The Clinical and Molecular Heterogeneity of 17βHSD-3 Enzyme Deficiency

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**Introduction**

The development of the male internal and external genitalia in an XY fetus requires a complex interplay of many critical genes, enzymes and cofactors \[1, 2\]. Wolffian ducts (mesonephric ducts) and mullerian ducts (paramesonephric ducts) are both present in early fetal life in the bipotential embryo. The wolffian ducts are the embryological structures that form the epididymis, vas deferens and seminal vesicles. Testosterone is produced by Leydig cells as early as 8 weeks of gestation and acts on the androgen receptor to stabilize the wolffian ducts \[3, 4\]. Testosterone and its 5α-reduced end product, dihydrotestosterone (DHT), induce the formation of male external genitalia, including the urethra, prostate, penis and scrotum \[1\]. The mullerian ducts should regress in a male with the presence of the mullerian inhibiting substance produced by Sertoli cells in the testes. In addition, multiple other factors are necessary for the male phenotype to be congruent with a 46,XY genotype. The enzyme 17β-hydroxysteroid dehydrogenase type 3 (17βHSD-3) is present almost exclusively in the testes and converts Δ4-androstenedione (Δ4) to testosterone (T). The diagnosis can be easily missed in early childhood as the clinical presentation may be subtle. Any young girl with an inguinal hernia, mild clitoromegaly, single urethral opening or urogenital sinus should raise suspicion. If not diagnosed early, patients present with severe virilization and primary amenorrhea in adolescence and may undergo a change from a female to male gender role. A low T/Δ4 ratio on baseline or hCG (human chorionic gonadotropin)-stimulated testing is suggestive of 17βHSD-3 deficiency. The diagnosis can be confirmed with molecular genetic studies. This review summarizes the clinical presentations, reported mutations, diagnosis, treatment and clinical course of this disorder. The Arg80 site in exon 3 is the most common location of repeated mutations and can be considered a hot spot in certain Arab populations.

**Key Words**

46,XY disorder of sex development · 17-β-Hydroxysteroid dehydrogenase type 3 deficiency · Δ4-Androstenedione · Testosterone · Human chorionic gonadotropin

**Abstract**

17β-hydroxysteroid dehydrogenase type 3 (17βHSD-3) deficiency is a rare, but frequently misdiagnosed autosomal recessive cause of 46,XY disorder of sex development (DSD). 17βHSD-3 enzyme is present almost exclusively in the testes and converts Δ4-androstenedione (Δ4) to testosterone (T). The diagnosis can be easily missed in early childhood as the clinical presentation may be subtle. Any young girl with an inguinal hernia, mild clitoromegaly, single urethral opening or urogenital sinus should raise suspicion. If not diagnosed early, patients present with severe virilization and primary amenorrhea in adolescence and may undergo a change from a female to male gender role. A low T/Δ4 ratio on baseline or hCG (human chorionic gonadotropin)-stimulated testing is suggestive of 17βHSD-3 deficiency. The diagnosis can be confirmed with molecular genetic studies. This review summarizes the clinical presentations, reported mutations, diagnosis, treatment and clinical course of this disorder. The Arg80 site in exon 3 is the most common location of repeated mutations and can be considered a hot spot in certain Arab populations.
17βHSD-3, can lead to a child with a 46,XY disorder of sex development (DSD).

DSD is defined as a congenital condition in which development of chromosomal, gonadal or anatomical sex is atypical [5–7]. These disorders are now classified into three major categories: sex chromosome DSD, 46,XX DSD and 46,XY DSD. The last category refers to a condition in which a child has a 46,XY karyotype, but in whom gonadal or anatomical sex is atypical. This designation was proposed to replace the former term of male pseudohermaphroditism, according to the consensus statement on management of intersex disorders [5–7, 21]. 46,XY DSD can have multiple etiologies, most commonly involving a disruption in androgen production and/or action. The ability to synthesize testosterone can be impaired by a mutation in any of the five critical enzymes that are involved in conversion of cholesterol to testosterone (fig. 1).

More patients with 46,XY DSD with normal testicular development have androgen insensitivity syndrome. Less commonly, 46,XY DSD results from deficiencies in 5α-reductase type 2 or 17βHSD-3 enzymes, or from defects in transcriptional factors like steroidogenic factor-1. The exact cause of the 46,XY DSD can be missed if the rare forms are not actively sought. Our review will only focus on 17βHSD-3 deficiency in the context of 46,XY DSD.

A deficiency in the 17βHSD-3 enzyme leads to an autosomal recessive form of 46,XY DSD, which was first described in 1971 [8, 9]. 17βHSD-3 enzyme deficiency (OMIM No. 264300), previously termed 17-ketosteroid reductase deficiency, is the most common testosterone biosynthesis defect of 46,XY DSD [10, 11]. The family of 17-β-hydroxysteroid dehydrogenase enzymes, now totaling 14 known isoenzymes, plays a key role in the final stages of all androgen and estrogen synthesis (table 1) [12–17]. Therefore, these enzymes are critical in both male and female reproductive organ development and function. Deficiency in the 17βHSD-3 enzyme can be caused by either homozygous or compound heterozygous mutations in the HSD17B3 gene [18]. Mutations in the HSD17B3 gene confers a spectrum of 46,XY disorders of sexual organ development ranging from completely undervirilized external female genitalia (Sinnecker type 5), predominantly female (Sinnecker type 4), ambiguous (Sinnecker type 3), to predominantly male with micropenis and hypospadias (Sinnecker type 2) [19–21]. The most frequent presentation of 17βHSD-3 deficiency is a 46,XY individual with female external genitalia, labial fusion and a blind ending vagina, with or without clitoromegaly (table 2; Sinnecker types 5 and 4).

