DOI: 10.4274/jcrpe.galenos.2020.2020.0249

Case report

46, XY Sex Development Defect due to a Novel Homozygous (splice site) c.673_1G>C Variation in the *HSD17B3* Gene: Case Report

Short Title: Novel Variation in *HSD17B3* Gene

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What is already known on this topic?
The 17-beta hydroxysteroid dehydrogenase type 3 (17β-HSD3) enzyme is primarily found in the testes and is involved in transforming Δ4-androstenedionun(A), a weak androgen, to the more biologically active form testosterone (T) . Defects in the HSD17B3 gene, which encodes this enzyme, causes17β-HSD3 deficiency.

What this study adds?
In this article, we describe a previously unreported c.673_1G>C homozygous variation **that** was identified in the HSD17B3 gene of the 46, XY patient.

ABSTRACT
The enzyme 17-β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) catalyzes the biosynthesis of testosterone from Δ4-androstenedione, and plays an important role in the final steps of androgen synthesis. 17β-HSD3 deficiency originates from mutations in the HSD17B gene, causing an autosomal recessive 46, XY sex developmental disorder (DSD). Patients with 46, XY karyotype can exhibit a wide phenotypic spectrum varying from complete external female genitalia to male genitalia with hypospadias. This study reports a case of 17β-HSD3 deficiency diagnosed in the infantile period who was later found to have a novel HSD17B3 gene **variation**. The 14-month old patient, who exhibited a female phenotype, presented with a bilateral lump in the inguinal area. Imaging revealed bilateral testicular gonads in the inguinal area. Hormonal evaluation showed low levels of basal and stimulated serum testosterone (T), a high level of androstenedione (A), and a low T/A ratio. Chromosomal analysis showed 46, XY karyotype. The patient's sequence analysis of the HSD17B3 gene revealed a c.673_1G>C homozygous class 2 (splice site) variation in intron 9. The father and mother were heterozygous carriers of the same variation. This **variation** has not been previously reported in the literature. In conclusion, a 46, XY DSD should be considered in patients with a female phenotype who exhibit gonad(s) in the inguinal area at an early age; in patients with insufficient testosterone synthesis and high levels of androstenedione, 17β-HSD3 should be considered, and molecular analysis should be done for a definitive diagnosis and subsequent genetic counseling.
Keywords: 17 beta-hydroxysteroid dehydrogenase type 3, 46,XY disorders of sex development, HSD17B3 Gene

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INTRODUCTION
The 17-beta hydroxysteroid dehydrogenase (17β-HSD) enzyme family includes at least 14 isoenzymes identified to date; these contribute to the development of reproductive organs by taking part in the final steps of androgen and estrogen synthesis. The 17-beta hydroxysteroid dehydrogenase type 3 (17β-HSD3) enzyme is primarily found in the testes and is involved in transforming Δ4-androstenedionun(A), a weak androgen, to the more biologically active form testosterone (T) (1). Defects in the *HSD17B3* gene, which encodes this enzyme, causes17β-HSD3 deficiency.
17β-HSD3 enzyme deficiency was initially described in 1971 and shows an autosomal recessive inheritance (2,3). Although not known clearly, the incidence rate has been reported to be 1/147,000 live births and the rate of heterozygosity to be 1/135 (4). Furthermore, in populations with a high rate of consanguineous marriage such as the Gaza Strip Arabs, the incidence has been reported to be as high as 1/100-300 (5,6). Problems in testosterone synthesis during fetal development result in insufficient development of male external genital organs. Although testosterone synthesis is insufficient, the production of anti-mullerian hormone (AMH) continues normally and prevents the development of mullerian structures(7).
These patients with a 46, XY karyotype can exhibit a wide phenotypic spectrum varying from female external genitalia to male external genitalia with hypospadias, or ambiguous genitalia with microphallus (8). During puberty, the increase in gonadotropin levels lead to an increase in A levels and extra-testicular conversion of A to T, which ultimately leads to evident virilization. The degree of virilization can vary depending on 17β-HSD3 isoenzyme residue in the testes and the activity of other isoenzymes, such as 17β-HSD5 (9,10,11). Individuals with a 46, XX karyotype generally have normal female genitalia and are asymptomatic, making the condition difficult to diagnose (5,12).

In the laboratory analysis of 17β-HSD3 deficiency, low serum T levels and high serum A levels are observed. A human chorionic gonadotropin (hCG) stimulation test generally results in a serum T/A ratio of below 0.8 (13). A final diagnosis is made through molecular genetic testing.
In this article, we describe a case who presented with a history of a bilateral lump in the inguinal area during the infantile period, the patient's physical examination revealed external genitalia of female phenotype and palpable gonads; the evaluations were consistent with 17β-HSD3 deficiency, and a previously unreported c.673_1G>C homozygous variation was identified in the *HSD17B3* gene of the 46, XY patient.

