8. Jordan S, Toyoda M, Vo A. Intravenous immunoglobulin a natural regulator of immunity and inflammation. *Transplantation* 2009; 88: 1–6.
9. Grois N, Prayer D, Prosch H, Lassmann H. Neuropathology of CNS disease in Langerhans cell histiocytosis. *Brain* 2005; 128: 829–38.
10. Ephrem A, Chamat S, Miquel C, Fisson S, Mouthon L, Caligiuri G, et al. Expansion of CD4+CD25+ regulatory T cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis. *Blood* 2008; 111: 715–22.
11. Imashuku S, Okazaki N, Nakayama M, Fujita N, Fukuyama T, Koike K, et al. Treatment of neurodegenerative CNS disease in Langerhans cell histiocytosis with a combination of intravenous immunoglobulin and chemotherapy. *Pediatr Blood Cancer* 2008; 50: 308–11.
12. Imashuku S. High dose immunoglobulin (IVIG) may reduce the incidence of Langerhans cell histiocytosis (LCH)-associated central nervous system involvement. *CNS Neurol Disord Drug Targets* 2009; 8: 380–6.
13. Wnorowski M, Prosch H, Prayer D, Janssen G, Gadner H, Grois N. Pattern and course of neurodegeneration in Langerhans cell histiocytosis. *J Pediatr* 2008; 153: 127–32.
14. Gadner H, Grois N, Pötschger U, Minkov M, Arico M, Braier J, et al. Improved outcome in multisystem Langerhans cell histiocytosis is associated with therapy intensification. *Blood* 2008; 111: 2556–62.
15. Grois N, Pötschger U, Prosch H, Minkov M, Arico M, Braier J, et al. Risk factors for diabetes insipidus in Langerhans cell histiocytosis. *Pediatr Blood Cancer* 2006; 46: 228–33.

WT1 mutation as a cause of 46 XY DSD and Wilm's tumour: a case report and literature review

Yang-li Dai[1], Jun-Fen Fu (fjf68@yahoo.com.cn)[1], Fang Hong[1], Shan Xu[2], Zheng Shen[3]

1.Division of Endocrinology, The Children's Hospital of Zhejiang University School of Medicine, Hangzhou, China
2.Division of Urology, The Children's Hospital of Zhejiang University School of Medicine, Hangzhou, China
3.Division of Laboratory, The Children's Hospital of Zhejiang University School of Medicine, Hangzhou, China

Keywords
Disorder of sexual development, Wilms' tumour

Correspondence
Jun-Fen Fu, M.D., Ph.D., Pediatrician, Department of Endocrinology of Children's Hospital, Zhejiang University School of Medicine, 57 Zhugan Avenue, Hangzhou, 310003 China.
Tel: +86 571 8706 1007, ext 61412 |
Fax: +86 571 8703 3296 |
Email: fjf68@yahoo.com.cn

Received
7 September 2010; revised 20 December 2010; accepted 12 January 2011.

DOI:10.1111/j.1651-2227.2011.02167.x

ABSTRACT

Aim: The Wilms' Tumour gene is thought to have tumour suppressor activity and to play an important role in nephrogenesis, genitourinary development, haematopoiesis and sex determination. WT1 mutations will impair gonadal and urinary tract development and have been demonstrated to cause syndromes of WAGR, Denys–Drash and Fraiser.

Methods: To elucidate the role of constitutional mutations of WT1, in the expression of the different clinical feature, we describe a 14-year-9-month nonmosaic XY sex-reversed woman with pure gonadal dysgenesis (46, XY karyotype, completely female external genitalia, normal Mullerian ducts, absence of Wolffian ducts, streak gonads) who had right kidney removed at 7 months of age because of Wilms' tumour and was diagnosed as secondary thrombocytopenia (Plt 60–80 × 10^9/L) since she was 4 years old. We sequenced the genomic DNA of all the 10 exons of the WT1 in which mutations may occur in proposita.

Results: A new *de novo* insertion mutation in the first exon was found. A *'GCCGCCTCACTCC'* is inserted between codon 138 and 139, resulting in the creation of a stop codon and a truncated protein.