To our knowledge, 27 mutations in the HSD17B3 gene have been reported to date. These include intronic splice sites, exonic deletions and missense mutations (table 2) [22]. A few clusters of these mutations have been described in the Arab population living in the Gaza Strip [21, 23, 24]. The most frequent mutation in this Arab cohort is p.Arg80Gln, which is a point mutation in exon 3 at codon 80 leading to arginine (CGG) being switched to

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**Fig. 1.** Androgen synthesis pathway, with the defect of 17βHSD-3 deficiency denoted by a red cross. Steroidogenic pathway and role of 17βHSD-3. Production of androgens is controlled by various enzymes. 17βHSD-3 converts Δ4-androstenedione to T by a reductive process.
Table 1. The various types of identified 17βHSD with corresponding locations and function [13–17]

<table>
<thead>
<tr>
<th>Type of 17βHSD (Gene Name)</th>
<th>Locations</th>
<th>Function</th>
<th>Cofactor/ reactions</th>
<th>Gene location</th>
</tr>
</thead>
<tbody>
<tr>
<td>17βHSD type 1 (HSD17B1)</td>
<td>liver, ovary, mammary glands and placenta</td>
<td>catalyzes the interconversion of E1 to E2</td>
<td>NADPH/ reduction</td>
<td>17q21.2</td>
</tr>
<tr>
<td>17βHSD type 2 (HSD17B2)</td>
<td>placenta, liver, intestine, endometrium, kidney, prostate, pancreas</td>
<td>inactivates both E2 into E1 and T into Δ4</td>
<td>NAD+/ oxidation</td>
<td>16q23.3</td>
</tr>
<tr>
<td>17βHSD type 3 (HSD17B3)</td>
<td>mainly testis, adipose tissue, brain, sebaceous glands and bone</td>
<td>converts Δ4 to T</td>
<td>NADPH/ reduction</td>
<td>9q22.32</td>
</tr>
<tr>
<td>17βHSD type 4 (HSD17B4)</td>
<td>liver, heart, prostate, testis, lung, skeletal muscle, kidney, pancreas, thymus, ovary, intestine, placenta and breast cancer lines</td>
<td>inactivates both E2 into E1, and 5-diol into DHEA-β; oxidation of FA</td>
<td>NAD+/ oxidation</td>
<td>5q23.1</td>
</tr>
<tr>
<td>17βHSD type 5* (AKR1C3)</td>
<td>placenta, testes, prostate, adrenals and liver</td>
<td>converts Δ4 to T in peripheral tissues; bile acid production and detoxification; eicosanoid synthesis</td>
<td>NADPH/ reduction</td>
<td>10p15.1</td>
</tr>
<tr>
<td>17βHSD type 6 (HSD17B6/RODH)</td>
<td>not determined</td>
<td>only retinoid metabolism identified in humans</td>
<td>NAD+/ oxidation</td>
<td>12q13.3</td>
</tr>
<tr>
<td>17βHSD type 7 (HSD17B7)</td>
<td>not determined</td>
<td>cholesterol synthesis; catalyzes the interconversion of E1 to E2</td>
<td>NADPH/ reduction</td>
<td>2 genes: 10p11.2 1q23</td>
</tr>
<tr>
<td>17βHSD type 8 (HSD17B8)</td>
<td>widespread, liver, kidney, ovary, testis</td>
<td>possible role in fatty acid metabolism; inactivates both E2 into E1 and androgens</td>
<td>NAD+/ oxidation</td>
<td>6p21.32</td>
</tr>
<tr>
<td>17βHSD type 9 (HSD17B8/RDH5)</td>
<td>not determined</td>
<td>only retinoid metabolism identified in humans</td>
<td>not determined</td>
<td>12q13.2</td>
</tr>
<tr>
<td>17βHSD type 10 (HSD17B10)</td>
<td>widespread, liver, CNS, kidney, testis</td>
<td>oxidation of fatty acids; catalyzes the synthesis of DHT from 5α-androstane-3α, 17β-diol; ability to oxidize the 21OH groups on C21 steroids</td>
<td>NAD+/ oxidation</td>
<td>Xp11.22</td>
</tr>
<tr>
<td>17βHSD type 11 (HSD17B11)</td>
<td>steroidogenic tissues, pancreas, liver, kidney, lung and heart</td>
<td>converts 5α-androstane-3α, 17β-diol to androsterone; lipid metabolism</td>
<td>NAD+/ oxidation</td>
<td>4q22.1</td>
</tr>
<tr>
<td>17βHSD type 12 (HSD17B12)</td>
<td>not determined</td>
<td>fatty acid synthesis; 3-ketoacyl-CoA reductase</td>
<td>NADPH/ reduction</td>
<td>11p11.2</td>
</tr>
<tr>
<td>17βHSD type 13 (HSD17B13)</td>
<td>not determined</td>
<td>enzymatically not characterized</td>
<td>not determined</td>
<td>4q22.1</td>
</tr>
<tr>
<td>17βHSD type 14 (HSD17B14)</td>
<td>CNS, kidney</td>
<td>inactivates both E2 into E1 and T into Δ4; β oxidation of FA</td>
<td>NAD+/ oxidation</td>
<td>19q13.33</td>
</tr>
</tbody>
</table>

