Proximal Hypospadias and a Novel WT1 Variant: When Should Genetic Testing Be Considered?

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We present a case of an infant with proximal hypospadias, penoscrotal transposition, and bilaterally descended testes found to have a clinically significant WT1 gene alteration on a customized disorder of sex development genetic panel in which 62 genes associated with 46, XY disorders of sex development were evaluated. This diagnosis led to early screening for and diagnosis of treatment of Wilms tumor. Patients with proximal hypospadias are not routinely evaluated by genetic testing, and when initial hormonal analyses are within normal ranges for a typical male patient, the genital atypia is usually attributed to an isolated anatomic abnormality. There is no consensus among urologists, endocrinologists, or geneticists regarding when genetic testing is warranted in these patients or the extent of genetic testing that should be pursued. However, given advances in genetic testing and the discovery of more genetic variants, the genetic evaluation of infants with proximal hypospadias should be considered on an individual patient basis. Only with continued evaluation and the identification of further genetic variants can we establish future parameters for genetic evaluation in patients with proximal hypospadias and more appropriately counsel patients and their families regarding the implications of these variants.

Disorders of sex development (DSDs) are congenital conditions caused by atypical sexual ontogenesis secondary to chromosomal, gonadal, or anatomic abnormalities. DSDs affect ~1 in 2000 to 5000 individuals and encompass an array of phenotypes and genotypes, from atypical genitalia in infancy to primary amenorrhea in adolescence to infertility in adulthood. Determining an etiology in infants with atypical genitalia is challenging. Historically, ~10% to 15% of patients with a 46, XY DSD received a genetic diagnosis via testing of SRY and NR5A1; however, through exome sequencing panels, diagnostic yield increases up to 35%. In the past, genetic testing had been reserved for the most severe cases. As genetic technology advances, testing becomes more affordable and new pathogenic mutations are discovered; there is now increasing consideration of genetic evaluation in less severe forms of genital anomalies. Disorders once considered purely anatomic abnormalities, such as isolated hypospadias or isolated cryptorchidism, have not been routinely evaluated with genetic testing. Among patients with hypospadias, there are no clear guidelines regarding when genetic laboratory evaluation is indicated or the extent of evaluation.

We present a patient with hypospadias, penoscrotal transposition, and bilaterally descended testes found to have a clinically significant WT1 gene alteration on a customized disorder of sex development genetic panel in which 62 genes associated with 46, XY disorders of sex development were evaluated. This diagnosis led to early screening for and diagnosis of treatment of Wilms tumor. Patients with proximal hypospadias are not routinely evaluated by genetic testing, and when initial hormonal analyses are within normal ranges for a typical male patient, the genital atypia is usually attributed to an isolated anatomic abnormality. There is no consensus among urologists, endocrinologists, or geneticists regarding when genetic testing is warranted in these patients or the extent of genetic testing that should be pursued. However, given advances in genetic testing and the discovery of more genetic variants, the genetic evaluation of infants with proximal hypospadias should be considered on an individual patient basis. Only with continued evaluation and the identification of further genetic variants can we establish future parameters for genetic evaluation in patients with proximal hypospadias and more appropriately counsel patients and their families regarding the implications of these variants.

abstract

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Cases to See: The Case of the Unexplained 1

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transmitted, and bilaterally descended testes diagnosed with a novel WT1 variant identified through a customized, multigene panel (XomeDxSlice; GeneDx, Gaithersburg, MD). The WT1 gene, on chromosome 11p13, plays an important role in early embryonic development of the renal and urogenital systems. Clinical presentations of WT1 pathogenic variants encompass a spectrum of features, including genital anomalies, renal failure, Wilms tumor (WT), gonadoblastoma, and syndromes such as Denys-Drash syndrome (DDS), Frasier syndrome (FS), and isolated nephrotic syndrome. Full deletions of the WT1 gene result in WAGR syndrome (Wilms tumor, aniridia, genital anomalies, and intellectual disability). We demonstrate how identification of a novel, likely pathogenic WT1 variant led to early screening, detection, and treatment of WT.

**CASE PRESENTATION**

A 7-day-old infant was referred to our multidisciplinary DSD clinic. At the 20-week fetal ultrasound, sex was indeterminate; the pregnancy was otherwise uneventful. The infant was delivered without incident via induced vaginal delivery. A detailed family history was noncontributory, albeit maternal history was limited by adoption. An initial examination revealed a healthy appearing infant who was appropriate for gestational age in weight, length, and head circumference with no apparent anomalies other than on the genitourinary examination, which revealed penoscrotal transposition with a well-formed phallic structure and perineal hypospadia. The labioscrotal folds were rugated and fused posteriorly; testes were palpable in the scrotum bilaterally. The karyotype was 46, XY, and an ultrasound revealed bilaterally descended testes and no Mullerian structures.

