A Novel Mutation Causing 17-β-Hydroxysteroid Dehydrogenase Type 3 Deficiency in an Omani Child: First Case Report and Review of Literature

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ABSTRACT

This is the first case report in Oman and the Gulf region of a 17-β-hydroxysteroid dehydrogenase type 3 (17-β-HSD3) deficiency with a novel mutation in the *HSD17B3* gene that has not been previously described in the medical literature. An Omani child was diagnosed with 17-β-HSD3 deficiency and was followed up for 11 years at the Pediatric Endocrinology Clinic, Royal Hospital, Oman. He presented at the age of six weeks with ambiguous genitalia, stretched penile and bilateral undescended testes. Ultrasound showed no evidence of any uterine or ovarian structures with oval shaped solid structures in both inguinal regions that were confirmed by histology to be testicular tissues with immature seminiferous tubules only. The diagnosis was made by demonstrating low serum testosterone and high androstenedione, estrone, and androstenedione:testosterone ratio. Karyotyping confirmed 46,XY and the infant was raised as male. Testosterone injections (25mg once monthly) were given at two and six months and then three months before his surgeries at five and seven years of age when he underwent multiple operations for orchidopexy and hypospadias correction. At the age of 10 years he developed bilateral gynecomastia (stage 4). Laboratory investigations showed raised follicle-stimulating hormone, luteinizing hormone, androstenedione, and estrone with low-normal testosterone and low androstendiol glucurunide. Testosterone injections (50mg once monthly for six months) were given that resulted in significant reduction in his gynecomastia. Molecular analysis revealed a previously unreported homozygous variant in exon eight of the *HSD17B3* gene (NM_000197.1:c.576G>A.Trp192*). This variant creates a premature stop codon, which is very likely to result in a truncated protein or loss of protein production. This is the first report in the medical literature of this novel *HSD17B3* gene mutation. A literature review was conducted to identify the previous studies related to this disorder.

S ex steroids of gonadal and adrenal origin have a critical role in sexual development during the different stages of life from early embryonic development to adulthood. Of the enzymes catalyzing the biosynthesis of these steroid hormones, there are enzymes that are shared in both the adrenal cortex and gonads whose defects cause congenital adrenal hyperplasia, and enzymes that exist mainly in gonads, which are responsible for the synthesis of sex steroids (testosterone in the testes and estrogens in the ovaries).[1] Disorders of sex development, including ambiguous genitalia, may develop due to gonadal dysgenesis, androgen biosynthesis defect (17-beta-hydroxysteroid dehydrogenase (17-β-HSD) deficiency or 5α-reductase deficiency), androgen

action defect (androgen insensitivity syndrome) or congenital adrenal hyperplasia (21α-hydroxylase, 17α-hydroxylase, 17,20 lyase, 3β-hydroxysteroid dehydrogenase, and cholesterol 20,22 desmolase).[1,2]