CASE

A 14-month old female patient presented with a history of a bilateral lump in the inguinal area. Her mother and father were first-degree cousins. Physical examination showed female looking external genitalia with the absence of clitoromegaly. Gonads were palpable in both inguinal regions.Ultrasound imaging revealed gonads that were compatible with testes in both inguinal areas, and no mullerian structures were observed in the pelvis. It was speculated that the patient may have 46, XY DSD. Laboratory testing was used to assess gonad functions, showing that the serum follicle stimulating hormone (FSH) level was 2,29 IU/L (0,26-3), luteinizing hormone (LH) was 1,19 IU/L (0,02-0,3), and T level was <0,693 nmol/L. Her serum AMH level was >164.29 pmol/L (70.7-3171.4) and inhibin B level was 388 ng/L (91-400). Evaluation of basal hormone levels revealed that testosterone synthesis was insufficient. In order to precisely evaluate testosterone synthesis ability, a hCG stimulation test was conducted. Both basal and stimulated serum T levels were found low; the serum T level did not increase following stimulation. The patient's testosterone synthesis defect was confirmed. Serum T/A ratio was 0.14 (Table 1). A standard dose (250µg) synacthen test was done to exclude disorders in which testosterone synthesis defect and adrenal insufficiency may be seen together (such as 17alpha hydroxylase deficiency). The test showed an adequate level of stimulated cortisol, and normal basal and stimulated serum progesterone and dehydroepiandrosterone sulphate levels. 17-alpha hydroxylase deficiency was ruled out. The karyotype was 46, XY, and FISH analysis showed the absence of SRY gene variations. Pelvic MRI was used to comprehensively examine internal gonadal structures. The MRI revealed structures proximal to both inguinal canals that were compatible with testes, and a 30x4 mm structure that was compatible with vaginal tissue between the bladder and rectum. Diagnostic cystoscopy and laparoscopy showed the presence of gonads identical to testes in both inguinal canals. The vagina was 2 cm long. Since AMH levels were high and imaging showed the absence of mullerian structures, gonadal dysgenesis was excluded.
A gonad biopsy was made for pathological evaluation; the pathology report stated "Bilateral testes containing seminiferous tubules and surrounded by the tunica albuginea were observed. Spermatogonia were not observed in the seminiferous tubules. Leydig cells were not present with Hematoxylin and Eosin staining. Of the immune markers used, Inhibin led to a strong positive immunoreaction in sertoli cells and a mild positive immunoreaction in a small number of leydig cells; Calretinin led to a mild, positive reaction in leydig cells; mild staining with PLAP; CD138 negative; OCT3/4 negative; LH receptor showed a negative reaction." Because of the patient's pathology report stated that the leydig cells were insufficient and LH receptors were absent with specific staining; leydig cell hypoplasia was considered but *luteinizing hormone/choriogonadotropin receptor (LHCGR) gene* analysis revealed no mutation. The patient, who had a testosterone synthesis defect as well as a low serum T/A ratio, and did not have adrenal insufficiency or gonadal dysgenesis, was considered to have 17β-HSD3 deficiency. A sequence analysis of the patient's *HSD17B3* gene revealed a c.673_1G>C homozygous class 2 (splice site) variation on intron 9 (Figure 1). Both parents exhibited an identical heterozygous variation. This variation has not been reported in the literature previously, and was most likely pathological according to in silico analyses (Table 2). It was reported that in 46, XY patients with 17β-HSD3 deficiency who exhibit a total female phenotype, it is possible to achieve a penis size within normal limits through treatment with 25-50 mg/dose of intramuscular T for 3-9 months during the infantile period (14). Accordingly, our patient was treated with 50 mg/month of intramuscular T and gender determination was made based on response to treatment.
The parents gave their written consent for sharing the patient's examination, laboratory, imaging, and genetic results in scientific publications, on the condition that the child remains anonymous.