Conclusion: The present data provide further evidence to support the role of WT1 in diverse cellular functions.

The Wilms' tumour gene, WT1, located on 11p13, encodes a zinc finger transcription factor involved in kidney and gonadal development. It is widely expressed in the condensing mesenchyme, the renal vesicle and developing podocytes of foetal kidney as well as in the genital ridge, foetal gonads and the mesothelium. Knockout of this gene in mouse resulted in the absence of renal and gonadal development (1). The gene has 10 exons, of which exons 1–6 encode a proline/glutamine-rich transcriptional regulation region and exons 7–10 encode the four zinc fingers of the DNA-binding domain. Defects in the WT gene account for only a minority (10–15%) of Wilms' tumour cases, but have been demonstrated to cause WAGR syndromes (OMIM 194072), Denys–Drash syndromes (DDS, OMIM 194080) and Fraiser syndromes (FS, OMIM 136680). The WAGR syndrome, which includes Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation, is caused by

Abbreviations
DSD, disorder of sexual development; WT1, Wilms' tumour; AMH, anti-Mullerian hormone.

constitutional deletion of one copy of the WT1 gene (2). DDS, which consists of variable degrees of genital dysgenesis, early-onset renal failure because of diffuse mesangial sclerosis and high risk of Wilms' tumour, is caused by heterozygous missense mutations in the zinc finger encoding exons (DNA-binding domain) of WT1 (3). FS, which is characterized by a female or ambiguous external genitalia phenotype in 46, XY patients, late-onset renal failure because of nonspecific focal and segmental glomerular sclerosis and high risk of gonadoblastoma development, is caused by heterozygous mutation occurring at an alternative splice site in intron nine between the region encoding the third and fourth zinc finger motifs (4). Mutations have also been described in male patients with cryptorchidism and/or hypospadias and WT or in patients presenting with WT only, either unilaterally or bilaterally (5,6). The reasons for heterogeneity of the clinical features of patients with WT1 mutations are still poorly understood. One way to elucidate the role of constitutional mutations, in the expression of the different clinical features, is to analyse WT1 in patients presenting with different combinations of these symptoms. Here, we report a case with a new *de novo* insertion mutation in the first exon of WT1 as a cause of 46, XY disorder of sexual development and Wilms' tumour.

CASE REPORT

A 14-year-9-month-old girl admitted to our clinic because of 'no breast development, no menarche and no other signs of puberty' in September 2009. She was the first girl of unrelated healthy parents, with normal history of birth and feed. When she was 7 months old, the right kidney was removed because of Wilms' tumour. And she was diagnosed with secondary thrombocytopenia (Plt $60–80 \times 10^9/L$) since she was 4 years old after about 80% of the people in the same village had got this diagnosis because of lead poisoning. Bone marrow aspiration was performed when she was 4 years old and was normal. There were no other congenital abnormalities and no family history of developmental abnormalities or renal problems. The parents' pubertal development was normal.

She was physically strong with height 166 cm and weight 47 kg. There was no webbed neck, no cubitus valgus, no pigmented naevus, no axillary hair, no pubic hair, no shield-like chest, no precordial bulge and no skin pigmentation. The posterior hairline was normal. Breasts were completely undeveloped. Heart and lung auscultation was normal. On examination of external genitalia, the appearance was female, genital naive, PH2, no clitoris hypertrophy. Spine and extremities were normal.

The laboratory results showed high basal serum gonadotrophin levels (LH 16.1 mIU/mL, FSH 87.9 mIU/mL) and low testosterone levels ($T < 20$ ng/dL). Estradiol (E2) was 20.3 pg/mL, prolactin (PRL) was 31.2 ng/mL, and human chorionic gonadotropin (HCG) was <1 mIU/mL, which was compatible with phenotypic woman with no signs of virilization. And the serum parameters of adrenal axis, liver function, kidney function (serum creatinine and BUN) and thyroid function were normal. The urinalysis was also normal (no proteinuria).

