

# KLINIKINIAI ATVEJAI

## Denys-Drash syndrome

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**Key words:** Denys-Drash syndrome, *WT1* gene, missense mutations.

**Summary.** Constitutional missense mutations in the *WT1* gene are usually associated with Denys-Drash syndrome. This rare syndrome is characterized by a rapid progressive nephropathy, male pseudohermaphroditism, and an increased risk for Wilms tumor. We report on a patient with incomplete Denys-Drash syndrome, which was evident by the clinical data and proved by molecular genetics methods. The patient has the mutation p.R394W in the *WT1* gene and clinical symptoms of Denys-Drash syndrome.

### Introduction

The Wilms tumor suppressor gene *WT1* is very important for development of the urogenital system and the gonads. Mutations of this gene can cause WAGR (Wilms tumor, aniridia, genital abnormality, mental retardation) syndrome, Denys-Drash (DDS) and Frasier syndromes (FS). Constitutional missense and splice site mutations in the *WT1* gene cause two similar clinical conditions: Denys-Drash and Frasier syndromes. DDS and FS diseases form two ends of the disorders, with clinical and mutational overlaps. We present here a girl, who has clinical symptoms of Denys-Drash syndrome. She carries the mutation p.R394W, which maps to exon 9, a sequence where mutations for DDS can be found frequently.

### Case report

The patient was born from a first uneventful pregnancy. The pregnancy, delivery and neonatal period were normal. At the age of 10 months she was hospitalized to Kaunas University of Medicine Hospital. The symptoms were vomiting and slight edema in the face. Hypertension was noticed. The blood analysis showed leucocytosis ( $18 \times 10^9/l$ ). Hypoproteinemia (40 g/l) with hypoalbuminemia (16 g/l) was found. Another biochemical analysis showed no abnormalities. The serum creatinine level was 38 mmol/l, urea 2 mmol/l. The glomerular filtration rate was 49 ml/min/1.73 m<sup>2</sup> by the Swartz formula. The urinalysis showed proteinuria more than 3g/l. The congenital nephrotic syndrome was suspected and ultrasound of

the kidneys was performed. The report of the kidneys ultrasound: kidneys collector system is not dilated; the tumor (8 cm × 6.5 cm × 5 cm) has been seen in the left kidney. In order to verify the ultrasound results a computed tomography (CT) scan was performed, which confirmed ultrasound examinations. According to the protocol of the International Society of Pediatric Oncology (SIOP) patient received one course of chemotherapy, but tumor was still growing. In December 2002 tumor biopsy was performed, but quality of sample was not satisfactory and histology of tumor was not determined. An emergency operation was performed December 11, 2002, because of partial ileus. The tumor was found 15 cm × 10 cm × 10 cm, with capsule, it was extended from retroperitoneal space and was successfully removed. The pathological examination showed that tumor was a neoplastic Wilms tumor.

Because of the suspicion of Denys-Drash syndrome, the patient was examined by children gynecologist and the biopsy of the right kidney was performed. The report of the children gynecologist: the phenotype is normal female, ultrasound examination showed internal genitals – normal uterus, however, it was not possible to see gonads.

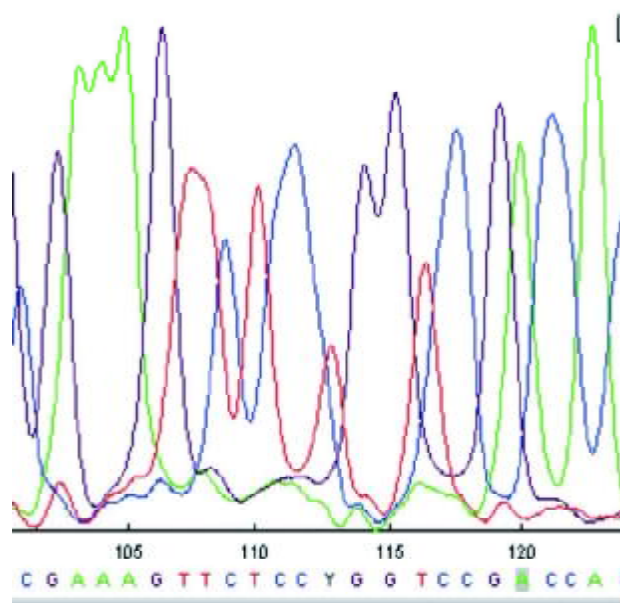
The light microscopy specimens of the kidney biopsy showed renal cortex with 30 glomeruli. Three globally sclerosed glomeruli were present. Four glomeruli were found with segmental capillary collapse, without synechia. Few glomeruli showed mild expansion of mesangial matrix. A few tubules showed mi-

cro cystic dilatation. Small arteries were unremarkable. Congo red staining for amyloid was not performed. Immunofluorescence on frozen sections using rabbit polyclonal antisera against human IgG, IgA, IgM, and C3 revealed weak segmental IgG and moderate staining for IgM in the mesangium. The diagnosis was FSGS (focal and segmental glomerulosclerosis) with collapsing glomerulopathy and signs of mesangiosclerosis. All data can be found in cases of Denys-Drash syndrome.

