Complexities of diagnosis in 17β-hydroxysteroid dehydrogenase-3 deficiency, and implementation of next generation sequencing in guiding management decisions – Case series of six patients

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Background

- 17β-HSD3 gene (HSD17B3) encodes the isoenzyme required for conversion of androstenedione to testosterone in the testis.
- Isoenzyme deficiency (17β-HSD3) is a rare, autosomal recessive 46XY disorder of sex development (DSD)[1].

- Phenotype: internal Wolffian duct urogenital structures and testes, genital appearance ranges from female to ambiguous[2].

- Human chorionic gonadotropin (hCG) stimulation test assesses testosterone biosynthesis.
- Biochemical results in confirmed 17β-HSD3 deficiency may overlap with androgen insensitivity syndrome (AIS) and gonadal dysgenesis, making diagnosis 17β-HSD3) deficiency challenging.

Case Series Description

- We report six 46XY DSD patients with confirmed 17β-HSD3 deficiency. [Please see table 1.]

- Four patients identified <12months of age due to either discordant karyotype and appearance, ambiguous genitalia, or inadvertently during elective herniotomy.
- Two adolescent patients presented with progressive virilisation causing significant emotional distress, and primary amenorrhoea.

- Time taken between DSD identification and molecular genetic diagnosis ranged 6weeks – 22months.
- Significantly longer times were experienced by patients presenting prior to 2014, when a targeted gene next generation sequencing (NGS) panel for DSD became available from a UK Genetic Regional Laboratory[3].

Table 1. Summary of clinical features and endocrine evaluation

	Age at presentation	Presentation	Palpable gonads	Karyotype	Initial screening testosterone (nmol/l)	Initial screening androstenedione (nmol/l)	Initial screening unstimulated T:A ratio (age at test)	Pre & Post hCG stimulation T:A ratio (age at test)	Genetic mutation in the HSD17B3 gene	Time from diagnosis to genetic confirmation	Sex of rearing
Patient 1 (in 2009)	1 day	Discordant karyotype & prenatal ultrasound. Ambiguous genitalia at birth	Yes - palpable gonads in labio-scrotal folds bilaterally	46 XY	12	-	-	N/A → 0.7* (7months)	Heterozygous pathogenic splice mutations 325+4 A>T and p.V205VE (T>TA)	22 months	Female
Patient 2 (in 2014)	7 days	Swollen labia from birth	Yes- Gonads palpable in labioscrotal folds	46 XY	1.3	-	-	0.2 → 0.07* (5 weeks)	Heterozygous pathogenic variant c.277+4A>T and missense c.645A>T	16 months	Female
Patient 3 (in 2016)	17 years	Primary amenorrhoea & hirsutism from 10yo	No	46 XY	8.3*	33.5	0.25 (17 years)		Heterozygous pathogenic missense c.194C<T and frameshift c.729_735delGATAACC	5 months	Female
Patient 4 (in 2018)	3 months	Left inguinal hernia	Yes – right inguinal hernia, left palpable inguinal/ labioscrotal fold area	46 XY	2.2	5.7	0.4 (3months)		Heterozygous pathogenic missense variant c.194C>T and c.695C>T	4 months	Female
Patient 5 (in 2019)	15 years	Clitoral enlargement from 13yo & primary amenorrhoea	Yes – right inguinal area	46 XY	7.9*	25.3	0.3 (15 years)		Homozygous pathogenic variant splice c.277+4A>T	9 weeks	Female
Patient 6 (in 2019)	1 day	Clitoromegaly noted at birth.	No	46 XY	<0.3*	-	-	0.4 → 0.36* (1 week)	Homozygous pathogenic splice-site variant c.277+4A>T	6 weeks	Female

*Tandem Mass Spectrometry

Choices Sex of Rearing & Gonadectomy

- Decisions around sex of rearing in the infants, and all surgery (including gonadectomy) has involved the local DSD multidisciplinary team (MDT). [Please see table 2.]

Sex of rearing:
- All 6 patients have been raised female.
- Neither adolescent case have voiced gender dysphoria/ disturbance.

Gonadectomy (5 out of 6 patients):
- Case 1 & 2 – parents elected to undergo bilateral gonadectomy.
- Case 3 –during reduction of an obstructive hernia unilateral gonadectomy performed when gonadal vessels and vas were inadvertently divided, the gonad removed and sent for histopathology.
- Both adolescent patients – were involved in extensive MDT discussions & counselling and elected to have initial treatment with combined GnRH analogue and oestrogen, and subsequent elective gonadectomy.
- No patient had histological evidence of Germ cell cancer.

Table 2. Summary of surgical outcomes

	Gonadectomy	Histology	Future surgery planned?
Patient 1	At 16 months, bilateral	Left gonad – normal prepubertal testis, no evidence carcinoma in situ or tumour	Yes examination under anesthetic vaginal cavity & consideration clitoral reduction
Patient 2	At 3 months, bilateral	Left & right prepubertal testes - no evidence of gonadoblastoma or malignancy	No further plans currently.
Patient 3	At 20 years of age bilateral gonadectomy – testes found at external inguinal rings	Left & right testes - tubular atrophy, no spermatogenesis, no in situ germ cell neoplasia, no malignancy	No further plans currently.
Patient 4	At 3 months, unilateral gonadectomy at time of herniotomy	Right gonad – normal prepubertal testis, no evidence of malignancy	Left gonad remains in situ. No further plans currently.
Patient 5	At 15.6 years bilateral gonadectomy	Left & right testis with epididymis – all seminiferous tubules have Sertoli cells, nil evidence of spermatogenesis, no atypical cells seen.	No further plans currently.
Patient 6	In situ	-	No further plans currently.

Discussion

Diagnosis:
- 17β-HSD3 deficient patients typically show low testosterone levels and an abnormal testosterone: androstenedione (T:A) ratio. T:A ratio should remain low <0.8 despite hCG stimulation[4].
- However, low T:A ratio can also occur in gonadal dysgenesis and AIS, especially in cases with abnormal testes[4,5].
- Molecular genetic diagnosis is required for certainty, and NGS can provide timely diagnoses for the patient and families.

Sex of rearing:
- There has been a trend towards male assignment for 17β-HSD3 cohort. However in 17β-HSD3, dissatisfaction with penile length if raised male and clinical distress due to virilisation if raised female are commonly reported[1].
- Optimal care for infants and adolescents with DSD requires an experienced MDT[5].

Gonadectomy:
- Multiple complex considerations, such as gender identity, sexual function, surgical options, gonadal malignancy risk, to prevent further virilisation, psychological (cultural, familial, social) and potential fertility[1,5,6].

Germ cell cancer (GCC):
- Individuals with testosterone biosynthesis defects have a lower risk of carcinoma in situ development (<1-15%) during childhood and a limited tendency towards invasive progression[1,5-7].

Take home messages

- 17β-HSD3 deficiency is a rare diagnosis which shares many similarities both AIS, 5-alpha reductase, and gonadal dysgenesis DSD phenotypes.

- We highlight the difficulty interpreting both initial and hCG stimulated plasma hormone levels, and how molecular genetic diagnosis through NGS is becoming integral to providing timely diagnostic information to patients and their families.

- Complex decisions regarding sex of rearing, gonadectomy and consideration for genital corrective surgery are best managed by an expert multidisciplinary team.

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