E1 = Estrone; E2 = 17β-estradiol; 5-diol = androst-5-ene 3β; DHEA = dihydroepiandrosterone; NADPH/NADP+ = nicotinamide adenine dinucleotide phosphate; Δ4 = androstenedione; T = testosterone; FA = fatty acids. * Only non short-chain dehydrogenase/reductase (SDR) member in the family.

a glutamine (CAG) [21, 23, 24]. A founder effect for this hot spot mutation has been demonstrated in this population [21, 24]. This site has been extensively studied by systematic replacement of the wild-type arginine at position 80 and has been shown to be extremely important for both forming the salt bridge with the terminal phosphate moiety of the NADPH, as well as providing for a hydrophobic pocket for the purine ring of the adenosine portion of the NADPH [25]. Along with p.Arg80Gln, three other mutations, c.325+4;A→T, p.Asn74Thr, c.655−1:G→A, have been traced back and have been found to be ancient mutations whose dispersion has been traced back historically [21]. The c.325+4;A→T and p.Asn74Thr mutations recurred in the Dutch populations suggesting a common founder effect in some European populations [21]. The c.655−1:G→A mutations found in Greeks, Turks and Syrians is attributed to the Ottoman Empire [21].
<table>
<thead>
<tr>
<th>Age of diagnosis</th>
<th>Phenotype/clinical presentation</th>
<th>Ethnicity</th>
<th>Mutation</th>
<th>Mutation type/effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 years</td>
<td>46,XY DSD; evaluated for hirsutism, clitoromegaly and failure to menstruate</td>
<td>Iranian</td>
<td>p.Ser65Leu</td>
<td>missense/inactivates enzyme</td>
<td>34</td>
</tr>
<tr>
<td>6 months, 11 years</td>
<td>46,XY DSD; female prepubertal external genitalia, pubertal virilization, severe hair growth, voice changes and clitoral enlargement (6 months, child diagnosed because of family history)</td>
<td>South Asian</td>
<td>p.Ala56Thr</td>
<td>missense/severe impairment of enzyme</td>
<td>30, 68</td>
</tr>
<tr>
<td>4–16 years</td>
<td>46,XY DSD; ambiguous genitalia, pubertal virilization</td>
<td>Dutch</td>
<td>p.Asn74Thr</td>
<td>missense</td>
<td>21</td>
</tr>
<tr>
<td>4–43 years (most extensively described)</td>
<td>46,XY DSD; ambiguous genitalia at birth to mild clitoromegaly, pubertal virilization, male gender role, and many reassigned as males if raised as girls</td>
<td>Arab, Dutch, Brazilian, Portuguese</td>
<td>p.Arg80Gln</td>
<td>missense/impaired enzyme activity (NADPH binding site)</td>
<td>11, 18, 21, 23, 40, 47</td>
</tr>
<tr>
<td>Newborn–12 years</td>
<td>46,XY DSD; female external genitalia, palpable gonads, clitoral enlargement and virilization at puberty</td>
<td>Spanish, Italian, Lebanese</td>
<td>p.Arg80Trp</td>
<td>missense/complete loss of enzyme activity (NADPH binding site)</td>
<td>25, 35, 69</td>
</tr>
<tr>
<td>4 months–15 years</td>
<td>46,XY DSD; normal female to ambiguous genitalia, palpable inguinal gonads</td>
<td>Dutch, Brazilian, German, American, Italian</td>
<td>c.325+4A→T</td>
<td>splice junction/disrupts splice acceptor site</td>
<td>11, 21, 34</td>
</tr>
<tr>
<td>8, 23, 34 years</td>
<td>15 years CH with R80 Q</td>
<td>Dutch</td>
<td>c.326–1G→C</td>
<td>splice junction</td>
<td>11, 18, 21, 34, 47, 68</td>
</tr>
<tr>
<td>14, 15 years</td>
<td>46,XY DSD; pubertal virilization, mild clitoromegaly, voice changes</td>
<td>English, German</td>
<td>p.Asn130Ser</td>
<td>missense/severe impairment of enzyme activity</td>
<td>30, 48, 68</td>
</tr>
<tr>
<td>Unknown</td>
<td>46,XY DSD</td>
<td>unknown</td>
<td>c.538–1G→A</td>
<td>splice junction</td>
<td>70</td>
</tr>
<tr>
<td>13 years</td>
<td>46,XY DSD; clitoromegaly and coarsening of voice, scrotalization of labia majora and inguinal masses</td>
<td>American (Italian, German, Irish)</td>
<td>p.Gln176Pro</td>
<td>missense</td>
<td>34, 68</td>
</tr>
<tr>
<td>12 years</td>
<td>46,XY DSD; female prepubertal development, clitoral enlargement at 12 years of age, testes in inguinal canal</td>
<td>German</td>
<td>c.608delT</td>
<td>downstream premature stop codon</td>
<td>36</td>
</tr>
<tr>
<td>10 years</td>
<td>46,XY DSD; prepubertal female external genitalia, inguinal mass</td>
<td>Turkish</td>
<td>p.Ala188Val</td>
<td>missense/inactivates enzyme</td>
<td>21</td>
</tr>
<tr>
<td>12 years</td>
<td>46,XY DSD; pubertal virilization, facial hair, 4–8 cm phallus and labioscrotal folds</td>
<td>Afghan</td>
<td>p.Met197Lys</td>
<td>missense/alters secondary protein structure</td>
<td>30</td>
</tr>
<tr>
<td>10, 16, 17 years</td>
<td>46,XY DSD; prepubertal female external genitalia, pubertal virilization, male gender role</td>
<td>Syrian, Turkish, Dutch, Greek-American</td>
<td>c.655–1G→A</td>
<td>splice junction/disrupts splice acceptance site</td>
<td>18, 21, 34, 68, 71</td>
</tr>
<tr>
<td>13, 18, 21, 26 years</td>
<td>46,XY DSD; absence of menses, failure of breast development, facial and chest hair and clitoral enlargement, male and female gender identity in siblings</td>
<td>African-Brazilian, Italian</td>
<td>p.Ala203Val</td>
<td>missense/inactivates enzyme</td>
<td>11, 18, 47, 68</td>
</tr>
<tr>
<td>Unknown</td>
<td>46,XY DSD; pubertal virilization</td>
<td>Southern Italian</td>
<td>p.Ala203Glu</td>
<td>missense</td>
<td>11, 43</td>
</tr>
<tr>
<td>Newborn, 20 years</td>
<td>46,XY DSD; prepubertal female external genitalia to perineoscrotal hypospadias, primary amenorrhea, mild clitoromegaly</td>
<td>White American, English</td>
<td>p.Val205Glu</td>
<td>missense/inactivates enzyme</td>
<td>30, 34</td>
</tr>
<tr>
<td>Newborn</td>
<td>46,XY DSD; born with ambiguous genitalia, clitoromegaly (1.5 cm) and posterior fusion and scrotalization of the labia majora which contained palpable masses</td>
<td>German</td>
<td>p.Phe208Ile</td>
<td>missense/inactivates enzyme</td>
<td>34</td>
</tr>
</tbody>
</table>
Epidemiology and Demographics