DISCUSSION

The clinical signs of 17β-HSD3 deficiency may vary due to its wide phenotypic spectrum. These 46, XY patients may have differing external genital appearances depending on the residual activity of enzymes. Patients most frequently have a complete external female genital structure, usually with separate urethral and vaginal openings; however, some patients have been reported to only have a short urogenital sinus (3,11,15). Patients with complete external female genitalia are usually diagnosed late, and are often raised as female individuals. These patients usually present during puberty with primary amenorrhea and varying degrees of virilization. In patients with evident lumps in the inguinal canals or labioscrotal folds, the palpation of gonads may lead to an early diagnosis, similar to our patient (4, 11, 16). 46, XY patients may less frequently present with micropenis and hypospadias, in which case the patient is generally raised as a male individual (5).
Due to their female phenotype and evident virilization during puberty, 46, XY patients with 17β-HSD3 deficiency are clinically similar to other conditions such as androgen insensitivity syndrome (AIS), partial 5-α-reductase type 2 deficiency, or steroidogenic factor 1 (SF1) deficiency (17). Boehmer and colleagues reported that 19 patients who were initially believed to have androgen insensitivity syndrome were diagnosed with 17β-HSD3 deficiency after further investigation (4). Leydig cell aplasia is also included in the differential diagnosis of 46, XY female patients who have been diagnosed at an early age.
The typical hormonal findings for 17β-HSD3 deficiency includes reduced T and increased A levels. While it is possible to diagnose patients through basal hormone levels during adulthood, puberty or minipuberty, a hCG stimulation test must be performed in the other age periods, or the diagnosis may be dismissed (11,13). In our patient, T levels did not increase with the hCG stimulation test and the T/A ratio was found to be low, suggesting 17β-HSD3 deficiency. Through imaging techniques, the observation of wolffian structures and the absence of mullerian structures are supportive in diagnosing 17β-HSD3 deficiency; however, since these findings are also present in both 5α reductase deficiency and androgen receptor mutations, they are inadequate for a definitive diagnosis. In individuals with 17β-HSD3 deficiency, while histological examination can reveal near

normal testicular structure at early ages, patients who reach adulthood with undescended testes usually display characteristics of testicular atrophy (exaggerated thickening of the basement membrane, evident decrease in the seminiferous tubule germinative epithelium, interstitial fibrosis, increased leydig cells) (18). According to the literature, in the pathological examination of gonads that were removed for prophylactic measures, 2-3% of cases had germ cell tumors (19). In 40 patients diagnosed with 17β-HSD3 deficiency histological examination of testicular tissue stained with hematoxylin eosin revealed that 5% of cases had germ cell tumors(18). On medical imaging, our patient had gonads in both inguinal canals that were compatible with testes and mullerian structures were absent; on the pathology report, the gonad biopsy was described as testicular tissue.
17β-HSD3 deficiency arises from the compound heterozygous or homozygous mutation of the *HSD17B3* gene. 17β-HSD3 deficiency shows an autosomal recessive inheritance pattern and is a frequent cause of 46, XY DSD among populations with high rates of consanguineous marriage. The *HSD17B3* gene consists of 11 exons and is located on chromosome 9q22. To date, more than 30 mutations have been identified in this gene, including insertion, exonic deletion, missense, and nonsense mutations (8,20-23). Most of these mutations have been identified in the Arab population of the Gaza strip. The most widespread mutation in the Arab population is the p.Arg80Gln mutation on exon 3. (4).In the Turkish population, c655-1;G-A, p.Ala188Val, and c.777-783del_GATAACC mutations have previously been identified (24). In a study by Ozen and colleagues, 20 patients being followed-up for 46, XY DSD, who did not have mutations in genes SRD5A2 and AR, were analyzed using targeted new generation sequence (TNGS) analysis for 56 potential genes which may be involved in the etiology of 46, XY DSD; mutations were identified in the *HSD17B3* gene in 30% of patients. It was reported that two patients had a homozygous p. Y287X variation, one patient had combined heterozygous p.R80Q and p.E93K variations, and three patients each had one homozygous p.T54A, p.R175T, or p.R80Q variation (25).
The literature has reported no genotype-phenotype correlation in 17β-HSD3 deficiency (26). Our patient exhibited a c.673_1G>C homozygous class 2 (splice site) variation on intron 9 of the *HSD17B3* gene; this is believed to be a novel variation as it has not been previously reported in the literature and is likely to be pathogenic according to in silico analyses.
Similar to other DSDs, gender selection proves to be a difficult decision in individuals with 17β-HSD3 deficiency, especially in cases diagnosed at an early age. The 2006 report of the Chicago Consensus Meeting recommends discussing both the fertility potential (unclear) and the development of sexual identity (mostly male) while determining sex in patients with 17β-HSD3 deficiency diagnosed during infancy (27). Male individuals with cryptorchidism and 17β-HSD3 deficiency show regression in spermatogenesis over time. These patients have an uncertain fertility potential, and a fertile 46,XY patient has not been previously identified. Gonads that are preserved should be lowered into the scrotum and routinely checked for malignancy (18,27). However, prepubescent gonadectomy is recommended for patients that are raised female because of the potential risks of germ cell tumors and virilization caused by a pubertal increase in androgens.
A significant proportion of female individuals (39-64%) who did not undergo gonadectomy and experienced virilization during their adolescence, later transitioned to the male sex (28). However, females who did undergo gonadectomy during their childhood were usually satisfied and very few individuals exhibited a desire for future sex change (4,8,29). It was reported that no individual with male-dominant phenotypes who was raised as a male desired a change in sex(28).
It is crucial that every case is evaluated individually while trying to determine sex. Since our patient was diagnosed early and evaluations showed that the patient and family embraced the individual's male identity, testosterone-based treatments were given and the patient awaited the response of the external genital structures to androgen therapy. We planned to determine the sex based on the patient's response to the treatment.