The enzyme 17-β-HSD is part of a group of isoenzymes that are involved in both the synthesis and metabolism of the sex hormones, androgens, and estrogens. 17-β-HSD1 is the major isoenzyme in the ovarian granulosa cells that catalyses the conversion of the less active estrogen, estrone, to the more active estradiol and so promotes maturation of the ovarian follicle. 17-β-HSD2 is an oxidative isoenzyme that inactivates estradiol to estrone and catalyses androgen inactivation in the target tissues. 17-β-HSD3 is expressed almost exclusively in the testis and where it helps to produce the male sex
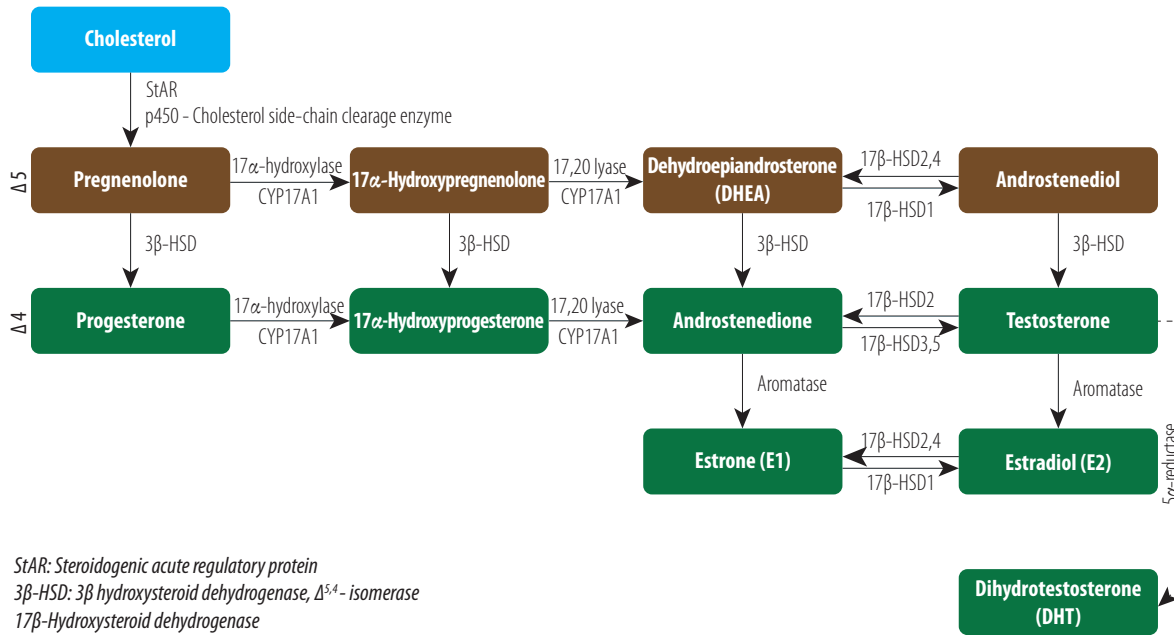
Figure 1: Biosynthesis of steroid hormones in the gonads (testes and ovaries).

hormone by converting the inactive Δ4C19 steroid androstenedione to the active androgen testosterone. 17-β-HSD4 inactivates both estradiol into estrone and Δ5 androstenediol into dehydroepiandrosterone (DHEA). 17-β-HSD5 catalyzes the formation of testosterone from androstenedione in the peripheral tissues [Figure 1].[3,4]

17-β-HSD3 deficiency (OMIM: 264300) is a rare autosomal recessive disorder of male sex differentiation (pseudohermaphroditism) resulting from a defect in the last reversible step of steroidogenesis in testosterone biosynthesis in the testes in which androstenedione is converted into testosterone. A mutation in the *HSD17B3* gene blocks the synthesis of testosterone in the fetal testis resulting in normal male Wolffian duct structures but with female external genitalia at birth. Homozygous or compound heterozygous 46,XY individuals are characterized by the absence or presence of hypoplastic internal male genitalia (prostate and testes). The diagnosis may be delayed until adolescence in phenotypic females with inguinal hernia, mild clitoromegaly, or urogenital sinus when presented with virilization and primary amenorrhea.[5,6]

Here we present the first clinically, biochemically, and genetically proven case of androgen biosynthesis defect due to 17-β-HSD3 deficiency in a 46,XY child in Oman and the Gulf region. The diagnosis of this type of pseudohermaphroditism needs a high

level of clinical suspicion with proper utilization of laboratory tools for demonstrating the different hormones, their precursors, and metabolites that characterize this disorder with confirmation by molecular mutation analysis.

CASE REPORT

This case report presents the clinical and laboratory course of an 11-year-old boy who was originally referred at the age of six weeks for expert evaluation and management of ambiguous genitalia. He was referred from Nizwa Hospital, a secondary-care regional hospital, to the Royal hospital, a tertiary-care hospital in Oman. The child's prenatal history was not significant, being born as a full-term baby of a consanguineous parents, through vaginal delivery with good weight. Systemic examination was normal apart from genitalia abnormalities. Examination of the genitalia showed a stretched penile length of 3cm, with undescended testes that were felt bilaterally in the groin. The child was assigned as boy since his delivery. There was no history of similar problems in the family.

At the age of six weeks, laboratory investigations revealed results within the reference ranges for core blood tests including electrolytes, renal, liver, bone, glucose, and thyroid profiles. The results for adrenal and gonadal steroids, pituitary hormones, and karyotyping are shown in Table 1. The low

testosterone and androstenediol glucuronide, high androstenedione and estrone levels are consistent with testosterone synthetic defect due to 17-β-HSD3 deficiency. Urine steroid profile revealed a normal quantitative pattern, which excludes 5α-reductase deficiency, which usually has comparable presentation. Ultrasound of the groin and pelvis showed no obvious evidence of any uterine or ovarian structures. There were oval shaped solid structures in both inguinal regions, each measuring about 1.5cm, which looked like testicular structures. A biopsy was performed and the histology report revealed immature seminiferous tubules only. No ovarian tissues were seen. He was referred to pediatric surgery for surgical correction of the hypospadias.