Ultrasonography revealed that there were prepubertal uterus ($2.5 \times 1.4 \times 0.8$ cm) and ovary-like materials (L $2.55 \times 1.2 \times 0.9$ cm, R $2.3 \times 1.1 \times 0.9$ cm) on which small follicles could be seen in pelvis. Bone age was 12 years old at the chronological age of 14 years and 9 months. MRI images of hypothalamus, pituitary, left kidney and adrenal glands were normal. Chromosome analysis from peripheral lymphocytes revealed that the karyotype was 46XY and the SRY gene was positive.

On suspicion of the existence of testis, laparoscopy was performed; however, no testicular-like tissue or ovotestis was found. There was only ovarian-like tissue and small uterus. Histopathologic study showed oviductal-like tissue of the left gonad and vascular and fibrous tissues of the right gonad (Fig. 1).

The association of male to female sex reversal with Wilms' tumour prompted us to perform a molecular analysis of the WT1. We sequenced the genomic DNA of all the

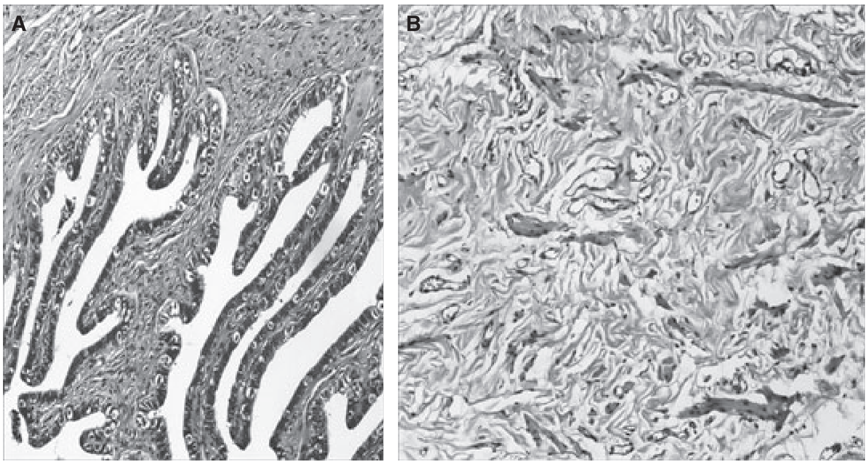

Figure 1 Histopathologic study showed oviductal-like tissue of the left gonad (A) and vascular and fibrous tissues of the right gonad (B) (HE ×100).

Table 1 Primers of the PCR

WT1 P1F	GGAGTTCAAGGCAGCGCCCACA
WT1 P1R	CCGCTATCCTCACGGCCCTTGG
WT1 P4F	acttaatttgccaggggttcg
WT1 P4R	cctttgcctaatttgctgtgg
WT1 P5F	ctgctaggggaatgcaaagtg
WT1 P5R	tcagagaccagggagatcagc
WT1 P6F	tatccagcctgtgttgccttt
WT1 P6R	cacagagagctttgccctttc
WT1 P7F	gaggggcttttcactggattc
WT1 P7R	ttcccatccaccaaatgctac
WT1 P8F	cgcagaaccctgcatctaaag
WT1 P8R	agcggttaattcaagcccaat
WT1 P9F	tgctcactctccctcaagacc
WT1 P9R	ctgctctgcctttctggaaga
WT1 P10F	aggcattttgggatctgttca
WT1 P10R	tgtcttgggaacaaccattcc
WT1 P11F	ggaatggttgttcccaagaca
WT1 P11R	tttccaccctccccttcttta
WT1 P12F	agaggtgctgctgtttcaagg
WT1 P12R	gaccaactcttccaggcacac

10 exons of the WT1 gene, in which mutations may occur. The protocol was approved by the Medical Ethics Committee of The Children's Hospital of Zhejiang University School of Medicine. Written informed consent from parents and the child was obtained. Genomic DNA was extracted from peripheral blood leucocytes using standard techniques. The PCR amplification of the 10 exons of the WT1 gene and purification of the amplified fragments were performed as previously described. Primers (Table 1) were designed using Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Sequencing reactions were performed on amplification products using the BigDye3.1 kit (Applied Biosystems, Foster city, CA, USA) and examined on ABI3130XL DNA sequencer (Applied Biosystems) using sequencing analysis software (Applied Biosystems).

