

Case Report

Denys Drash Syndrome, What Paediatrician Should Know About?

AKH KWOK, SMY WONG, MT LEUNG, WKY CHAN

Abstract We report on a boy with known bilateral undescended testes who was incidentally detected to have proteinuria on a routine pre-operative assessment. Further investigations confirmed childhood nephrotic syndrome. Because of atypical features, an early ultrasound kidney was arranged and it showed bilateral nephroblastomatosis. His karyotype was 46XY. Further DNA analysis showed a heterozygous mutation in Wilms tumour suppressor gene 1 (*WT1* gene). A diagnosis of Denys Drash syndrome was made and he underwent treatment for Wilm's tumour.

Key words Denys-Drash syndrome; Genes, Nephrotic syndrome; Pseudohermaphroditism; Wilms tumour; *WT1* proteins

Case Report

LCY, an 18-month-old boy, was born full term with normal spontaneous delivery. His neonatal history was uneventful except he was noted to have bilateral undescended testes. His scrotum was well formed and penile length was normal. Both testes were high in the inguinal canals. He was well until 14 months of age. He was admitted for bilateral orchidopexy. Pre-operative assessment showed puffy eyelids with blood pressure 130/90 mmHg and urine

albumin 3+ for protein. His body weight was above 97th percentile and body height was 75th percentile. Subsequent work up showed serum albumin 18 gram/L (normal range: 38-54 gram/L). 24-hour urine protein was 3.49 gram/day (normal range: <0.1 gram/day). Cholesterol was 4.9 mmol/L. Serum urea was 6.0 mmol/L (normal range: 1.8-6.4 mmol/L), serum creatinine was 36 $\mu\text{mol/L}$ (normal range: 21-36 $\mu\text{mol/L}$). Anti-nuclear antibody, anti-neutrophil cytoplasmic antibody and anti-dsDNA were negative. C3 and C4 levels were normal. Ultrasound of kidneys showed multiple hypoechoic nodules on bilateral kidneys suggestive of nephroblastomatosis. CT abdomen showed multiple hypodense hypoenhancing mass lesions in both kidneys, the largest hypodense lesion measures 3.8 cm in right lower pole. The findings were consistent with bilateral nephroblastomatosis. In view of early onset nephrotic syndrome with bilateral renal tumour and undescended testes, karyotype study and genetic work up for *WT1* mutation were performed. It showed heterozygous mutation in *WT1* gene (*NM_024426.3(WT1):c.1384C>T*, changing codon 462 from arginine to tryptophan, i.e. p.Arg462Trp) (Figure 1). Karyotyping of patient was 46XY. *WT1* mutation screenings for parents were negative. Our patient was started on angiotensin-converting enzyme inhibitor, calcium channel blocker and vasodilator for hypertension and heavy proteinuria. Options of prophylactic bilateral nephrectomy,

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versus renal salvage approach for nephroblastomatosis with adjuvant chemotherapy were discussed with parents.

In view of the rapid growth in renal mass, LCY underwent 4 cycles of neo-adjuvant chemotherapy with intravenous Actinomycin D 550 microgram and intravenous Vincristine 0.75 mg once per week. MRI abdomen afterwards showed resolved lesions over left kidney but mild enlargement in right lower pole lesion. Right total nephrectomy was done 2 months after presentation, an 8x7 cm right renal tumour with multiple lymph nodes on the inferior vena cava (IVC) excised. Pathology showed triphasic nephroblastoma with clear margins. There were no malignant cells in the lymph nodes. Renal histology confirmed diffuse mesangial sclerosis

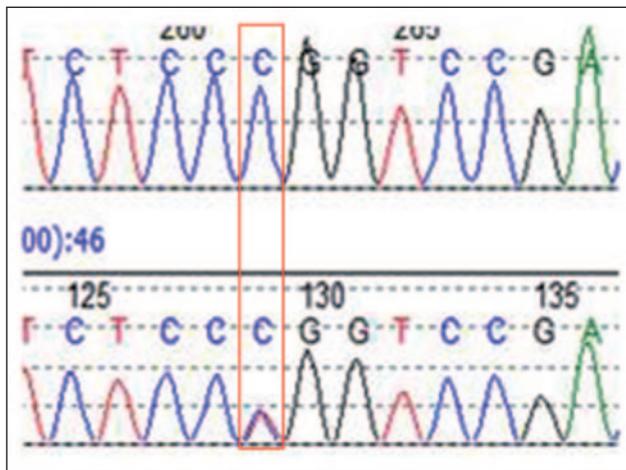


Figure 1 Showed the Missense mutation in codon 462 at exon 9 of *WT1* gene of the patient.

