Case report

Unusual Presentation of a Denys-Drash Syndrome Girl with Undisclosed Assumption of Biotin

Short Title: Misdiagnosed Hyperandrogenism in Denys-Drash Syndrome Girl

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What is already known on this topic?

WT1 gene mutations are associated with Denys-Drash syndrome, characterized by steroid-resistant nephrotic syndrome, Wilms tumor, disorder of sex development (DSD) with dysgenetic gonads and gonadoblastoma risk in males. The renal manifestations are generally the only pathological condition in females. Hormone assays support the endocrinological assessment and the suspicion of gonadal dysgenesis.

What this study adds?

We describe a girl with an unusual presentation of a Denys-Drash syndrome (DDS), with end stage renal failure, severe genital abnormalities, signs of hyperandrogenism, and suspected dysgenetic gonads.

Recent clinical history revealed that the patient assumed biotin, and the high levels of testosterone were due to an analytical interference of laboratory immunoassay.

Questioning for biotin supplementation should be conducted, since patients may not consider biotin as a medication and therefore may not mention it in their medication list.

Abstract

We describe a 15 year-old girl with Denys-Drash syndrome (DDS), showing both kidney disease and genital abnormalities, in whom a misdiagnosis of hyperandrogenism was made.

A 15 year-old girl was affected by neonatal nephrotic syndrome, progressing to end stage kidney failure. Hair loss and voice deepening were noted during puberty. Pelvic ultrasound and MRI showed utero-tubaric agenesis, vaginal atresia and urogenital sinus, with inguinal gonads. Gonadotrophin and estradiol levels were normal, but testosterone levels increased up to 285 ng/dL at Tanner stage 3. She underwent prophylactic gonadectomy and histopathology reported fibrotic ovarian cortex containing numerous follicles at different maturation stages and rudimental remnants of Fallopian tubes. No features of gonadoblastoma were detected.

Unexpectedly, testosterone levels were found elevated 4 months after gonadectomy (157 ng/dL). Recent medical history revealed a chronic assumption of a high daily dose of biotin, as a therapeutic support for hair loss. Laboratory immunoassay instruments used streptavidin-biotin interaction to detect hormones and, in competitive immunoassays, high concentrations of biotin can result in false high results. Total testosterone, measured using liquid chromatography tandem mass spectrometry (LC-MS/MS), was found within reference intervals. Similar testosterone levels were detected repeating the immunoassay two weeks after biotin uptake interruption.

Discordance between clinical presentation and biochemical results in patients taking biotin, should rise the suspicion of erroneous results. Improved communication among patients, health care providers, and laboratory professionals is required concerning the likelihood of biotin interference with immunoassays.

Keywords: Denys-Drash Syndrome, testosterone, biotin, DSD

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Introduction

Wilms’ tumor suppressor gene 1 (WT1, OMIM *607102) is essential for kidney and gonadal development [1-2] and WT1 gene mutations are associated with Denys-Drash syndrome. In 46,XY subjects, WT1 mutations are associated with steroid-resistant nephrotic syndrome, Wilms tumor, disorder of sex development (DSD) with dysgenetic gonads and gonadoblastoma risk. On the contrary, the impact of WT1 gene on the genital development of 46,XX subjects is not clear and most affected subjects show the renal manifestations of the condition only [1-2].

We describe a girl with end stage renal failure, severe genital abnormalities, signs of hyperandrogenism, and suspected dysgenic gonads. Recent clinical history revealed that the patient assumed biotin, and the high levels of testosterone were due to an analytical interference of laboratory immunoassay.

Case Description

A 15 year-old Caucasian Italian girl had manifested steroid-resistant nephrotic syndrome in the first month of life. Kidney biopsy at onset showed mesangial proliferative glomerulonephritis with focal segmental glomerulosclerosis. End-stage renal failure was reached at 2 years of age. Cytogenetic analysis showed a normal 46,XX female karyotype. Sanger sequencing of WT1 gene, performed at 5 years of age in another Institution, showed the missense mutation c.1097G>A of exon 8, causing the amino acid change Arg366His affecting the zinc finger 2. The mutation was de novo and in heterozygous state. She underwent left nephrectomy at 1 year of age. Right nephrectomy and a first renal transplantation were performed at 13 years of age. Chronic primary EBV infection was diagnosed early after transplantation and did not respond to immunosuppression reduction and Rituximab. In the following years progressive chronic allograft nephropathy developed and renal function worsened. At the age of 12 years hemodialysis was restarted and 10 months later the transplanted kidney was removed.