Based on the laboratory finding, the child was diagnosed as having 17-β-HSD3 deficiency. During the course of his management, testosterone injections (25mg once monthly) were given: at two and six months then at three months before his surgeries at the age of five and seven years when he underwent multiple operations for orchidopexy and hypospadias correction. He showed good response that was reflected in terms of an increase in phallas length.

Patient was followed-up in the Pediatric Endocrinology Clinic annually, until the age of 10 years when he presented with gynecomastia that was progressive and associated with voice change as well as bad body odor. On examination, his weight was 41.8kg (which had increased from the previous year by approximately 10kg), height was 141.9cm, and BMI was 22kg/m². On examination the gynecomastia was bilateral, stage 4, with pubic hair Tanner 3 (p3) and testicular volume 8ml. Laboratory investigations showed normal serum liver profile, beta-human chorionic gonadotropin (β-HCG) and α-fetoprotein levels, which excluded other causes of gynecomastia. He had also raised follicle-stimulating hormone (FSH), luteinizing hormone (LH), androstenedione, and estrone with low-normal testosterone, low androstendiol glucurunide, and normal DHEA levels [Table 2]. At this age, wrist x-ray examination showed a normal bone age compared with his chronological age. The magnetic resonance imaging showed normal pituitary gland and hypothalamic structures with no brain abnormality.

The gynecomastia could be explained by 17-β-HSD3 deficiency and the high FSH and LH

Table 1: Plasma steroids and pituitary hormones at time of presentation at the age of six weeks (compared to recommended age and sex matched reference ranges, if age and sex dependent).

Test	Result	Reference range (age range)
Testosterone (nmol/L)	2.5	0.0–6.5 (2–5 weeks)
Luteinizing hormone (IU/L)	32.6	0.3–2.8 (2 weeks–10 years)
Follicle-stimulating hormone (IU/L)	7.0	0–2.5 (2 weeks–3 years)
Androstenedione (nmol/L)	12.6	<1.7
Androstanediolglucuronide (nmol/L)	6.0	8.5–80
Dehydroepiandrosterone sulfate (umol/L)	1.0	0.2–8.6 (≤1 month)
Estrone (pmol/L)	62.9	<40 (prepubertal range)
Estradiol (pmol/L)	109	37–117 (prepubertal range)
Progesterone(nmol/L)	2.1	2.7–10.7 (0–12 months)
Chromosomal analysis	46,XY	normal male pattern

Table 2: Plasma steroids and pituitary hormones at age of 10 years (compared with recommended age and sex matched reference ranges).

Test	Result	Reference range
Testosterone (nmol/L)	3.6	0.1–2.4 (prepubertal) 9.0–31.0 (adult)
Luteinizing hormone (IU/L)	25.2	1.5–9.0
Follicle-stimulating hormone (IU/L)	59.3	1–12
Androstenedione (nmol/L)	24.4	<1.7
Androstanediolglucuronide (nmol/L)	6.0	8.5–80
Dehydroepiandrosterone sulfate (umol/L)	2.88	0.5–6.6
Estrone (pmol/L)	280	30–220
Estradiol (pmol/L)	66.0	20–95 (Tanner 3)
Chromosomal analysis	46,XY	normal male pattern