DSD affects 1 in 5,000–5,500 people (0.018%) [26, 27]. Although the precise incidence of 17βHSD-3 deficiency is unknown, a recent study from the Netherlands estimated the incidence around 1 in 147,000 newborns, with a calculated heterozygote frequency of 1 in 135 [21]. The frequency of proven complete androgen insensitivity syndrome from the same population was 1 in 99,000, which indicates that the frequency of 17βHSD-3 deficiency is 0.65 times that of complete androgen insensitivity syndrome [21]. In areas of high consanguinity, such as among the Gaza Strip Arab population, the incidence of 17βHSD-3 deficiency has been reported to be as high as 1 in 100–300 [23, 24]. A proper diagnosis is imperative in 46,XY DSD, yet only 50% of the children in this group receive a definitive diagnosis [6].

In a recent study from a gender assessment team in the United States that looked at DSD over a 25-year period, no patient with 17βHSD-3 deficiency was diagnosed [28]. Of the known cases of 17βHSD-3 deficiency, most of the patients have been reported in Europe, Asia, Australia and South America, whereas only 11 cases have been reported in the United States [13, 22]. We are unsure whether 17βHSD-3 deficiency is truly rare in the United States or whether it is frequently missed. In one study, patients who were later confirmed to have 17βHSD-3 deficiency were initially misdiagnosed with androgen insensitivity syndrome (AIS), and the rate of misdiagnosis was calculated to be as high as 67% [29]. The risk of misdiagnosis is especially problematic because the clinical findings in 17βHSD-3 deficiency may mimic AIS in childhood and 5α-reductase deficiency in puberty [30]. Thus, correct diagnosis should be made in a timely manner to ensure appropriate medical care and social support for affected individuals.

### Table 2 (continued)

<table>
<thead>
<tr>
<th>Age of diagnosis</th>
<th>Phenotype/clinical presentation</th>
<th>Ethnicity</th>
<th>Mutation</th>
<th>Mutation type/effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 years, 3 months</td>
<td>46,XY DSD; inguinal mass, mild clitoromegaly compound heterozygous with M235V</td>
<td>Italian</td>
<td>p.Leu212Gln</td>
<td>missense/inactivates</td>
<td>18, 39</td>
</tr>
<tr>
<td>14, 15, 21 years</td>
<td>46,XY DSD; female or ambiguous genitalia at birth, male behaviors in childhood, pubertal virilization, absence of menses, male gender role</td>
<td>White Brazilian, English</td>
<td>p.Glu215Asp</td>
<td>missense/inactivates enzyme</td>
<td>11, 30, 34</td>
</tr>
<tr>
<td>2 months, 2, 6, 17 years CH with S232L</td>
<td>46,XY DSD; clitoromegaly, primary amenorrhea, inguinal masses</td>
<td>African-American, South Asian</td>
<td>p.Ser232Leu</td>
<td>missense/inactivates enzyme</td>
<td>18, 30, 68</td>
</tr>
<tr>
<td>17 years CH with M235V</td>
<td>46,XY DSD; clitoromegaly, primary amenorrhea, inguinal masses</td>
<td>African-American, Italian</td>
<td>p.Met235Val</td>
<td>missense/inactivates enzyme</td>
<td>18, 39, 68</td>
</tr>
<tr>
<td>15 years</td>
<td>46,XY DSD; testis in herniorrhaphy sac, failure to menstruate</td>
<td>Polish</td>
<td>c.777–783delGATAACC</td>
<td>deletion/frame shift truncates protein</td>
<td>34</td>
</tr>
<tr>
<td>5, 18 months, 2–4 years</td>
<td>46,XY DSD; prominent clitoris, palpable inguinal gonads</td>
<td>Pakistani</td>
<td>p.Cys268YTyr</td>
<td>missense/inactivates enzyme</td>
<td>30, 72</td>
</tr>
<tr>
<td>unknown</td>
<td>46,XY DSD</td>
<td>French</td>
<td>p.His271Arg</td>
<td>missense/inactivates enzyme</td>
<td>73</td>
</tr>
<tr>
<td>12, 14 years</td>
<td>46,XY DSD; clitoromegaly, failure of breast development and deepening of voice</td>
<td>White American, Dutch</td>
<td>p.Pro282Leu</td>
<td>missense/inactivates enzyme</td>
<td>21, 34</td>
</tr>
<tr>
<td>6 months</td>
<td>46,XY DSD; normal female prepubertal genitalia, bilateral inguinal hernia at sonography</td>
<td>Italian, West Indian</td>
<td>p.Gly289Ser</td>
<td>polymorphism/unknown</td>
<td>21, 22*, 43, 74*</td>
</tr>
</tbody>
</table>

* Other phenotypic manifestations seen in patients with 17βHSD-3 mutation.
early so that treatment, management and genetic counseling can be specifically directed toward 17βHSD-3 deficiency [31, 32].