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Mini Kit according to the manufacturer's instruction (Qiagen). Exons 1-10 of the *WT1* gene and their flanking intron sequences were amplified individually by PCR, performed in the GeneAmp PCR System 9700 (Applied Biosystems). All reagents used in the PCR reactions are supplied by Applied Biosystems. Sequencing reactions were performed forward and reverse, by Applied Biosystems Genetic Analyser 3500. Nucleotide changes and allelic variants were detected using the Mutation Surveyor software as well as manual inspection. One heterozygous missense mutation was identified in this patient. This mutation is situated in codon 462 at exon 9 and causes a CGG to TGG transformation (the red rectangle), changing the amino acid from arginine to tryptophan. This occurs within a zinc finger domain of the mature *WT1* protein and has been reported in about half of the patients with complete Denys-Drash syndrome.

with mild global glomerulosclerosis and mild chronic tubulointerstitial injury (Figure 2).

Patient was put on chemotherapy according to the SIOP (International Society of Pediatric Oncology) nephroblastoma clinical trial 2001 protocol, intermediate risk arm. His chemotherapy comprises of weekly injection of intravenous actinomycin D and/or vincristine. He suffered from several infective complications. His nephrotic syndrome was treated with regular albumin transfusion and ACEI. Renal function however, was noted to be gradually deteriorating over a few months. His creatinine rose from baseline of 25-35 $\mu\text{mol/L}$ to 100-110 $\mu\text{mol/L}$ over 5 months. His latest estimated glomerular filtration rate by Schwartz formula is 28 ml/min/1.73 m^2 . His condition remained stable initially but progressive renal failure is anticipated and he is having his scheduled chemotherapy with close monitoring of renal function.

Discussion

Denys-Drash syndrome (OMIM 194080) was first described in 1967 as a triad of 46XY pseudohermaphroditism, nephrotic syndrome due to diffuse mesangial sclerosis and predisposition to unilateral or bilateral Wilms tumour and gonadoblastomas.^{1,2} All of the patients were infants with heavy proteinuria progressing rapidly to renal failure. In 1991, Pelletier et al described a direct role for *WT1* in Denys-Drash syndrome (DDS).³

The *WT1* gene is located on chromosome 11p13 and comprises 10 exons which encode a zinc-finger DNA-binding protein that acts as a transcriptional activator or repressor. The gene is expressed in a wide variety of embryonic tissue including the mesenchymal cells of the foetal kidney, and the stromal cells of the gonads and spleen. It is thus essential in normal formation of the kidney and genitourinary system.⁴ Different mutations in *WT1* gene lead to a spectrum of different phenotypes including DDS, Frasier syndrome (FS) or isolated steroid-resistant nephrotic syndrome.^{2,5}

Classically, DDS and FS are two separate entities with different clinical features. DDS patients have early onset nephrotic syndrome during infancy due to diffuse mesangial sclerosis, with rapid progression to renal failure. In contrast, FS patients have nephrotic syndrome due to focal segmental glomerulosclerosis in the second to third decade of life. DDS patients are prone to Wilms' tumour whilst FS patients are prone to gonadoblastoma. DDS and FS are both known to be related to gonadal dysgenesis. DDS patients have 46XY

partial gonadal dysgenesis, with variable external phenotype depending on the degree of testicular function. The phenotype can range from ambiguous genitalia, under-virilized male to normal male phenotype with reduced sperm production. In contrast, FS patients have 46XY complete gonadal dysgenesis. They have normal Mullerian structures with bilateral streak gonads due to the lack of gonadal steroid production.^{6,7} Our DDS patient is genetically XY and he had bilateral undescended testes. In a local case report by Chan et al,⁸ a post-renal transplant patient presented with delayed pubertal development at the age of 15 years. She had a normal female phenotype but karyotype was 46XY. DNA analysis confirmed diagnosis of Frasier syndrome.