levels, which indicated primary gonadal failure. As this patient had significant gynecomastia with low testosterone, he was given testosterone injections, 50mg once a month, for a total of six months. He was followed-up and reviewed three months after commencing testosterone therapy. There was a
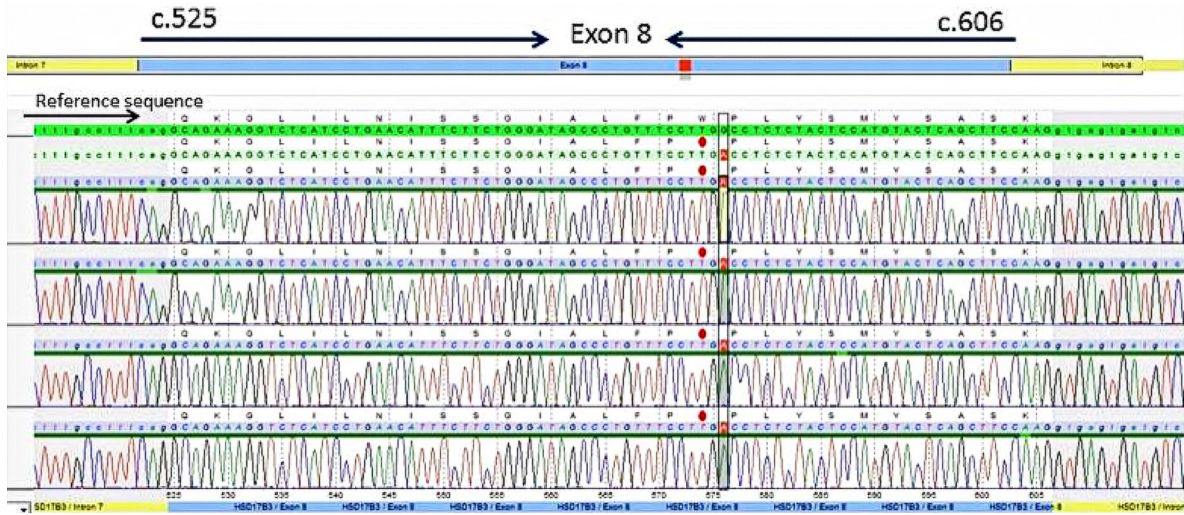
Figure 2: Electropherogram of sequencing data of exon eight of the *HSD17B3* gene (NM_000197.1).

significant reduction in his gynecomastia with much improvement in his psychological status. He remains under follow-up every six months to check for virilization during puberty, which is expected in his case. His fertility status will also be evaluated in the future as he is at risk of infertility.

Molecular genetic analysis
DNA was extracted from the patient's peripheral blood sample using QIAsymphony kit (Qiagen, USA). All 11 exons of the *HSD17B3* gene were analyzed using the polymerase chain reaction (PCR) and sequencing of both DNA strands of the entire coding region was carried out, including the highly conserved exon-intron splice junctions. The *HSD17B3* gene provides instructions for making an enzyme called 17-β-hydroxysteroid dehydrogenase 3. Mutations in the *HSD17B3* gene result in a 17-β-hydroxysteroid dehydrogenase 3 enzyme with little or no activity, thus reducing testosterone production.

The sequencing analysis revealed a previously unreported homozygous variant in exon 8 of the *HSD17B3* gene (c.576G>A.Trp192*) [Figure 2]. This variant creates a premature stop codon, which is likely to result in a truncated protein or loss of protein production. Parental samples were not available in order to confirm the homozygosity in place of compound heterozygosity for a large deletion. However, given the parent's consanguinity (they are first cousins) and the fact that so far no large deletion/duplication has been described in the *HSD17B3* gene[7] being homozygous is the most likely scenario for the detected mutation.

DISCUSSION
This is the first case of 17-β-HSD3 deficiency to be reported in Oman and the Gulf region. The clinical and laboratory course of an 11-year-old boy diagnosed with 17-β-HSD3 deficiency at the age of six-weeks old following his presentation with ambiguous genitalia (stretched penile and bilateral undescended testes) is described. The clinical, imaging and laboratory findings (adrenal and gonadal steroids, pituitary hormones, and karyotype) were consistent with 17-β-HSD3 deficiency. The diagnosis was confirmed by genetic analysis due to presence of a novel homozygous variant in exon eight of the *HSD17B3* gene (c.576G>A.Trp192*). This nonsense variant creates a premature stop codon, which is very likely to result in a truncated protein or loss of protein production. This novel mutation has not been described so far in the literature for the *HSD17B3* gene.