**Clinical Presentation**

**Birth**

Patients with mutations in the HSD17B3 gene may go unnoticed at birth since female external genitalia is common [24, 30, 33]. These children are usually assigned the female gender and raised as such [11, 21, 24, 30, 33–35]. In these patients the diagnosis may be missed until adolescence. Those who come to medical attention in childhood have some degree of virilization or inguinal hernia with testes present along the inguinal canals or labioscrota1

With testes present along the inguinal canals or labioscrota1

Less frequently, micropenis or hypospadias has also been reported [19, 23, 24, 36, 37]. In these patients, the male sex is assigned at birth and they are raised accordingly [23]. The degree of virilization can vary from Sinnecker stage 5 to stage 2 as mentioned above. This is speculated to be due to the partial activity of 17βHSD-3 in the testes and extratesticular testosterone conversion by other members of the family, such as 17βHSD-5 [30, 38].

On examination, a separate urethral and vaginal opening is noted in many, although a short urogenital sinus is reported in some [8, 30, 39]. Blind ending vaginas that have lengths ranging from 1 to 7 cm have been reported in this condition [11, 35]. Although, these findings are not specific for 17βHSD-3 deficiency and can be seen in other 46,XY DSD, they should raise suspicion for 17βHSD-3 deficiency.

**Pubertal**

At the time of puberty, patients initially reared as females who have not undergone gonadectomy present with primary amenorrhea, varying degrees of virilization including development of male body habitus, increased body hair and deepening of the voice; in some individuals, it prompts a change to a male gender role [11, 22, 23, 30, 35, 40]. The clitoris can enlarge to as much as 5–8 cm in length due to peripheral conversion of testosterone [11, 33], but still remains smaller than a normal-sized penis and may be affected by chordee [41]. A late onset form of 17βHSD-3 deficiency causing breast development was reported in up to 6% of the patients with idiopathic pubertal gynecomastia [42]. It appeared to be related to the functional inactivity of 17βHSD-3 during puberty and increased aromatization of Δ4-androstenedione to produce excessive estrogens; however, the HSD17B3 gene was not studied for defects in this study [33, 42]. The diagnosis was made based on T/Δ4 ratios instead.

**Prenatal**

Recently, the first case of prenatally identified 17βHSD-3 deficiency was reported in a child with discordance between 46,XY karyotype and female external genitalia with phallic structure [43]. Therefore, patients have been diagnosed with 17βHSD-3 deficiency from infancy to adulthood with varied clinical phenotypes.

**Genotype-Phenotype Correlation**

No phenotype to genotype correlation has been noted in 17βHSD-3 deficiency, as exemplified by members of the same family who have different phenotypes despite the same genotype [30]. A variable T/Δ4 ratio after human chorionic gonadotropin (hCG) stimulation was also seen despite the same homozygous mutation in different subjects of the same pedigree [30]. This can be attributed to the extratesticular ability of some subjects to convert Δ4-androstenedione to testosterone by other enzymes such as 17βHSD-5 [38].

**Gender Role**

Cultural influences and virilization at puberty are the most common reasons for reassignment of gender role. If gonadectomy is not performed, affected individuals undergo marked virilization due to extratesticular conversion of Δ4-androstenedione to testosterone secondary to some residual function of the enzyme and increased substrate availability in Δ4-androstenedione at puberty [44, 45]. These females may adopt a male gender role due to this marked virilization at puberty [46]. The female-to-male gender role change generally occurs in late adolescence to early adulthood and is relatively frequent, at the rate of 39–64% [32, 46]. This has been commonly seen among the Arab cohort from Israel, probably due to social and cultural influences [23, 24]. In individuals who were raised as boys, no gender changes were reported and genital appearance at birth was not related to gender changes later in life [46]. The severity of the enzymatic defect shows no relationship to the decision about adult social gender role; thus, why gender changes occur in some is not completely understood [11]. Most female patients who underwent gonadectomy in childhood (prepubertally) have been satisfied with their female gender role, and very little gender reversal has been noted [43].
Since 17βHSD-3 deficiency is an autosomal recessive disorder, 46,XX females could have it as well with equal chance as 46,XY males. However, 46,XX subjects are difficult to diagnose with 17βHSD-3 deficiency since they have a normal female phenotype, normal gender role and normal endocrine function [23, 47].