The sequence analysis showed an insertion mutation in the exon 1. A novel '*GCCGCCTCACTCC*' sequence was inserted between codon 138 and 139, resulting in the replacement of the phenylalanine by the serine and creation of a stop codon after 64 new amino acids, and thus to the production of a truncated protein(shown in Fig. 2).

After the mutation being detected, sequence analysis was taken by her parents, younger brother and other 25 unrelated normal controls, but no WT1 mutation was detected, indicating that it occurred *de novo*.

The patient did well in the post-operative period. She was put on estradiol replacement and is now doing well. (Follow-up of renal function was also performed and showed no abnormality).

DISCUSSION

Although the WT1 gene was identified over a decade ago, many aspects of its function and roles in development and tumourigenesis remain obscure. One reason for this is that the WT1 gene encodes numerous protein isoforms that are closely related in terms of structure and function (7). Mutations in exon 8 or exon 9, encoding zinc finger 2 or zinc finger 3, were mostly found in patients with DDS, and mutations in intron 9 in FS patients (8–10). There was very limited previous literature about mutations in exon 1 that encodes a transcriptional regulation region. A deletion mutation in exon 1 was reported by Suzanne Little in a patient with DDS, and a point mutation in exon 1 by vertical transmission by Regev in a patient with genitourinary anomalies, gonadal dysgenesis and intra-abdominal Mullerian derivatives (11,12). The major finding of this study is that a new *de novo* insertion in the exon 1. A '*GCCGCCTCACTCC*' is inserted between codon 138 and 139, resulting in the replacement of the phenylalanine by the serine and creation of a stop codon after 64 new amino acids and a truncated protein. As the insertion mutation has not been described before and was not detected in the patient's parents and male siblings and other 25 normal controls, the mutations in the WT1 gene seem to occur almost *de novo* and lead to 46,XY unambiguous women. This mutation leads to a 46,XY unambiguous female

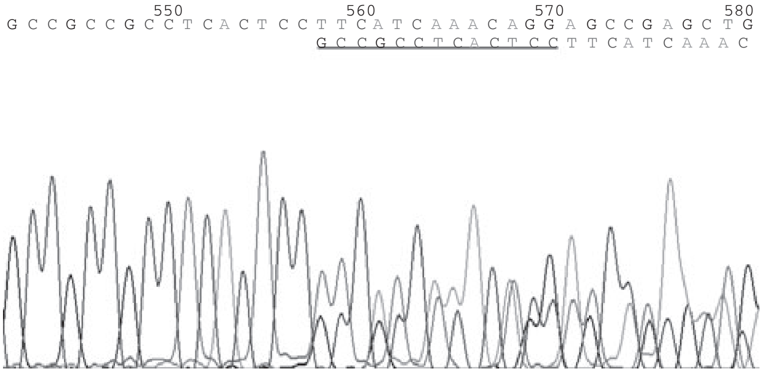

Figure 2 Sequence analysis of exon 1 of the WT1 gene: a 'gccgcctcactcc' inserted between codon 138 and 139, resulting in the replacement of the phenylalanine by the serine and creation of a stop codon after 64 new amino acid.

phenotype and may also explain the Wilms' tumour, suggesting that protein structures encoded by exon 1 of the WT1 gene are important for cellular functions in nephrogenesis, genitourinary development, haematopoiesis and sex determination (13).

In addition to its function in early gonad development and sex determination, a novel function for WT1, namely, in Mullerian duct regression through activating the anti-Mullerian hormone receptor gene (*Amhr2*) has been reported (14). An important step after mammalian sex determination is the differentiation of the Mullerian and the Wolffian ducts. The Mullerian duct serves as the primordium of the oviduct, uterus and upper vagina in women, whereas in men, the Wolffian duct develops into the epididymis and vas deferens. Our patient with a 46XY karyotype, exhibited complete sex reversal of female internal genitalia, which demonstrated the failure of the Mullerian duct regression, a process that is essential for normal male development to occur. The key factor in the regression of the Mullerian duct is the anti-Mullerian hormone, which is secreted by Sertoli cells of the testis (15). In our patient, the new *de novo* insertion mutation in exon 1 of WT1 gene may also lead to the failure of Mullerian duct regression. The absence of male internal genitalia suggested the failure of the Wolffian duct development because of the lack of testerone secreted from Leydig cells.