CONCLUSION

17β-HSD3 deficiency is an autosomal recessive form of 46, XY DSD. Although the diagnosis can be made with the appropriate endocrinological evaluations, it is confirmed by molecular genetic analysis. Our case showed a novel variation (c.673_1G> C homozygous) in the HSD17B3 gene. 46, XY DSD should be considered in females who present with inguinal lumps and/or mild clitoromegaly during infancy or childhood, and in adolescent females who experience virilization during puberty. For a definitive diagnosis and subsequent genetic counseling, molecular analysis should be performed on cases with insufficient testosterone synthesis and high androstenedione levels who are suspected of 17β-HSD3 deficiency. An early and accurate diagnosis is important for determining sex, patient management, and genetic counseling.

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Figure 1:*HSD17B3*gene ,c.673_1G>C homozygous class 2 (splice site) variation on intron 9

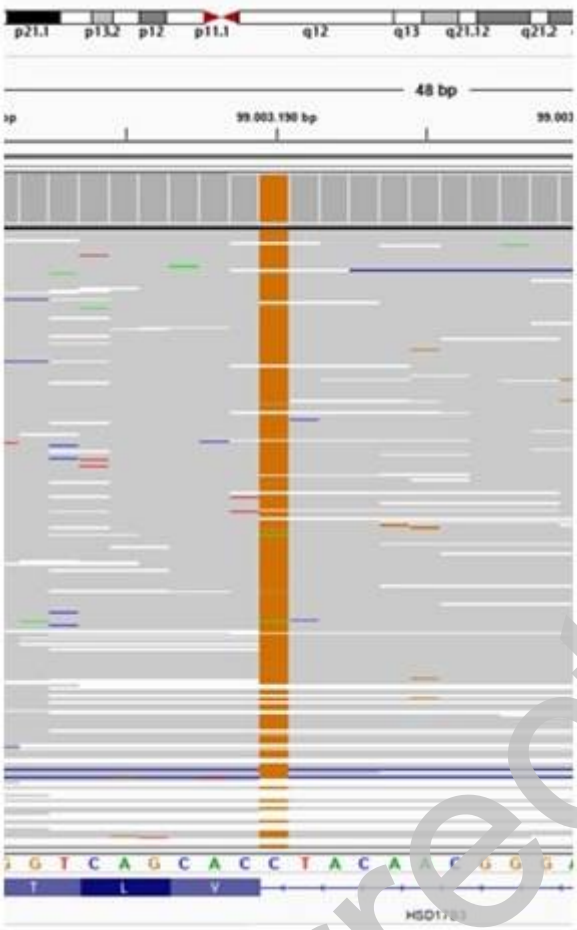

Table1:Serum androgen concentrations before and after human chorionic gonadotropin stimulation.
T: testosterone, DHT: dihydrotestosterone, hCG: human chorionic gonadotropin

Serum hormone levels	Pre-hCG	Post-hCG
T (**nmol/L**)	<0,693	<0,693
DHT (**nmol/L**)	0,11	0,3
Androstenedione (Δ4) (**nmol/L**)	<0,83	3,83
T/DHT	5	1,81
T/Δ4	0,66	0,14

Table 2: The in silico analysis result, revealing the genetic variation in the *HSD17B3*gene of our patient.

GENE	HSD17B3
Genbank transcript ID	NM-000197.2
Chromosomal Locus	9q22.32
DbSNP	novel
Variant	c.673-1G>C
Variant Location	Intron9
Variant Type	Splice-site
Mutation Taster	Disease causing
Polyphen-2	Damaging
Varscak Splice –site Prediction	Class5(Splicing Effect)
Eigen score	Pathogenic
ExAC(allele frequency)	Not found
GnomAD exomes	No entry
ClinVAR	-
Conservation	Conserved
DANN score	0,9952
ACMG Classification	Likely pathogenic
ACMG Pathogenity Criteria	PVS1, PM2, PP3