Diagnosis of 17βHSD-3 Deficiency: Endocrine, Imaging and Molecular Studies

The phenotype of 17βHSD-3 deficiency is clinically indistinguishable from that of androgen receptor mutation or 5α-reductase 2 deficiency. 17βHSD-3 deficiency, however, can be reliably diagnosed by systematic endocrine evaluation and the diagnosis confirmed by molecular genetics study (fig. 2). The characteristic hormonal profile of 17βHSD-3 deficiency is of increased concentrations of Δ4-androstenedione and reduced levels of testosterone [29]. The basal levels can be quite variable at different ages [29]. At baseline in adults, the precursor Δ4-androstenedione is usually elevated with a borderline low or normal testosterone [11]. This translates to a low T/Δ4 ratio of <0.8 [29] (table 3). The DHT levels in 17βHSD-3 deficiency can be decreased, normal or high, while the dehydroepiandrosterone (DHEA) levels are typically high [11]. In a recent analysis of data collected from patients with mutations proven in the HSD17B3 gene, it was

![Table 3. Selected T/Δ4 ratio in basal and hCG-stimulated values in reported cases](image)

### Table 3. Selected T/Δ4 ratio in basal and hCG-stimulated values in reported cases

<table>
<thead>
<tr>
<th>Age of patients</th>
<th>Range of basal T/Δ4 ratio</th>
<th>hCG stimulation test</th>
<th>Range of stimulated T/Δ4 ratio</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1–17 years</td>
<td>0.07–1.08</td>
<td>dose not reported</td>
<td>0.09–0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21</td>
</tr>
<tr>
<td>5 months–20 years</td>
<td>0.12–0.75</td>
<td>1,000–2,000 IU/day for 3 days (70% of the cases)</td>
<td>0.12–3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td>11–14 years</td>
<td>0.4</td>
<td>1,000–2,000 IU/day for 3 days (70% of the cases)</td>
<td>0.3</td>
<td>29</td>
</tr>
<tr>
<td>2.3 years</td>
<td>Unable to determine</td>
<td>5,000 IU/m² once</td>
<td>0.54</td>
<td>39</td>
</tr>
<tr>
<td>15 years</td>
<td>0.26</td>
<td>1,500 IU/day for 5 days</td>
<td>0.12</td>
<td>22</td>
</tr>
<tr>
<td>39 weeks</td>
<td>0.006</td>
<td>1,000 IU/day for 3 days</td>
<td>0.1</td>
<td>69</td>
</tr>
<tr>
<td>4 weeks–13 years</td>
<td>0.11–1.64</td>
<td>1,500 IU every 2 days for 2 weeks</td>
<td>0.13–0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36</td>
</tr>
<tr>
<td>15 years</td>
<td>0.21</td>
<td>1,500 IU every 2 days for 2 weeks</td>
<td>0.20</td>
<td>8</td>
</tr>
<tr>
<td>6 months–21 years</td>
<td>&lt;0.4, 0.63</td>
<td>dose not reported</td>
<td>0.16–0.45</td>
<td>34</td>
</tr>
<tr>
<td>16–28 years</td>
<td>Not reported</td>
<td>500–1,000 IU twice a week for 2–6 weeks</td>
<td>0.04–0.11</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup> 2 patients with values of <0.8 only on stimulated (age: 6 years and 10 years). 1 patient with no value <0.8 in basal or stimulated, but both <1.0 (age: 13 years).

<sup>b</sup> 2 mutation-proven patients with value of <0.8 on basal but stimulated to 1.5 and 3.4 (age: 2 months and 2 years, respectively).

<sup>c</sup> 1 patient who had a value of >0.8 later became <0.8 with the prolonged hCG test.
seen that in infants younger than 6 months, the diagnosis can be arrived at with a basal low T/Δ4 ratio of <0.8, with a sensitivity of 100% [48]. This can be due to the mini-puberty of infancy. However, the diagnosis of 17βHSD-3 can be easily missed after this mini-puberty (but before true puberty) unless hCG stimulation testing is performed and the T/Δ4 ratio is calculated [48]. In the same HSD17B3 gene mutation proven cohort of prepubertal children, hCG stimulation increases the sensitivity of diagnosis from 57 to 90% when a cutoff of <0.8 is used for the T/Δ4 ratio [48]. In a study that looked at all causes of undermasculinization, of the 114 patients that had testosterone and Δ4-androstenedione before and after hCG stimulation, none of the cases with AIS with a proven AR mutation had a low T/Δ4 ratio of <0.8 [29]. Only 4 out of 84 cases of ‘presumptive’ AIS diagnosis had a low T/Δ4 ratio <0.8. One of the 4 cases responded to a prolonged hCG test, with a ratio of 3.5 [29]. A normal ratio above 0.8 on hCG stimulation raises the suspicion of other diagnoses such as androgen receptor mutation. Not all cases of low (<0.8) baseline or hCG-stimulated T/Δ4 ratios can be considered to be 17βHSD-3 deficiency, as this may also be encountered in conditions with abnormal testes. In the same undermasculinized group, the median T/Δ4 ratio in cases with abnormal testes was 0.4 (0.1–5.6) at baseline to a stimulated median ratio of 0.6 (0.1–3.6), which was in the range of T/Δ4 ratio in their comparison 17βHSD-3 deficiency with a mean ratio of 0.4 (SD: 0.2) [29]. Although clinically indistinguishable, 5α-reductase type 2 deficiency can be reasonably suspected when an elevated T/DHT ratio is noted (fig. 2).

Imaging studies that reveal the absence of Mullerian structures and persistent Wolffian structures also point to the diagnosis of 17βHSD-3 deficiency, but this is not pathognomonic as 5α-reductase type 2 deficiency will also have similar findings. Histological evidence from gonadal tissue may show normal testicular structures, which can help exclude any structural abnormalities (testicular dysgenesis) as the cause for the 46,XY DSD. Despite early orchidopexy, absent spermatogenesis has been seen in patients raised as males with 17βHSD-3 deficiency rendering them infertile [49]. Although being reared as males, and well adjusted to the male gender role, to date no male with 17βHSD-3 deficiency has been fertile, thus infertility appears to be the rule in adulthood [23, 48].