Puberty started at 13 years. A few weeks later, hair loss and voice deepening were observed. Repeated hormone assays, measured by chemiluminescence (ADVIA Centaur XPT Immunoassay System, Siemens Healthcare) showed normally increasing pubertal female levels of gonadotrophins and estradiol, but testosterone level progressively increased up to abnormally high concentrations (285 ng/dl) when the girl reached Tanner stage 3 of breast and pubic hair development. The levels of adrenal androgens and precursors (delta4 androstenedione, DHEAS and delta5 P4 progesterone) were in the normal range for a pubertal female, excluding the adrenal origin of hyperandrogenism. These data suggested the presence of dysgenic gonads.

Pelvic ultrasound and MRI showed absence of uterus and Fallopian tubes, vaginal atresia and urogenital sinus. Both gonads were located at the internal inguinal ring. The right gonad appeared small, with a relatively homogeneous (streak-like) structure and rare anechoic areolas. The left gonad was larger and showed an anechoic area consistent with a dominant follicle. As a second step, a targeted next generation sequencing was performed using a customized panel of disorders of sex development, including all coding exons and flanking introns of the following genes: AR, FOXL2, FST, HSD17B2, NR5A1, RSP01, SOX3, SOX9, SRY, WNT4, WT1. Sequence enrichment was performed using the NimbleGen SeqCap Target Enrichment kit (Roche) and sequenced on the Illumina NextSeq550 platform (Illumina, San Diego, CA). The WT1 Arg366His mutation was confirmed, while no others mutations were found (Figure 1).

The neoplastic risk associated with WT1 mutations, the need to plan a second renal transplantation with the related long-term immunosuppressive therapy, the absence of Mullerian structures with potentially dysgenic gonads producing testosterone led to consider prophylactic gonadectomy. She underwent gonadectomy at the age of 14 years. Gross examination revealed small multicystic ovaries. Microscopy showed fibrotic ovarian cortex containing numerous follicles in different maturation stages, from primordial to secondary ones (Figure 2); some follicles were cystic and scattered corpora lutea were observed. No features of gonadoblastoma were detected. Rudimental remnants of Fallopian tubes were present. Four months after gonadectomy hair loss appeared improved but hormonal tests unexpectedly showed elevated testosterone levels (157 ng/dL).

In-depth interview on recent medical history revealed daily assumption of high dosage of biotin started 8 months before, as a therapeutic support for hair loss. Plasmatic level of biotin was higher than 800 mg/L. The patient’s total testosterone levels, collected during biotin intake and after its cessation, were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) and were both found within reference intervals (respectively 4 and 5 ng/dL). Similar testosterone levels were confirmed using immunoassay testing two weeks after biotin uptake interruption.

Discussion

WT1 encodes a DNA binding protein containing 4 zinc fingers, which is essential for normal mammalian urogenital development [3]. WT1 knockout mice lack gonads in both sexes, suggesting a role of this gene during the formation of the genital ridge, an early stage of genital development when the gonad is still undifferentiated [4]. Classically, its pathogenic variants are associated with abnormal tests development, leading to 46,XY DSD; while 46,XX subjects generally show normal female genitalia [5-7].

WT1 mutations have been described in two 46,XX patients with premature ovarian insufficiency [8]. Minor genital abnormalities, such as streak ovaries or bicornuate uterus have been reported sporadically [2]. Steroid-resistant nephrotic syndrome associated with absence of both ovaries has been described in a single case [9]. A 46,XX woman showing adult onset of both focal segmental glomerulosclerosis and hypergonadotropic hypogonadism has been recently reported.