The patient received a second treatment (chemotherapy by SIOP protocol), but because of damage of the liver, treatment was not prolonged. The patient all time received antihypertensive therapy and albumin infusions. She was checked for the presence of metastases several times. The bone scintigraphy, myeloid punctures, pulmonary X ray, did not show any signs of metastases. A karyotype analysis was performed. Normal female karyotype 46,XX was found. The performed analysis of hormonal status showed hypothyreosis and patient received thyroxin. The girl several times had sepsis, which was successfully treated. The renal functions worsened and she has now end stage renal disease, and peritoneal dialysis is performed because of renal insufficiency. At the age of 2 years, blood was taken and molecular genetic analysis was performed in two exons of the *WT1* gene. The exons 8 and 9 were PCR-amplified. The primers were wt1e8f-bio (gi:207755) 5'TCC CAT TCA TTT GTA ACT TG 3' and wt1e8r 5'GGG TGT TTT CCT TTT CTT TC 3' for amplification of the exon 8. The primers for amplification of exon 9 were wt1e9f 5'AAA GAA TGA GGG TGA TGG AG 3' and wt1e9r-bio 5'CAC GCA CTA TTC CTT CTC TC 3'. The conditions for amplification of exon 8: 1.5 mM MgCl<sub>2</sub> mix, 50°C annealing temperature, 35 cycles. For amplification of exon 9 were 1.5 mM MgCl<sub>2</sub> mix, 53°C annealing temperature, 35 cycles. After amplification, PCR products were washed and DNA strands were separated using a Dynabeads kit. Only one single strand was sequenced with an ALFexpress sequencer, using primers labeled Cyanine 5 in 5' end: wt1e8r-cy5 5'GGA AAA TAC TGG AAA AAC 3' (exon 8) and wt1e9f-cy5 5'GAA ATG CTG GGC TCC TCC 3' (exon 9). The missense mutation p.R394W was found in exon 9. The sequence result is shown in Figure.

### Discussion

*WT1* gene was isolated from chromosome region 11p13 (1). *WT1* is a 10 exons gene with four zinc finger domains in the C terminal end, a proline/glutamine-



**Fig. Sequence analysis of Exon 9 of the *WT1* gene**

The Y (position 113 of the graph) stands for pyrimidine (C or T) and is the result of heterozygosity at this position.

rich tract in the N terminus, and tumor suppressor activity (2). Two alternative splicing sites in exons 5 and 9 create 4 different isoforms. Splicing at the second alternative site (exon 9) results in the inclusion or exclusion of 3 amino acids (lysine, threonine, serine (KTS)) and has a great biological importance. The precise ratio of the KTS-positive/negative forms seems to be very important for the normal function of the *WT1* gene.

The WT1 protein, a transcription factor, is expressed mainly in the genital ridge, fetal gonad, mesothelium and in epithelia of the developing kidney (3). The WT1 protein mediates the mesenchymal-epithelial transition and differentiation during morphogenesis of the kidney and gonad by repressing genes that encode cell proliferation factors and by activating genes that encode markers of epithelial cell differentiation.

*WT1* gene mutations affect the stability of DNA-binding domain and alter the ability of WT1 protein to regulate transcription of target genes (4, 5). This causes nephropathy and genital abnormalities in patients with 46,XY karyotype. Individuals with 46,XX show less severe or no genital abnormalities. Development of the Wilms tumor is a consequence of 2 independent events, which lead to loss of function of both alleles of the *WT1* gene. A germline mutation in a single allele of the *WT1* gene (first hit) leads to persistence of an undifferentiated mesenchyme. A somatic mutation (second hit) or loss of heterozygosity

in the second allele causes uncontrolled cell proliferation and Wilms tumor formation.

Drash et al described two unrelated children with Wilms tumor, pseudohermaphroditism, and nephropathy (6). However, the constellation of anomalies was first described by Denys et al in French literature (7). Since then 150 cases of Denys-Drash syndrome have been described in literature (8).

Nephropathy of DDS is a constant feature. In incomplete forms of the syndrome, nephropathy exists with either Wilms tumor or intersex disorders, but the majority of the patients are destined to develop Wilms tumor. The nephropathy of the Denys-Drash syndrome is characterized by the early onset of proteinuria, which in most cases is sufficiently severe to cause nephrotic syndrome. Impairment of renal function commonly progresses to end-stage renal failure before the age of 3 years. Histologically, the kidneys show varying degrees of focal or diffuse mesangial sclero-

sis. Deposition of fibrillary material in the cytoplasm leads to mesangial cell expansion.

Among the intersex disorders, pure gonadal dysgenesis with male pseudohermaphroditism is the classic presentation, although another variety of gonadal abnormalities can also be encountered.

Our patient represents a typical incomplete case of Denys-Drash syndrome. She was hospitalized at the age of 10 months. She had nephrotic syndrome and tumor in the left kidney. The operation was performed and pathology showed Wilms tumor. The dysfunction of the kidney was progressing, and now she needs peritoneal dialysis. Molecular genetic analysis was performed and the sequencing result showed a typical mutation in the "hot spot" of the *WT1* gene. We suggest to consider mutations in the *WT1* gene in those girls with idiopathic nephropathy and Wilms tumor and to check, whenever possible, this suspected finding by molecular genetics methods.

### Denys-Drash sindromas

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**Raktažodžiai:** Denys-Drash sindromas, *WT1* genas, keičiančioji mutacija.

**Santrauka:** Keičiančioji mutacija *WT1* gene paprastai yra susijusi su Denys-Drash sindromu. Šiam retam sindromui būdinga greitai progresuojanti nefropatija, vyriškasis pseudohermafroditizmas ir padidėjusi rizika Vilmsio navikui. Straipsnyje aprašoma pacientė, kuriai kliniškai nustatytas ir molekulinės genetikos metodais patvirtintas Denys-Drash sindromas. Pacientei nustatyta p.R394W mutacija *WT1* gene ir klinikiniai Denys-Drash simptomai.

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