Since the advent of molecular diagnosis, HSD17B3 gene sequencing has allowed the precise determination of the mutation causing the disorder. A national cooperative study from the Netherlands showed that 67% of patients with 17βHSD-3 deficiency were misdiagnosed as having AIS [21]. This is much higher than previously thought and underscores the high number of mislabeled 46,XY DSD patients. The correct diagnosis is paramount as the therapeutic decisions and outcomes differ based on the type of 46,XY DSD. In patients suspected of having this disorder because of an abnormally low T/Δ4 ratio, genetic analysis of the HSD17B3 gene should be sought.

The Arg80 Site and Founder Effect

The Arg80 site in exon 3 has been a frequent site for mutation in the HSD17B3 gene, especially among Mediterranean and Palestinian populations [18, 23]. The arginine (CGG) at this site is usually mutated to a glutamine (CAG), resulting in a Arg80Gln change that is the most commonly reported mutation causing 17βHSD-3 deficiency. This mutation was originally reported in a Palestinian family from the Gaza Strip [18, 23], but also in Brazilian families with no known Palestinian ancestry [21]. Extensive in vitro functional studies have been done on mutations at this specific site, arginine 80, and have confirmed the importance of this location in enzyme function [18, 34]. It was also proven that this location is important for optimal binding of the NADPH cofactor, which is necessary for 17βHSD-3 function [25]. A founder effect for the p.Arg80Gln mutation, common among the Arabs in various parts of Israel, has been speculated to be from Druze ancestors from Lebanon and Syria [21]. The same mutation is found in the Dutch, Portuguese and white Brazilians, leading to the speculation that the mutation was introduced by the Phoenicians, the ancient traders who migrated from present day Syria, Lebanon and Israel around 750 B.C. toward Portugal and Spain in search of metal and timber. It may have subsequently been passed on to the white Brazilians by Portuguese colonists and to the Dutch during Spanish rule in the 16th and 17th centuries A.D. [21]. Another theory implicates the Moors, who might have introduced the mutation from the Middle East to Spain and Portugal.

Family of 17βHSDs: 17βHSD-3

The 17βHSD class of enzymes controls the production of androgens and estrogens by controlling the final step of their biosynthesis in multiple tissues. At present, 14 mammalian 17βHSDs have been characterized, and with the exception of 17βHSD-5, which is an aldo-keto reductase, all are members of the short-chain dehydrogenase/
reductase family [14, 15, 17]. They can be grouped into in vivo oxidative enzymes (17βHSD types 2, 4, 6, 8, 9, 10, 11 and 14) and in vivo reductive enzymes (17βHSD types 1, 3, 5 and 7). Primates, unlike other mammals, are unique in their ability to synthesize sex steroids from adrenal sources of DHEA [sulfate, DHEA(S)], independently of gonadal sources, which is then localized to active sex steroids [50]. 17βHSDs, along with other steroid metabolizing enzymes such as aromatase, steroid sulfatase, 3αHSD and 3α-reductases are able to produce their own hormones at the peripheral cells, and this has given the term ‘intracrinology’ [51]. The 17βHSD-3 isoenzyme is 310 amino acids long and has a molecular mass of 35 kDa. The gene encoding the enzyme is HSD17B3. It has been localized to chromosome 9q22, is known to consist of 11 exons ranging in size from 35 bp to 264 bp, and is expressed almost exclusively in the fetal and adult testes [18]. In the Leydig cells, it catalyzes the reductive conversion of the less active Δ4-4-androstenedione into the biologically active androgen, testosterone, in the presence of a cofactor, NADPH [52]. Extranodal tissues such as bone, adipose tissue, sebaceous glands and brain have also been shown to express this enzyme [12, 15].

Other Family Members of 17βHSDs

The main function of 17βHSD-1 is the catalysis of the reduction of estrone to estradiol, with its highest concentration in the ovaries and placenta [53]. 17βHSD-2 plays a major role in inactivation of the sex steroid hormones by oxidizing estradiol and testosterone to estrone and Δ4-androstenedione, respectively [54], and has a broad tissue distribution [55]. 17βHSD-3 plays a predominant role in male testosterone production from Δ4-androstenedione [18]. Although found mainly in testes, there are reports of it being found in adipose tissue, brain, sebaceous glands and bone. Unlike other members of the 17βHSD family, 17βHSD-4 is expressed in liver [56] and intracellularly in the peroxisomes [57], and its major function has been described in murine models in the metabolism of fatty acid oxidation. It plays a minor role in steroid metabolism. Human mutations have been described that lead to severe disease and death of the patient within the first year of life [58]. 17βHSD-5, which is highly expressed in the testes, prostate, adrenals and liver, is thought to play a major role in conversion of Δ4-androstenedione to testosterone and may explain some of the virilization seen despite a mutation in 17βHSD-3. 17βHSD-7 has been shown to play a role in cholesterogenesis [59]. 17βHSD-8 has been linked to a recessive form of polycystic kidney disease [60]. Several of the 17βHSD enzymes show overlap with enzymes involved in lipid metabolism. Since most of the 17βHSD enzymes are steroid metabolizing enzymes, they are possible drug targets in many cancers, such as breast and prostate cancer, as well as common diseases, such as obesity and metabolic syndrome (table 1).

