Dear Editor,

Denys–Drash syndrome (DDS) is characterized by the triad of progressive nephropathy, urogenital malformation and Wilms' tumor. Incomplete variants present with nephropathy with either intersex disorders or Wilms' tumor. Most DDS patients carry WT1 mutations in exon 8 or 9. Here, we report an incomplete DDS child who carried a novel WT1 missense mutation in exon 6 and had unique clinical manifestations.

The subjected child’s age was 11 years and 9 months. His father is healthy and his mother died suddenly after giving birth to his younger brother. The patient was born at full-term birth without asphyxia. He was found to have abnormal external genitalia after birth and had been raised as a girl. He learned to speak between 2- and 3-years-old and learned to walk between 4- and 5-years-old. He also had difficulties in learning at school. The child was not sent to hospital until his penile development was noticed at 10 years and 11 months. Ultrasound revealed microcalcifications in the left testis, right cryptorchidism and a right testis in the right iliac fossa. Magnetic resonance imaging revealed that prostate and seminal vesicles dysplasia could not be ruled out. Karyotype was 46,XY. Right testicular descent surgery was performed. His urinary protein was observed positive (+ – ++) 5 months later. He was admitted to our department for the unexplained proteinuria at 11 years and 9 months. Regular rhythm and a precordial systolic murmur were heard. The penis was developing and scrotum was symmetrical dehisced. The urethral opening was located in the rifi, with little pubes on the symphysis pubica. The volume of the left testis was approximately 6ml, and the volume of the right one was approximately 0.5 ml. Ultrasound revealed kidneys of normal size and echoes. Echocardiography revealed a persistent left superior vena cava and mitral valve prolapse with mild regurgitation as well as an enlargement of the left atrium, left ventricle and right ventricle (diameter of right atrium 31 mm, right ventricle 18 mm, left atrium 29 mm, left ventricular 39 mm and ejection fraction of left ventricular was 68%). Urinary protein was 10.6 mg kg\(^{-1}\) d\(^{-1}\), serum creatinine 50 µmol l\(^{-1}\) and albumin 41.8 g l\(^{-1}\). The hormones test revealed follicle-stimulating hormone 22.25 IU l\(^{-1}\) (1–12 IU l\(^{-1}\)); luteinizing hormone 12.34 IU l\(^{-1}\) (2–12 IU l\(^{-1}\)); and prolactin, estradiol, testosterone and progesterone were all in normal range. Renal histopathology revealed mild segmental hyperplasia in mesangial cells and matrix and minimal change disease was indicated (Figure 1a and 1b). The child has not received specific therapy except for fosinopril. Urinary protein is still positive (+ – ++) at present and renal function is within normal range.

WT1 mutation in exon 6, c.893A>T (p.Q298L) was identified in the present patient (Figure 1c and 1d), which was not found in his father (Figure 1e). This mutation is absent from 100 control chromosomes.

Alignment of the WT1 protein across six different species revealed that glutamine is conserved at the 298\(^{th}\) position in human, chimpanzee, dog, rat, mouse and zebrafish (Figure 1f). The missense mutation p.Q298L is predicted to cause the loss of an α helix in vicinity of the mutant position (Figure 1g and 1h).

WT1 is located at human chromosome 11p13 and contains 10 exons. Exons 1–6 of WT1 encode an amino-terminus rich in proline and glutamine, which regulates gene expression. Exons 7–10 encode four contiguous Cys2-His2 zinc finger regions, which function as DNA- or RNA-binding domains. Mutant WT1 can lead to glomerular maturation disorders and genitourinary malformations. Mutations in exon 8 and 9 of WT1 have been reported the most. There have been only a few reports of truncate mutations in exons 1–6 that cause DDS. Missense mutation c.903A > G (p.Q298L) in exon 6, which was detected in the present patient, is a novel mutation. This mutation causes a substitution of leucine for glutamine which leads to a helix lost in the peptide chain.

DDDS patients usually present with congenital or early onset proteinuria and progress to end-stage renal disease before 4 years. The most common histology of DDS is diffuse mesangial sclerosis; whereas, minimal change disease can also be observed occasionally. In the present case, proteinuria was discovered at 11 years and 4 months, and renal histopathology was minimal change disease. It has been reported that WT1 nephropathy caused by WT1 mutations in exons 1–6 had a mild phenotype. Although renal function is currently normal in this child; proteinuria is persistent with the administration of angiotensin converting enzyme inhibitor (fosinopril). Because WT1 nephropathy is steroid resistant, calcineurin inhibitors (cyclosporine A or tacrolimus) is on consideration for next treatment in this child based on the benefits observed in literature and our previous experience.