Cytoscopy showed myomas of uterus and cervix, and streak gonads. Both tubes were lying face up with missing fimbrian funnel [10]. None of the aforementioned patients showed abnormalities of the external genitalia. Recently, a novel frameshift WT1 variant (c.1453_1456del; p.Arg485Glyfs*14) has been reported in a SRY-negative 46,XX girl with clitoridomegaly, single perineal opening, and short blind-ending vagina. At 10 years of age, basal gonadotrophins were low, but GnRH analog stimulation test showed a significant elevation of testosterone levels, without an increase in estradiol levels. She underwent bilateral gonadectomy, confirming bilateral testes with seminiferous tubules containing predominantly Sertoli cells and rare germ cells. An immature right uterine tube was also identified [11].

The Arg366His mutation found in our patient, was first described in a 46,XY subject with early onset renal disease, female external genitalia with right dysgenetic testis, left streak-gonad and absence of both Mullerian and Wolffian structures. Histopathology of the removed gonads showed a gonadoblastoma [2]. The Arg366His mutation has been subsequently described in several 46,XY subjects with Denys-Drash syndrome, while 46,XX patients with this mutation generally show normal female genitalia and normal pubertal development [5-7]. An
exception are the 2 identical twins described by Vikas [12]. Both were phenotypically females and died a few weeks after birth due to multiorgan failure. At autopsy, the gonads were normal sized ovaries in both twins. Twin A had a complete duplication of uterus and vagina. Mesonephric remnants were prominent in the mesovarium of both twins. Twin B had a microscopic cluster of tubules within the mesovarium consisting of germ cells and supporting cells, reminiscent of testicular architecture. Fluorescent in situ hybridization (FISH) analysis for detection of the Y chromosome was negative in both twins. Biotin (also known as vitamin H, vitamin B7, and coenzyme R) is a water-soluble vitamin, naturally present in some foods, with urinary excretion and plasmatic levels between 100–250 µg/L. In Western populations, dietary biotin intake is estimated to be 35 to 70 µg daily, a level in line with the recommended dietary allowance. In the last years, high-dose supplementation (doses greater than 1 mg/d) has played a role in the treatment of several diseases, including biotinidase deficiency, mitochondrial metabolic disorders, and multiple sclerosis. Furthermore, advised doses up to 10 mg/day are frequently encountered in nutritional supplements taken to improve hair, skin, and nail health. Many common blood tests employ a biotin-streptavidin reaction as part of the test procedure. While the amount of usual dietary biotin intake is not expected to be high enough to affect these tests, biotin supplementation at doses greater than 1 mg per day can either falsely lower or falsely high test results, depending on the analyte and platform used for testing [13]. Briefly, excess biotin in blood competes with biotinylated antibody of the assay, which produces falsely decreased hormone concentrations in sandwich or non-competitive immunoassays and falsely increased hormone concentrations in competitive immunoassays. Several reports have shown analytical biotin interference, especially in thyroid function tests, but only one included total testosterone measurement [14]. Our analytical platform measured testosterone by a competitive immunoassay; elevated concentrations of biotin compete with biotin-antibody (labeled) analyte complexes for binding to the streptavidin-coated well, leading to the detection of a diminished signal causing a falsely high analyte result. Our case confirms that the clinical phenotype of subjects with WT1 mutation and 46,XX karyotype may include a severe DSD. The clinical signs of hyperandrogenism, partially improved after gonadectomy, suggest that the girl had a hypersecretion of ovarian androgens during puberty, but the “male” levels of testosterone mostly resulted from the analytical interference. Unfortunately, we don’t know the “true” testosterone levels before gonadectomy, because we don’t have any testosterone measurement without biotin assumption. Several different factors primarily impacted the decision-making process leading to prophylactic gonadectomy: the potential risk of gonadoblastoma associated with WT1 mutations; the need of a second renal transplantation with long-term immunosuppressive therapy; the absence of Mullerian structures associated with abnormally located and potentially dysgenetic gonads. However, the spuriously elevated testosterone levels apparently confirmed clinical hyperandrogenism, and supported the suspicion of gonadal dysgenesis. Surprisingly, histology showed normal appearance of ovarian tissue, but we cannot exclude the presence of abnormal clusters of androgen secreting cells. Experimental studies on mouse models demonstrated that WT1 gene expression controls the differentiation of genital ridge somatic cells into granulosa or Sertoli cells in genetically female and male gonads, respectively. When WT1 is deleted, these somatic cells turn into steroidogenic cells, hyper-expressing enzymes involved in androgen synthesis, without any sex dimorphism [15]. Questioning for biotin supplementation should be conducted, since patients may not consider biotin as a medication and therefore may not mention it in their medication list. In the presence of discordance between clinical presentation and biochemical results in patients taking biotin-containing medications, we recommend to repeat specimen collection after at least 48 hours of interruption.