The Role of Other 17βHSDs: 17βHSD-5

An enigma of 17βHSD-3 deficiency that remains to be fully elucidated is the paradox of the failure of intrauterine virilization, but virilization in puberty. A limited capacity of the extragonadal tissues to convert Δ4-androstenedione to testosterone in embryonic life might explain the lack of virilization at birth [19]. This might then be overcome at puberty, when the levels of Δ4-androstenedione are elevated beyond any lower limit threshold and thus activate peripheral conversion into testosterone. The aromatization of Δ4-androstenedione by the placenta might prevent the extragonadal conversion of Δ4-androstenedione to testosterone in the fetus [8, 23].

In a study looking at tissue-specific transcription profiles of sex steroid biosynthesis enzymes, expression of 17βHSDs 1, 2, 3, 4, 5, 7 and 10 were all noted in the genital skin fibroblasts (both scrotal and foreskin) and only 17βHSD-2 was not seen in the peripheral blood [61]. Nearby androgen-dependent tissues, such as the foreskin and scrotum both express 17βHSD-3 and -5, and may exert local effects in addition to systemic effects. All except 17βHSD-1 showed a significantly higher mRNA expression concentration in the foreskin compared to the scrotal tissue, which points to a tissue-specific local control of steroid hormone synthesis and action [61]. 17βHSD-5 transcripts are increased with aging in scrotal skin fibroblasts, while 17βHSD-3 mRNA expression is noted to be higher in the younger age groups [61]. Age-specific differences in the 17βHSD-5 transcription profile and activity in peripheral blood mononuclear cells have also been noted [62]. This implicates 17βHSD-3 with a more important role in childhood, which later is taken over by the 17βHSD-5 after puberty. It is precisely this increase in 17βHSD-5 that could play a large role in the virilization seen in puberty in patients with 17βHSD-3 deficiency. A large interindividual variation of the 17βHSD enzyme transcript levels has also been shown to exist [61]. Microarray investigation of multiple blood samples taken on different days from the same individual showed time-dependent differences in gene clustering [63]. In light of such intra- and interindividual variability, baseline and stimulated levels of the steroid hormones can vary within a wide range of normals.
Psychological Aspects

Sex assignment of children with DSD is a subject of intense debate. The early pioneers in this field coined the term 'optimal gender policy', which advocated for early corrective surgery to help the affected children and their parents to facilitate stable gender identity and appropriate gender role behavior [64]. Opponents of early surgery argue for a 'full consent policy', in which surgery is not performed in nonemergency situations before full consent may be obtained from the child [65]. While both arguments have important pros and cons, in the case of 17βHSD-3 deficiency, we believe that a decision must be made relatively early. To wait for major intervention before puberty begins is not always feasible in 17βHSD-3 deficiency, as a child with 17βHSD-3 deficiency who is severely undervirilized (Sinnecker stage 5 or 4) and assumes a female gender role and identity, when left alone without gonadectomy, can undergo virilization throughout childhood especially at the time of puberty. As laid out in the recent comprehensive guidelines for clinicians, patients and their families by the Ethics Workgroup of German Network of DSD, sound ethical justification for treatment decisions in early childhood is the key to decision making in DSD [66]. With this approach, parents take the first-line responsibility in defining what might be best for the child, and this might vary according to their individual experience and lifestyle, cultural expectations and religious beliefs. The child, according to his or her developmental level, is granted partnership status to express preferences and vetoes [66]. Each case must be weighed on its own merits. When there is a doubt, the psychological and social support of the child and the parent is to be ranked higher than the creation of biological normalcy [66], The authors of this review subscribe to the ethical principles and recommendation for medical management laid out by this expert committee for 17βHSD-3 deficiency.

Treatment and Monitoring

Patients with 17βHSD-3 deficiency who are not diagnosed in childhood and are raised as females will invariably virilize at the onset of puberty. The gonads are involved in this virilization, as shown by spermatogenic venous blood sampling, which revealed the presence of higher testosterone levels and production [34]. This is one of the reasons for removing the testes prepubertally in a patient who will be raised as a female. Individuals who are raised as males are able to undergo male development without much medical intervention, although corrective surgery for better cosmetic appearance of the male external genitalia might be warranted. The males may virilize on their own or with the help of testosterone treatment [45]. In female patients in whom the gonads are removed, appropriate intervention with estrogen therapy should be instituted at the time of puberty to induce secondary sexual characteristics. Vaginal dilation using the modified Frank’s procedure or vaginal reconstruction surgery may be necessary to create a vaginal cavity with adequate capacity for sexual relations [42]. The patient and family will need appropriate psychological counseling to accept the diagnosis and the infertility that accompanies it [67].

Malignancy Risk

The external genitalia are mostly female in 17βHSD-3 deficiency, but the internal structures are derivatives of wolffian structures. The testes are usually positioned in the inguinal canal, sometimes at the labia majora and rarely in the abdominal cavity [11]. The consensus statement for management of DSD puts the risk of germ cell malignancy at 28% in 17βHSD-3 deficiency [5–7]. This puts it in the intermediate risk group for malignancies and close monitoring is recommended for someone who is raised as a male rather than having gonadectomy at the time of diagnosis [5–7].

Conclusion

17βHSD-3 deficiency causes an autosomal recessive form of 46,XY DSD that can be clinically indistinguishable from other forms. The correct diagnosis can be arrived at by systematic endocrine evaluation and, most importantly, by the calculation of the T/Δ4 ratio. Molecular genetic testing confirms the diagnosis and provides the orientation for genetic counseling. A high index of suspicion should be present for any female who presents with inguinal hernias or mild clitoromegaly in infancy or early childhood. Virilization in the adolescent girl should also arouse suspicion. Since there are unique clinical implications based on the diagnosis of this condition, it is important to be as prompt and accurate as possible.
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