Urogenital abnormalities, including male pseudohermaphroditism, cryptorchidism, hypospadias and penile hypoplasia, mainly occur in DDS patients with 46,XY karyotype. Urogenital malformations are prominent in the present case, though his nephropathy is mild.
Gonadotropin disorders have been reported in a few patients with WT1 mutations. Most of these patients had genitourinary malformations and significantly elevated luteinizing hormone and follicle-stimulating hormone, but normal range testosterone similar to the present case. These disorders may indicate that the child's gonads had a low response to gonadotropins, as seminal vesicles dysplasia has not been ruled out in this child by magnetic resonance imaging, abnormal gonadal development should be considered.

Wilms' tumor has been observed in all reported WT1 nephropathy cases with WT1 mutations in exons 1–6. Thus, the present boy is at high risk of developing Wilms' tumor and should receive periodic medical examination.

Cardiovascular abnormalities have been observed in the present case. It was reported that WT1 is expressed in the epicardium and subepicardial mesenchymal cells and is required for normal vascularization in heart development. There has been no report
on mental retardation in DDS patients to date. Whether the heart abnormalities and mental retardation are attributed to the detected \textit{WT1} mutation deserves more study.

In summary, this study reports an incomplete DDS boy with unique manifestations and an atypical \textit{WT1} mutant site. This study may help to clarify the genotype to phenotype relations of DDS patients.

\textbf{AUTHOR CONTRIBUTIONS}
HYW and ZHY carried out the experiments and clinical data collection and drafted the manuscript. JCM, YM and JJS helped collecting some clinical data and the blood samples. LZS conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

\textbf{COMPETING INTERESTS}
All authors declare no competing interests.

\textbf{ACKNOWLEDGMENT}
The authors thank all participants for their support. The study was supported by the Fundamental Research Funds for the Central Universities (No. 09ykpy40), Science and Technology Planning Project of Guangdong Province (No. 2011B031800126) and the Natural Science Foundation of Guangdong Province (No. S2012010009405).

\textbf{REFERENCES}
1. Yue Z, Pei Y, Sun L, Huang W, Huang H, et al. Clinical pictures and novel mutations of WT1-associated denys-drash syndrome in two Chinese children. \textit{Ren Fail} 2011; 33: 910–4.
2. Jeanpierre C, Denamur E, Henry I, Cabanis MO, Lucé S, et al. Identification of constitutional WT1 mutations, in patients with isolated diffuse mesangial sclerosis, and analysis of genotype/phenotype correlations by use of a computerized mutation database. \textit{Am J Hum Genet} 1998; 62: 824–33.
3. da Silva T, Nishi MY, Costa EM, Martin RM, Carvalho FM, et al. A novel WT1 heterozygous nonsense mutation (p.K248X) causing a mild and slightly progressive nephropathy in a 46,XY patient with Denys-Drash syndrome. \textit{Pediatr Nephrol} 2011; 26: 1311–5.
4. Chiang PW, Aliaga S, Travers S, Spector E, Tsai AC. Case report; WT1 exon 6 truncation mutation and ambiguous genitalia in a patient with Denys-Drash syndrome. \textit{Curr Opin Pediatr} 2008; 20: 103–6.
5. Auber F, Lortat-Jacob S, Sarnacki S, Jaubert F, Salomon R, et al. Surgical management and genotype/phenotype correlations in WT1 gene-related diseases (Drash, Frasier syndromes). \textit{J Pediatr Surg} 2003; 38: 124–9.
6. Cherinin G, Vega-Warner V, Schoeb DS, Heeringa SF, Ovunc B, et al. Members of the GPN Study Group. Genotype/phenotype correlation in nephrotic syndrome caused by WT1 mutations. \textit{Clin J Am Soc Nephrol} 2010; 5: 1655–62.
7. Stefanidis CJ, Querfeld U. The podocyte as a target: cyclosporin A in the management of the nephrotic syndrome caused by WT1 mutations. \textit{Eur J Pediatr} 2011; 170: 1377–83.
8. Wang HY, Sun LZ, Yue ZH, Yang J, Jiang XY, et al. Clinical and pathological features of Denys-Drash syndrome: report of 3 cases. \textit{Zhonghua Er Ke Za Zhi} 2012; 50: 855–8.
9. Melo KF, Martin RM, Costa EM, Carvalho FM, Jorge AA, et al. An unusual phenotype of Frasier syndrome due to IVS9 + 4C > T mutation in the WT1 gene: predominantly male ambiguous genitalia and absence of gonadal dysgenesis. \textit{J Clin Endocrinol Metab} 2002; 87: 2500–5.
10. Wagner N, Wagner KD, Theres H, Englert C, Schedl A, et al. Coronary vessel development requires activation of the TrkB neurotrophin receptor by the Wilms’ tumor transcription factor WT1. \textit{Genes Dev} 2005; 19: 2631–42.

\textbf{How to cite this article:} Wang HY, Yue ZH, Sun LZ, Mo JC, Mo Y, Sun JJ. Clinical features and an atypical WT1 mutant site in a child with incomplete Denys-Drash syndrome. \textit{Asian J Androl} 07 March 2014. doi: 10.4103/1008-682X.125396. [Epub ahead of print]