with classical 3 beta-hydroxysteroid dehydrogenase deficiency showing spontaneous secondary sexual maturation. *Hormone Research*, *48*(2), 83–87. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9251925.

Zachmann, M., Forest, M. G., & De Peretti, E. (1979). 3 beta-Hydroxysteroid dehydrogenase deficiency. Follow-up study in a girl with pubertal bone age. *Hormone Research*, *11*(6), 292–302. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/295036.

Zachmann, M., Vollmin, J. A., Murset, G., Curtius, H. C., & Prader, A. (1970). Unusual type of congenital adrenal hyperplasia probably due to deficiency of 3-beta-hydroxysteroid dehydrogenase. Case report of a surviving girl and steroid studies. *The Journal of Clinical Endocrinology and Metabolism*, *30*(6), 719–726. https://doi.org/10.1210/jcem-30-6-719.

Zhang, L., Mason, J. I., Naiki, Y., Copeland, K. C., Castro-Magana, M., Gordon-Walker, T. T., et al. (2000). Characterization of two novel homozygous missense mutations involving codon 6 and 259 of type II 3beta-hydroxysteroid dehydrogenase (3betaHSD) gene causing, respectively, nonsalt-wasting and salt-wasting 3betaHSD deficiency disorder. *The Journal of Clinical Endocrinology and Metabolism*, *85*(4), 1678–1685. https://doi.org/10.1210/jcem.85.4.6539.

Zhang, L., Sakkal-Alkaddour, H., Chang, Y. T., Yang, X., & Pang, S. (1996). A new compound heterozygous frameshift mutation in the type II 3 beta-hydroxysteroid dehydrogenase (3 beta-HSD) gene causes salt-wasting 3 beta-HSD deficiency congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology & Metabolism*, *81*(1), 291–295. https://doi.org/10.1210/jcem.81.1.8550766.

FURTHER READING

Bongiovanni, A. M. (1962). The adrenogenital syndrome with deficiency of 3 beta-hydroxysteroid dehydrogenase. *The Journal of Clinical Investigation*, *41*(11), 2086–2092. https://doi.org/10.1172/JCI104666.

Schneider, G., Genel, M., Bongiovanni, A. M., Goldman, A. S., & Rosenfield, R. L. (1975). Persistent testicular delta5-isomerase-3beta-hydroxysteroid dehydrogenase (delta5-3beta-HSD) deficiency in the delta5-3beta-HSD form of congenital adrenal hyperplasia. *The Journal of Clinical Investigation*, *55*(4), 681–690. https://doi.org/10.1172/JCI107977.

Zachmann, M., Kempken, B., Anner, I., & Pezzoli, V. (1988). 160 age-related differences in the excretion of delta 5 steroids (D5S) in two related girls with 3b-hydroxy steroid dehydrogenase deficiency (3b0HD). *Pediatric Research*, *24*(4), 543. https://doi.org/10.1203/00006450-198810000-00181.