Multiple laboratory evaluations were performed within the first month of life (see Table 1). Testosterone levels were typical of a male infant. Evaluation of the adrenal axis revealed no abnormalities consistent with congenital adrenal hyperplasia. The testosterone-to-dihydrotestosterone ratio was not consistent with a diagnosis of 5α reductase deficiency. Thus, more common causes of 46, XY DSDs were excluded. This, in conjunction with normal hormone levels for a male patient and the presence of bilaterally descended testes, led clinicians to believe the abnormal genitourinary findings were most likely due to an isolated anatomic abnormality. However, a genetic anomaly remained possible, and the family was counseled regarding testing options. They decided to proceed with a customized XomeDxSlice that evaluates 62 genes known to be associated with DSDs.

Testing identified a novel, heterozygous WT1 variant in exon 1 of the WT1 gene (NM_024426.4, GRCh37/UCSChg19), p.Glu153Ter (E153X): c.457G>T. This previously unreported WT1 variant resulting in a premature stop codon was predicted to cause loss of normal protein function via protein truncation or nonsense-mediated messenger RNA decay. Although our patient’s mutation was not previously described and therefore of unclear clinical significance, similar variants in the same region were reported as pathogenic with variable phenotypes. The clinical phenotype and likely pathogenic variant in our patient prompted further evaluation of known WT1 variant anomalies, including WT screening.

Initial ultrasound at 5 months of life revealed mild enlargement of the left kidney with nonspecific areas of increased echogenicity; a repeat ultrasound, 3 weeks later, revealed 2 focal areas of abnormal parenchyma in the left kidney and a new cluster of small cortical cysts in the right kidney. Through MRI, multifocal, bilateral renal lesions consistent with nephroblastomatosis as well as a dominant, spherical lesion in the upper-right kidney measuring 2.8 × 1.9 × 2.5 cm were shown, which were concerning for WT (Fig 1A). Repeat imaging before initiation of therapy revealed significant interval enlargement of multiple bilateral renal masses, the largest measuring 4.3 × 4.3 × 4.1 cm, suggestive of bilateral WT (Fig 1B); however, MRI cannot always differentiate between WT and nephroblastomatosis. Biopsy specimens from the dominant right renal lesion obtained after 6 weeks of chemotherapy revealed intralobar nephrogenic rests and favorable histology for WT (Fig 2). After another 6 weeks of chemotherapy, the patient underwent right radical nephrectomy, and biopsy specimens from the left renal lesions revealed nephrogenic rests. The patient continues on chemotherapy and is currently in complete remission.

**TABLE 1 Laboratory Results**

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Reference Range</th>
<th>4 d Old</th>
<th>7 d Old</th>
<th>25 d Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-hydroxyprogrenolone, ng/dL</td>
<td>10–829</td>
<td>227</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Androstenedione, ng/dL</td>
<td>10–279</td>
<td>25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DHEAS, µg/dL</td>
<td>—</td>
<td>49</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total testosterone, ng/dL</td>
<td>72–344 at 1–3 mo</td>
<td>—</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>Dihydrotestosterone, ng/dL</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>T/DHT ratio</td>
<td>—</td>
<td>6.66</td>
<td>3.33</td>
<td>—</td>
</tr>
<tr>
<td>LH, mIU/mL</td>
<td>—</td>
<td>—</td>
<td>7.89</td>
<td>—</td>
</tr>
<tr>
<td>FSH, mIU/mL</td>
<td>—</td>
<td>4.6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

DHEAS, dehydroepiandrosterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T/DHT, testosterone/dihydrotestosterone; —, not applicable.

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FIGURE 1
A. MRI (abdomen) revealing multiple bilateral renal lesions compatible with nephroblastomatosis and a lesion (arrow) in the upper-right kidney concerning for neoplastic rest or WT because of the dominant size and spherical shape. B, Repeat MRI before treatment (5.5 weeks later) revealing significant enlargement of multiple bilateral renal masses suggestive of bilateral WT.