In addition to its role in the development of a childhood kidney malignancy, the *WT1* has emerged as an important factor in normal and malignant haematopoiesis. But there is no previous literature about WT1 mutation with thrombocytopenia. The patient may be with lead poisoning (the level of the serum lead was 6.5 ug/dL) and without renal dysfunction, so it is hard to say that the WT1 mutation is implicated with the thrombocytopenia, a regular follow-up of renal function and platelet should be taken.

In conclusion, the present data may suggest that the insertion mutation in exon 1 led to the dysfunction of WT1 gene and resulted in multiple cellular disorders. But how a mutation in exon 1 leads to these dramatic phenotypic anomalies is still unclear, and further research is needed.

References

1. Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, et al. WT1 is required for early kidney development. *Cell* 1993; 74: 679–91.
2. Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GA. Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. *Nature* 1990; 343: 774–8.
3. Little M, Wells C. A clinical overview of WT1 gene mutations. *Hum Mutat* 1997; 9: 209–25.
4. Barbaux S, Niaudet P, Gubler MC, Grünfeld JP, Jaubert F, Kuttenn F, et al. Donor splice-site mutations in WT1 are responsible for Frasier syndrome. *Nat Genet* 1997; 17: 467–70.
5. Huff V. Genotype/phenotype correlations in Wilms' tumor. *Med Pediatr Oncol* 1996; 27: 408–14.
6. Schumacher V, Schneider S, Figgz A, Wildhardt G, Harms D, Schmidt D, et al. Correlations of germline mutations and two-hit inactivation of the WT1 gene with Wilms tumors of stromal-predominant histology. *Proc Natl Acad Sci USA* 1997; 94: 3972–7.
7. Scharnhorst V, Van der Eb AJ, Jochemsen AG. WT1 proteins: functions in growth and differentiation. *Gene* 2001; 273: 141–61.
8. Mueller RF. The Denys-Drash syndrome. *J Med Genet* 1994; 31: 471–7.
9. Pelletier J, Bruening W, Kashtan CE, Mauer SM, Manivel JC, Striegel JE, et al. Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. *Cell* 1991; 67: 437–47.
10. Dome JS, Coppes MJ. Recent advances in Wilms tumor genetics. *Curr Opin Pediatr* 2002; 14: 5–11.
11. Regev M, Kirk R, Mashevich M, Bistritzer Z, Reish O. Vertical transmission of a mutation in exon 1 of the WT1 gene: lessons for genetic counseling. *Am J Med Genet Part A* 2008; 146A: 2332–6.
12. Little S, Hanks S, King-Underwood L, Picton S, Cullinane C, Elizabeth Rapley E, et al. A WT1 exon 1 mutation in a child diagnosed with Denys-Drash syndrome. *Pediatr Nephrol* 2005; 20: 81–5.
13. Dallosso AR, Hancock AL, Brown KW, Williams AC, Jackson S, Malik K. Genomic imprinting at the WT1 gene involves a novel coding transcript (AWT1) that shows deregulation in Wilms' tumours. *Hum Mol Genet* 2004; 13: 405–15.
14. Klattig J, Sierig R, Kruspe D, Besenbeck B, Englert C. Wilms' tumor protein Wt1 Is an activator of the anti-Müllerian hormone receptor gene *Amhr2*. *Moll Cell Biol* 2007; 27: 4355–64.
15. Josso N, di Clemente N, Gouedard L. Anti-Mullerian hormone and its receptors. *Mol Cell Endocrinol* 2001; 179: 25–32.

Copyright of Acta Paediatrica is the property of Wiley-Blackwell and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.