Since the 1990s, more than 80 mutations have been reported in DDS patients. Missense mutations in exons

8 and 9 are the commonest identified mutations, with truncating mutations also described. There is significant evidence to suggest genotype/phenotype correlation in DDS mutation patients. Patients with missense mutations in exons have onset of proteinuria at median age of 6 months, which is much earlier than those with truncating mutations and intron mutations. They also progress more rapidly toward end stage renal disease (ESRD), with need for renal replacement therapy (RRT) at median age of 1.1 years.⁵

Our patient has the classical genotype and phenotype described in DDS. However, he presented to us with undescended testes which is a common paediatric problem which could hardly be linked to this rare disorder at the beginning. His nephrotic syndrome was subtle too as he was asymptomatic and was only picked up by a careful physical

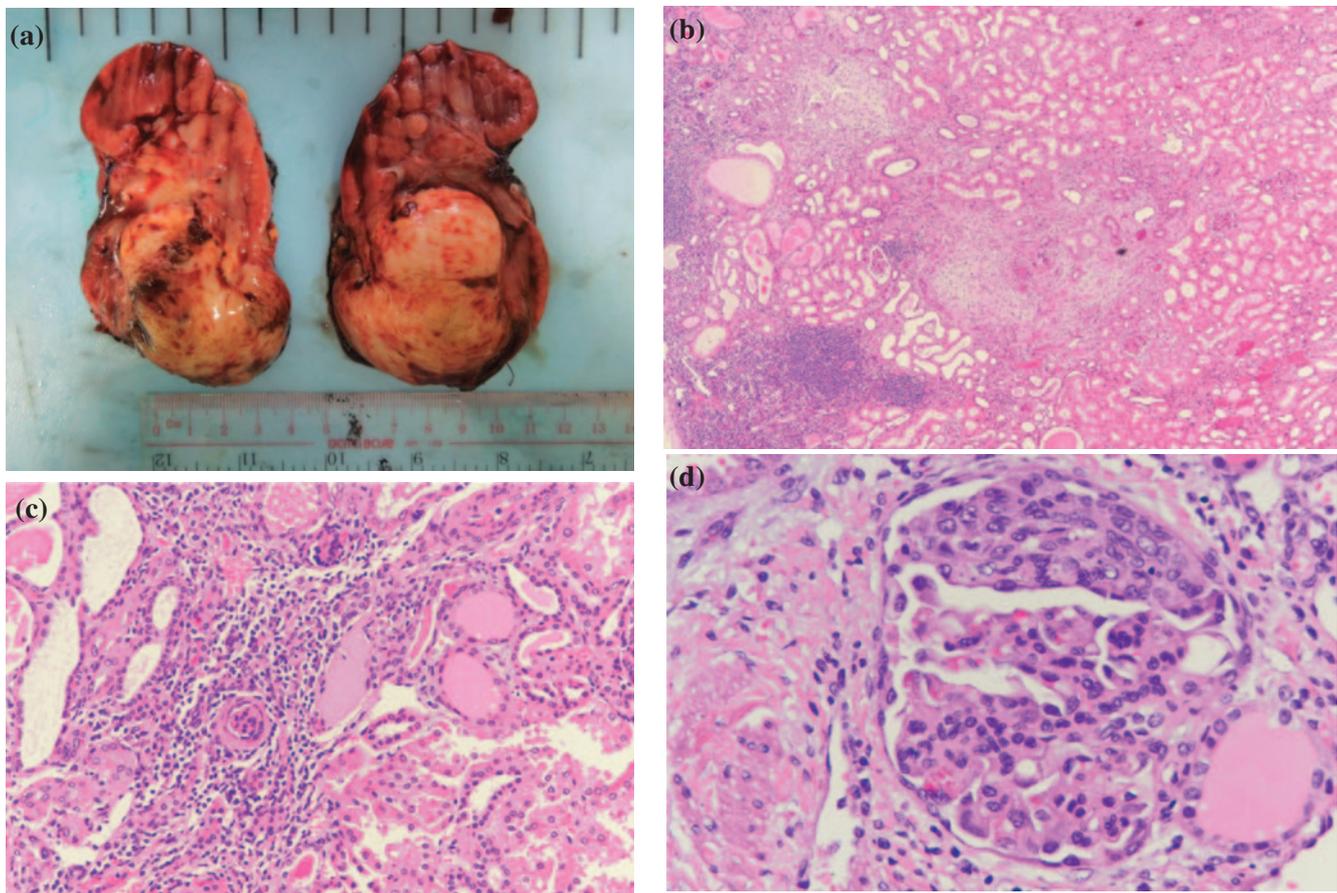


Figure 2 (a) The pale looking circumscribed tumour was seen in the lower pole of right bivalved kidney. (b) H&E 4x view showed the intralobar nephroblastomatosis which was a feature of nephroblastoma seen in patients with WT1 mutation. (c) H&E 10x view showed the immature glomeruli which were of smaller size as compared with "normally developed glomeruli" and had parietal epithelial cells with high nuclei-cytoplasm ratio in the periphery of the glomeruli. (d) H&E 20x view showed the diffuse mesangial sclerosis in this glomerulus with parietal epithelial cells proliferation and these cells composed of podocytes with vacuolated nuclei.

examination and laboratory test. DDS only came to our mind when he was detected to have early onset of nephrotic syndrome. The presentation was subtle and an early ultrasound helped to pick up the renal tumour which pointed to the rare diagnosis of DDS.

Genetic test was arranged after detection of nephrotic syndrome and bilateral nephroblastomatosis. Within a few days' time, DDS was confirmed by detection of the missense mutation c.1384C>T, which is one of the most described mutations amongst DDS patients.⁵ The genetic test result sufficed to imaging findings suggestive of Wilm's tumour. It also provides a realistic expectation towards management goal and parent counselling during the gradual deterioration of renal disease.

