

ONLINE MUTATION REPORT

A necropsy case of Denys-Drash syndrome with a *WT1* mutation in exon 7

R Fukuzawa, J Sakamoto, R W Heathcott, J-I Hata

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The Wilms tumour suppressor gene 1 (*WT1*) is located on chromosome 11p13, encodes zinc finger domains, and its product plays a role in the regulation of gene transcription.¹ Since expression of *WT1* is observed in the glomerular epithelium of the kidneys and the genital ridge during the embryonic period, *WT1* is thought to have a functional role in renal and gonadal organogenesis.^{2,3} Denys-Drash syndrome (DDS) is characterised by *WT1* mutations, early onset renal failure, abnormal sex differentiation, and a predisposition to Wilms tumour.^{4,5} It is thought that presence of a constitutional point mutation in the zinc finger domain of *WT1* in one allele causes diffuse mesangial sclerosis (DMS) and abnormal sex differentiation by a dominant negative effect, that is, loss of normal function of both alleles may result from a dysfunctional mutation in only one allele, while deletion of the normal *WT1* gene usually gives rise to Wilms tumour in children with DDS. Most DDS patients carrying *WT1* mutations have missense changes in exon 8 or 9 affecting zinc finger 2 or 3.⁶ Thus, zinc fingers 2 and 3 in particular are thought to have an important DNA binding capacity. Whether missense mutations in exon 7 altering *WT1* zinc finger 1 structure are responsible for DDS is not well understood. Although this patient has previously been reported,⁷ we describe here the pathological findings together with the clinical and biological significance of an altered *WT1* zinc finger 1.

CASE REPORT

The child was delivered vaginally at 42 weeks of gestation. The birth weight was 2620 g. There was no parental consanguinity or family history of renal disease. The patient initially presented at 11 months of age with rapidly progressive renal failure after repeated episodes of generalised afebrile convulsions. She was started on continuous ambulatory peritoneal dialysis (CAPD) the following day. Renal biopsy was not performed. The patient was followed up in the same hospital until the age of 8 years 5 months, at which stage the patient was referred to Tokyo Metropolitan Kiyose Children's Hospital for kidney transplant evaluation. On admission, the external genitalia were phenotypically normal female. Cytogenetic analysis showed a 46,XY karyotype. Molecular analysis of the *WT1* gene was performed on leucocytes. After obtaining informed consent from the parents, we collected peripheral blood from the patient. A *WT1* mutation in exon 7 (1025T>G, M342R) was detected. The patient's growth was retarded for her age. Her height was 108.8 cm (-3.2 SD) and weight was 15.1 kg (-2.5 SD). Repeat renal ultrasonography showed no evidence of a Wilms tumour by the age of 8 years. At 8 years 10 months, cardiac arrest occurred when general anaesthesia was administered to exchange the CAPD catheter and the patient did not recover despite repeated and thorough resuscitation.

NECROPSY FINDINGS

The internal sex organs remained as an immature form of the paramesonephric duct. Bilateral long tubes, which histologi-

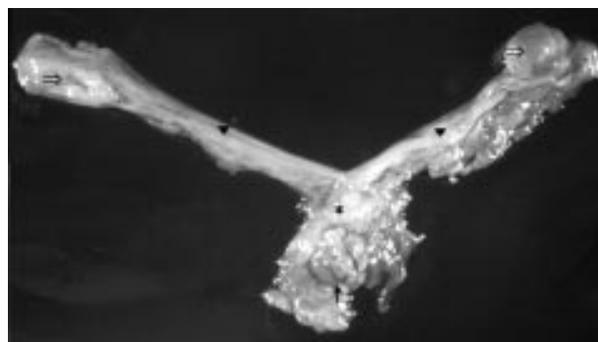


Figure 1 Gross appearance of the gonads and internal genitalia. Open arrows: gonads with cystic change; solid arrow: uterine cervix; arrowheads: fallopian tube; asterisk: uterine corpus.

cally showed fallopian tube characteristics, ran downwards and joined just before the vagina resulting in the formation of a rudimentary uterus (fig 1). Well developed mesonephric ducts (Wolffian derivatives) were microscopically visible, running downwards along the paramesonephric ducts. Bilateral gonads with cystic changes were noted in the lower part of the

Key points

- Denys-Drash syndrome (DDS) is characterised by constitutional *WT1* mutations, nephropathy with male pseudohermaphroditism, and an increased risk of tumours. The mutations observed in most DDS patients are heterozygous missense changes in exons 8 or 9 affecting zinc finger 2 or 3 of the *WT1* gene. So far, there have been no reports of missense mutations in exon 7 altering zinc finger 1 and contributing to sexual development in a 46,XY female.
- We report on an 8 year old, 46,XY female with a novel mutation in exon 7 (1025T>G, M342R) and renal failure. Necropsy showed common features of DDS with dysgenetic testes with small foci of gonadoblastoma and abnormal internal sexual development. No Wilms tumour was found.
- The clinical and pathological findings imply that *WT1* missense mutations in exon 7, which affect zinc finger 1, alter not only renal function but also male gonadal development in a DDS patient with a 46,XY karyotype.