DISCUSSION

Our patient’s novel WT1 variant, p.E153X, initially interpreted as likely pathogenic, was reclassified as pathogenic after parental test results were negative and genitourinary anomalies in association with WT development in our patient were identified. As discussed, there is a wide array of phenotypic variability associated with WT1 alterations, including isolated WT and syndromic and nonsyndromic disorders involving various degrees of genitourinary abnormalities, ocular abnormalities, intellectual disabilities, and nephrotic syndrome.9

WT1 mutations, once thought to cause distinct syndromes such as DDS or FS, are now considered to encompass a phenotypic spectrum that may correlate with the location and type of alteration.7,10 Historically, both DDS and FS present with ambiguous genitalia or complete sex reversal in XY individuals secondary to complete or partial gonadal dysgenesis. DDS has been reported in association with heterozygous germline missense variants in WT1 exons 8 or 9, which also have high rates of nephropathy.10 However, mutations of all types and in all exons have been reported in patients diagnosed with DDS or partial DDS.10–14 WT1 exon mutations are associated with WT in 36% to 88% of patients with bilateral WT, and these mutations are highest in truncating variants.13 Progressive renal failure occurs in 74% of those with WT, with milder phenotypes associated with mutations outside exons 8 and 9.7,10,15,16 FS is classically associated with intron 9 splice-site variants, resulting in gonadoblastoma and nephropathy without WT. However, both gonadoblastoma and WT have been reported in a single patient, indicating that DDS and FS are not isolated syndromes.7,17–19 Further supporting a diagnostic continuum rather than isolated syndromes, WT1 variants were identified in several 46, XY patients with genital malformations with and without WT and/or nephropathy and in patients with WT without genital malformations and/or nephropathy.7,11–14,20–23

Despite their clear association with genitourinary malformations, WT1 variants are an uncommon cause of isolated hypospadias.7,24,25 In a pediatric series, 210 male patients with 46, XY DSDs were assessed for genetic alterations and a WT1 variant was identified in 7. Two of these patients presented with penoscrotal hypospadias, normal phallus size, bifid scrotum, and unilateral cryptorchidism, abnormalities similar to the ones identified in our case patient except for the presence of cryptorchidism. One patient with a truncating mutation in exon 1 had WT in infancy and normal renal function at age 23. Another patient with a missense mutation in exon 2 had no reported renal abnormalities by age 12. All 7 patients had unilateral or bilateral cryptorchidism, and 6 had associated hypospadias; WT1 variants were not identified in any patient with bilaterally descended testes. The authors concluded that newborns with complex hypospadias and unilateral or bilateral cryptorchidism should be evaluated for WT1 mutations; in those with severe hypospadias and bilaterally descended testes, however, they proposed that WT1 analysis is not warranted.7

In other studies, researchers evaluating patients for WT1 variants reported that all patients with a WT1 alteration and hypospadias had a history of unilateral or bilateral cryptorchidism.7,10,26 Specifically, Lehnhardt et al10 described the phenotypes of 53 individuals with constitutional, heterozygous WT1 mutations treated at pediatric nephrology centers in Germany, Austria, and Switzerland and identified 32 male patients with genitourinary malformations. All male patients with hypospadias had unilateral or bilateral cryptorchidism with or without other anomalies. Lehnhardt et al10 noted that only 1 patient in this cohort fit a classic diagnosis for a WT1 disorder, supporting previous reports that these syndromes (DDS and FS) are the extremes of a spectrum of disorders.27 Wang et al25 evaluated 90 male patients with hypospadias for mutations in SRY, SOX9, WT1, AR, and SRD5A2 and identified WT1 variants in 3 adult patients with hypospadias and no other abnormalities.
Despite bilaterally descended testes, multigene panel testing resulted in identification of a WT1 variant in our patient. The multigene panel employed was created by an endocrinologist and genetic counselor after review of the current literature for use in cases of atypical genitalia without a clear etiology and broad differential diagnosis. In these situations, multigene panels increase diagnostic yield at a lower cost than sequential, single-gene testing; targeted gene testing; or whole-exome sequencing. Ultimately, identification of a WT1 variant prompted screening for and diagnosis of WT. This is significant because earlier diagnoses of WT may result in identification of smaller tumors and the potential for nephron-sparing surgical options.

With our case, we add to a small number of cases that can be used to contradict previous suggestions that WT1 testing is not indicated for isolated hypospadias without cryptorchidism. Although isolated proximal hypospadias is historically considered an anatomic abnormality, the association with genitourinary malformations (as evidenced above) should prompt consideration of further genetic testing. In light of technological advances and an unclear implication of genetic variants on phenotype, at this time, all patients with genitourinary anomalies should be carefully considered, and the utility of genetic evaluation should be determined on an individual basis. Inevitably, many newly discovered variants may have unclear clinical significance, and because of the rarity of these mutations, creation of national and international databases would be integral to establishing genotype-phenotype correlation.

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ABBREVIATIONS
DDS: Denys-Drash syndrome
DSD: disorder of sex development
FS: Frasier syndrome
WT: Wilms tumor

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