The gene *HSD17B3*, encoding 17β-HSD3, contains 11 exons and has been cloned and mapped to chromosome 9q22. To date, 35 mutations have been reported in the *HSD17B3* gene, three of which are STOP mutations.[7] Although mutations throughout the gene have been described, a mutation cluster region in exon nine with complete elimination of 17β-HSD3 activity was identified in many populations.[3,5,8-15] Moreover, so far, only three pathogenic mutations have been reported in patients with Middle Eastern ancestry as follows:
- P.R80Q mutation in exon 3 that has been identified in Palestinian, Turkish, Iranian and Brazalian populations.[16-18] Roster et al,[14] were the

first to identify this mutation in 24 subjects from nine Arab families from Gaza, Jerusalem, Lod, and Ramle.[5,10]

- A novel homozygous splice-site mutation (c.524 + 2T>A) in intron 7.
- A novel homozygous missense mutation in exon 11 with premature stop codon (p. Y287*), described in 46,XY Sudanese and Turkish, phenotypic females who presented with primary amenorrhea and virilism respectively.[19,20]

17-β-HSD3 deficiency is a genetic steroid disorder of testicular androgen synthesis that was first described by Saez et al.[21] As in our case, male newborns with 17-β-HSD3 deficiency usually have external genitalia with feminizing features together with undescended testes usually in the inguinal region or in a bifid scrotum.[5,10,11] The presence of Wolffian duct structures such as epididymis, seminal vesicles, vas deferens, and ejaculatory ducts may be explained by the low testosterone concentration, which appears to be sufficient for their development in utero. In addition, testosterone production through an alternative pathway catalyzed by other 17-β-HSD isoenzymes may contribute to the androgenization of these structures.[5,6]

The laboratory diagnosis of 17-β-HSD3 deficiency is usually made based on finding a characteristic biochemical pattern with predominance in 17-ketosteroids (namely androstenedione, DHEA, and estrone) compared with 17-hydroxylsteroids (namely testosterone, androstendiol, and estradiol) with consequent increase in androstenedione:testosterone and estrone:estradiol ratios in basal state or post-HCG stimulation. This biochemical profile was demonstrated in our case since six weeks of age when the diagnosis was made. The urine steroid profile was normal, which excludes 5α-reductase deficiency that usually has comparable presentation. Confirmation of the diagnosis and mutation type was done at the prepubertal age due to the importance of this age on virilization and fertility state.[1,5,6,9,11]

The decision for sex rearing in patients with 17-β-HSD3 deficiency is difficult especially that the majority of cases are diagnosed late in childhood or at puberty. Also, consensus guidelines do not clearly support one gender assignment although there is more support to male gender.[22] Sex rearing is usually revisited in these patients at the time of puberty when they develop marked virilization with penis enlargement, male pattern body hair, and muscle development.[12,13] Puberty-dependent virilization pushes many patients to change their social sex to male at puberty whether with or without surgical correction, otherwise they require bilateral orchiectomy if the female social sex is chosen. The consequent female-to-male gender reassignment has been reported in 39–64% of cases.[23] However, almost all diagnosed cases of Arab patients involve male social sex assignment.[15,17,19,23-27]

The excessive virilization at puberty and its discrepancy from intrauterine masculinization is not fully understood, but it is thought to occur by one of two mechanisms. The first is attributed to the peripheral conversion of androstenedione to testosterone by other 17-β-HSD isoenzymes particularly isoenzyme five in extragonadal tissues. The second mechanism is attributed to the raised LH in patients with 17-β-HSD3 deficiency, which in turn increases testicular testosterone production in patients with residual 17-β-HSD3 function.[3] In addition, the expression of aldo-keto reductase family 1 member C3 (AKR1C3; 17-β-HSD5) has been demonstrated in extragonadal tissues in response to high LH in both normal subjects and patients with 17-β-HSD3 deficiency.[28] The high concentration of androstenedione is converted to testosterone by the cells containing AKR1C3 (17-β-HSD5) which include extra gonadal tissues such as genital skin and adipose tissue as well as the Leydig cells of 17-β-HSD3 deficient patients.[29,30] Bilateral orchiectomy has been reported to result in a marked decrease in androstenedione as well as in virilization confirming the role of the testis as the main source of testosterone production in these patients.[3,31]

CONCLUSION

We report the first genetically proven case of 17-β-HSD3 deficiency in Oman and the Gulf region. The diagnosis is usually made by demonstrating a characteristic biochemical pattern in a profile of gonadal steroid levels together with confirmation by molecular testing. Mutation analysis in our case revealed a novel homozygous variant in exon 8 of the *HSD17B3* gene (c.576G>A.Trp192*), which is the first to be reported in the literature.

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