Abbreviations: DDS, Denys-Drash syndrome; *WT1*, Wilms tumour suppressor gene 1; DMS, diffuse mesangial sclerosis; *EGR1*, early growth response gene 1; CAPD, continuous ambulatory peritoneal dialysis

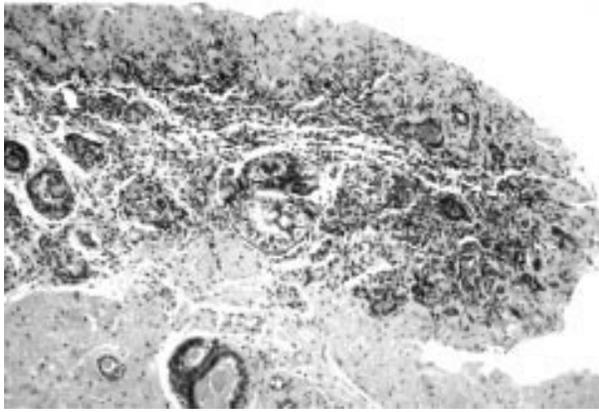


Figure 2 The dysgenetic testicular tissues: atrophic and abortive seminiferous tubules and a small focus of gonadoblastoma.

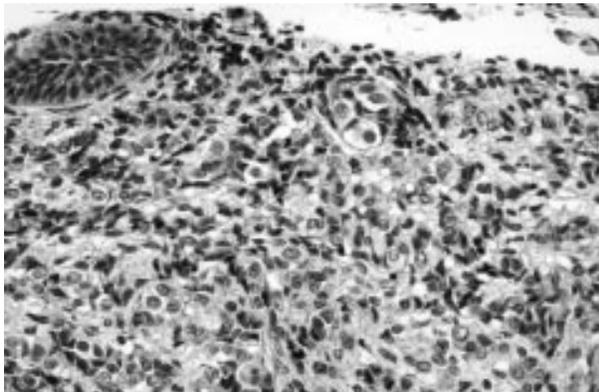


Figure 3 A compact area, which was composed of germ cells mixed with Sertoli cells, showed inconspicuous tubular formation.

peritoneal cavity. Both gonads showed almost the same histological findings. There was thickening of the tunica albuginea and the cortex with atrophic or abortive seminiferous tubules surrounded by fibrous stroma (fig 2). Most of the tubules were lined by Sertoli cells only, but occasional isolated germ cells were noted. Parts of the lumina of the seminiferous tubules were obstructed, similar to those of a normal fetal testis. One microscopic focus contained a well circumscribed nest of immature sex cord cells resembling Sertoli cells with an inconspicuous tubular formation, which were interspersed with large cells resembling germ cells (fig 3). Leydig cells were not evident. Gonadoblastomas were noted at some sites (fig 2). The right and left kidneys weighed 195 g and 160 g, respectively. Gross appearance of both kidneys showed atrophy with multiple cysts. The incised surface was almost flat without normal corticomedullary demarcation or any mass lesions. Histologically, no intralobar nephrogenic rests or Wilms tumour were recognised. Three strikingly enlarged parathyroid glands were identified, with a total weight of 1.6 g. Microscopic examination showed hyperplasia. Generalised calcification and osteomalacia owing to secondary hyperthyroidism were noted. Concentric left ventricular hypertrophy owing to hypertension was evident. The weight of the heart was 125 g. No other anomalies were detected.

DISCUSSION

This is the first case in which an exon 7 missense mutation caused dysgenetic testes and abnormal sexual development in a DDS patient with a 46,XY karyotype. The clinical and necropsy findings provided manifestations of incomplete DDS. Rapidly progressive renal disease in the first year of life

strongly suggests that our patient had DMS. However, no information on the primary kidney disease is available for our patient, since biopsy was not performed at presentation. Various histopathological findings of the gonads in DDS have been reported and include fibrous streaks, dysgenetic testes, as well as hypoplastic testes, which lead to various degrees of incomplete inhibition of Müllerian development and promotion of Wolffian development.⁸ Similarly, the histopathological findings of our case showed incomplete testicular formation and mixed Müllerian and Wolffian maldevelopment.

Germline mutations in *WT1* have been reported in the majority of DDS patients.^{2,6,9,10} Missense point mutations in exon 7 are very rare.^{6,10} Usually, *WT1* missense mutations are detected in exons 8 or 9 and affect zinc fingers 2 or 3, which show a high level of homology to the three zinc fingers of *EGR1* and are believed to be important for their binding capacity to *WT1* DNA targets.¹¹ To our knowledge, there are two other reported cases of a missense mutation in exon 7. One case is a 46,XX female with DMS.^{6,10} Another case is a normal 46,XY patient with normal sexual development and nephrotic syndrome,⁷ but in this patient the mutation in exon 7 (935G>A, R312Q) was situated upstream of zinc finger 1 and could not alter the structure of the zinc finger. Breuning *et al*¹² showed two separable nuclear targeting signals; one is in zinc finger 1 and the other in zinc fingers 2 and/or 3. In their transfection assay, they indicated that nuclear localisation was distinct from DNA binding, since deletion mutants that completely impaired DNA binding could still localise to the nucleus. They showed that deletion of zinc finger 1 produced a polypeptide, but it failed to concentrate in the nucleus, as a property shared by proteins containing missense mutations within zinc finger 2 or 3. It is possible that zinc finger 1 mutants may function by sequestering wild type WT1 protein in the cytoplasm similar to zinc finger 2 or 3 mutants. *WT1* missense mutations in exon 7, which affect zinc finger 1, might have altered not only renal function but also male gonadal development in our DDS patient with a 46,XY karyotype.

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Authors' affiliations

R Fukuzawa, J-I Hata, Department of Pathology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
R Fukuzawa, R W Heathcote, Cancer Genetics Laboratory, Department of Biochemistry, University of Otago, PO Box 56, Dunedin, New Zealand
J Sakamoto, Department of Paediatric Surgery, University of Tokyo Faculty of Medicine, Tokyo 113-0033, Japan

Correspondence to: Dr R Fukuzawa, Cancer Genetics Laboratory, Department of Biochemistry, University of Otago, PO Box 56, Dunedin, New Zealand; ryuji.fukuzawa@stonebow.otago.ac.nz

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