Ethics
Ethics Committee Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee

Informed Consent: Informed consent was obtained from all individual participants included in the study

Authorship Contributions
Surgical and Medical Practice: Carla Bizzarri, Luca Dello Strologo, Isabella Guzzo, Francesco Emma, Marco Cappa
Concept: Carla Bizzarri, Marco Cappa, Ottavia Porzio, Luca Dello Strologo, Isabella Guzzo, Francesco Emma
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Data Collection or Processing: Carla Bizzarri, Germana Antonella Giannone, Jacopo Gervasoni, Sabina Benedetti, Federica Albanese, Francesca Diomedi Carnassei
Analysis or Interpretation: Carla Bizzarri, Germana Antonella Giannone, Jacopo Gervasoni, Sabina Benedetti, Federica Albanese, Luca Dello Strologo, Isabella Guzzo, Mafalda Mucciolo, Ottavia Porzio
Literature Search: Carla Bizzarri, Ottavia Porzio
Writing: Carla Bizzarri, Ottavia Porzio

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Conflict of Interest: No conflict of interest

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REFERENCES


Table 1. Laboratory measurements before and after gonadectomy and with or without assumption of biotin

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<th>Before gonadectomy</th>
<th>After gonadectomy</th>
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<tr>
<td></td>
<td>With Biotin</td>
<td>With Biotin</td>
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<tr>
<td>Testosterone (ng/dl)</td>
<td>285</td>
<td>147</td>
</tr>
<tr>
<td>LH (mU/mL)</td>
<td>7.4</td>
<td>191.2</td>
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<td>FSH (mU/mL)</td>
<td>5.3</td>
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<tr>
<td>Estradiol (ng/dl)</td>
<td>136.3</td>
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Patient DNA was sequenced using a custom panel including genes involved in 46,XX DSD. Sequence enrichment was performed using the NimbleGen SeqCap Target Enrichment kit (Roche) and sequenced on the Illumina NextSeq550 platform (Illumina, San Diego, CA). VariantStudio software (Illumina, http://variantstudio.software.illumina.com/) was used for variants annotation. Each single variant has been evaluated for the coverage and the Qscore, and visualized via Integrative Genome Viewer (IGV) software. The variant was analyzed in silico using prediction pathogenicity software (Scale-Invariant Feature Transform - SIFT and Polymorphism Phenotyping v2 -Polyphen2) and database of variants frequency.

Figure 1. Next Generation Sequencing (NGS) analysis WT1: variant visualization on IGV (Integrative Genome Viewer).
Figure 2: Gonadal histology

A) Right ovary: multiple cystic follicles in the fibrotic cortex (Hematoxylin Eosin 2,5x).
B) (Higher magnification of the red insert in A) Follicles in different maturation stages: primordial (arrows), primary (arrowheads) and late stage secondary (asterisk) follicles (Hematoxylin Eosin 10x).
C) Left Ovary: fibrotic cortex containing some dilated follicles (asterisks) and a small corpus luteum (star) (Hematoxylin Eosin 2,5x).
D) Left Ovary: Follicles in different maturation stages: primordial (arrows), primary (arrowheads) and early stage secondary (asterisk) follicles (Hematoxylin Eosin 20x).