The management of renal condition in DDS patients is challenging. Diffuse mesangial sclerosis is reported to be resistant to steroids and immunosuppressive drugs. Careful monitoring of disease, adequate nutrition, medical treatment for hypertension and proteinuria are all important to slow the declining renal function.

Nephrectomy with adjuvant chemotherapy is the mainstay of treatment for DDS patients with Wilms' tumour. Bilateral nephrectomy is recommended for DDS patients with ESRD. For those without Wilm's tumour or ESRD upon diagnosis, timing for nephrectomy is debatable. Some authors prefer "early" bilateral nephrectomy in view of the high risk for Wilms' tumour. Other authors suggest delaying nephrectomy until onset of ESRD, with careful monitoring of patients every 4 to 6 months by abdominal ultrasonography.⁹

Both diffuse mesangial sclerosis and Wilms' tumour contribute to an inevitable progression to ESRD in DDS patients. Renal replacement therapy is eventually needed. Outcomes of children with Wilms' tumour and DDS who proceeded to renal transplantation are comparable with children with other diagnoses, with no graft failures because of recurrence.¹⁰ It is generally recommended to wait 1 to 2 years after completion of chemotherapy for Wilms' tumour. However, studies have shown a high early mortality before renal transplant. Questions have thus been raised upon the optimal timing of renal transplant.

DDS is a complex disease which requires good collaboration amongst various disciplines, including paediatrician, nephrologist, oncologist, endocrinologist, geneticist, and vast nursing and social support. Thorough counseling with the family on management options is mandatory.

Conclusion

DDS is a rare but important entity that should not be missed. Our patient illustrates that, while nephrotic syndrome is common in children, clinicians should be particularly aware of the possibility of rare causes like DDS, especially in those with early onset nephrotic syndrome and ambiguous external genitalia. DNA analysis is a useful means of confirming the diagnosis.

Declaration of Interest

All authors do not have any financial and personal relationships with other people or organisations that could inappropriately influence their work.

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