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

Nurdan Çiftci, Leman Kayaş, Emine Çamtosun, Ayşehan Akıncı
İnonu University Faculty of Medicine, Department of Pediatric Endocrinology, Malatya, Turkey

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, causes 17β-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

Corresponding Author
Nurdan Çiftci, Research Fellow in Pediatric Endocrinology, MD, Department of Pediatric Endocrinology, İnonu University Faculty of Medicine, Malatya, Turkey. Tel: +90 422 3410660 (5377)
0545321312
pediatrinurdan@gmail.com
ORCID no: 0000-0002-8203-3572

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-androstenedione(A), a weak androgen, to the more biologically active form testosterone (T) (1). Defects in the HSD17B3 gene, which encodes this enzyme, causes 17β-HSD3 deficiency.

17β-HSD3 enzyme deficiency was initially described in 1971 and shows an autosomal recessive inheritance (2,3). Although not well-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 may 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, XY 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. 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, an hCG stimulation test was conducted. Both basal and stimulated serum T levels were found low; the 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. 17alpha 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 vaginal tissue 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 reported bilateral testes containing seminiferous tubules and surrounded by the tunica albuginea were observed. Spermatogenesis were not observed in the seminiferous tubules. Leydig cells were not present with Hematoxylin and Eosin staining. Most 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, it was considered 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 normal 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 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 testicular masses 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 5α-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 micropuberty, 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 wolfian 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 characteristic features 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 gonadal 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, exon or 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, c.655-1G>A, p.T54A, p.T175T, 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 female 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 female 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 evaluated 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 genitalia 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. 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 female 46,XY patient has not been previously identified. Gonads that are preserved should be lowered into the scrotum and routinely checked for malignancy. 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 female 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 evaluated 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 genitalia structures to androgen therapy. We planned to determine the sex based on the patient’s response to the treatment.

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

Table 1: Serum androgen concentrations before and after human chorionic gonadotropin stimulation.
T: testosterone, DHT: dihydrotestosterone, hCG: human chorionic gonadotropin

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<tr>
<th>Serum hormone levels</th>
<th>Pre-hCG</th>
<th>Post-hCG</th>
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<tr>
<td>T (nmol/L)</td>
<td>&lt;0.693</td>
<td>&lt;0.693</td>
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<tr>
<td>DHT (nmol/L)</td>
<td>0.11</td>
<td>0.3</td>
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<tr>
<td>Androstenedione (Δ4) (nmol/L)</td>
<td>&lt;0.83</td>
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<td>T/DHT</td>
<td>5</td>
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<tr>
<td>T/Δ4</td>
<td>0.66</td>
<td>0.14</td>
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Table 2: The in silico analysis result, revealing the genetic variation in the \textit{HSD17B3} gene of our patient.

<table>
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<td>Variant Type</td>
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<td>Polyphen-2</td>
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