CASE REPORT

Atypical clinical presentation of a WT1-related syndrome associated with a novel exon 6 gene mutation

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SUMMARY

Wilms' tumour suppressor gene-1 (WT1) plays a critical role in kidney development and function. Several WT1 mutations can occur in exons 7, 8 and 9 and they have been associated with Denys-Drash syndrome. WT1 mutations of intron 9 have been reported too and associated with Frasier syndrome. However, overlapping and incomplete forms of both the syndromes have been described. We report a novel sequence variant (c.1012A>T) of the *WT1* gene in exon 6 (p.R338X) in a 18-year-old girl with a history of Wilms' tumour, minor gonadal changes and relatively late-onset nephropathy. WT1-related nephropathies should be suspected in every patient with proteinuria not associated to immunological changes when a congenital neoplasia or minor gonadal anomalies are present.

BACKGROUND

Wilms' tumour suppressor gene *WT1* plays a critical role in genital and kidney development. The gene maps on chromosome 11p13 and is composed of 10 exons. It encodes for a 449-amino acid protein with four carboxy-terminal zinc fingers which acts as a transcription factor. The normal gene function requires a balanced ratio of four isoforms of the protein resulted from alternative splicing at exon 5 and 9. *WT1* is involved in several diseases characterised by renal and genital anomalies. Classically, heterozygous mutations occurring in exon 8 or 9 were observed in children with Denys-Drash syndrome (DDS) while intron 9 splice donor site mutations were found in patients with Frasier syndrome (FS). DDS is characterised by the association of early-onset nephropathy, male pseudohermaphroditism and Wilms' tumour.¹ Diffuse mesangial sclerosis (DMS) is the typical histological lesion in the kidney tissue. DDS usually manifests within the first 2 years of life with steroid-resistant nephrotic syndrome and progresses to end-stage renal failure by the age of four.² Some authors suggest bilateral nephrectomy in end-stage renal disease (ESRD) to prevent the development of Wilms' tumour.³ FS includes the association of male pseudohermaphroditism, slow progressive nephropathy and gonadoblastoma.^{4 5} The nephropathy is usually detected in childhood or later with proteinuria which increases progressively with age, resulting in nephrotic syndrome and finally in end stage renal failure in the second or third decade of life. Renal biopsy shows focal and segmental glomerular sclerosis (FSGS).⁶ In both DDS and FS the

full-blown clinical picture is present only in XY subjects as patients carrying a XX karyotype usually have normal genitalia.

However, in the last years' patients mixing clinical and molecular findings of each syndrome have been described, as well as sporadic cases of WT1-related DMS or FSGS.⁷⁻¹¹ So far, the sole DDS has been associated to more than 80 germline mutations, most of them occurring 'de novo'. WT1-related nephropathies have been almost exclusively associated to changes in intron 9 or exon 7, 8 or 9 (table 1).

We report here a novel exon 6 variant in an 18-year-old girl presenting with ESRD and only minor gonadal changes.

CASE PRESENTATION

The proband is an Italian 18-year-old girl who was referred to our unit for chronic uraemia. At age of three she underwent right nephrectomy because of Wilms' tumour. One year later left kidney surgical biopsy was performed for questionable Wilms' tumour but the histological examination showed renal dysplastic tissue. At the age of seven, the left kidney was reported morphologically regular on ultrasound examination. Hyppuran and diethylene triamine pentaacetic acid (DTPA) clearances were respectively, 698 and 155 ml/min/1.73 m² on a radionuclide study and urinalysis was normal. In the following years the patient was healthy and did not perform any further clinical ascertainment. She had menarche at age of 13 and menstrual cycles irregular in length. Repeated ultrasound examination of pelvis showed right ovarian hypertrophy and normal findings of the other pelvic organs. At the age of 15 serum creatinine was 0.88 mg/dl and urinalysis showed proteinuria 300 mg/dl on occasional laboratory investigations. Two years later proteinuria was unchanged but serum creatinine was 5.8 mg/dl and the girl was referred to our unit. On admission her arterial blood pressure was 155/105 mm Hg, weight and height were respectively 52.5 kg and 165 cm. Cardiac, pulmonary and abdominal physical findings were normal as well her secondary sexual characters. Laboratory tests showed haematocrit 26%, haemoglobin 9.0 g/dl, serum urea 250 mg/dl, serum creatinine 11 mg/dl. Creatinine clearance was 4 ml/min/1.73 m². Protein excretion rate was about 3 g/24 h in both orthostatic and clinostatic urinary collections. Antinuclear and antiphospholipid antibodies, circulating immune complexes, serum immunoglobulins, and C3/C4

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Table 1 WT1-related syndromes

	WT1 mutations	Clinical features	Histological changes in kidney
Denys-Drash	Exons 8 and 9, rarely exons 1, 4, 6 and 7	Wilms' tumour, steroid-resistant nephrotic syndrome and variable genitourinary abnormalities	Diffuse mesangial sclerosis or rarely focal segmental sclerosis
Incomplete Denys-Drash	Denys-Drash: Exons 7, 8 and 9; rarely exons 1, 4 and 7 Incomplete Denys-Drash: Exons 6, 7, 8 and 9	Proteinuria/nephrotic syndrome and Wilms' tumour or genitourinary abnormalities.	Diffuse mesangial sclerosis or rarely focal segmental sclerosis
Frasier	Intron 9	Proteinuria/nephrotic syndrome, genitourinary abnormalities, Gonadoblastoma. Wilms' tumour (very rare)	Focal segmental glomerular sclerosis or focal glomerular sclerosis
WAGR	Deletion of a allele	Wilms' tumour, aniridia, genitourinary abnormalities, mental retardation. Late-onset renal failure (rare)	???
Isolated focal segmental glomerular sclerosis	Intron 9	Familial nephrotic syndrome	Focal segmental glomerular sclerosis
Isolated diffuse mesangial sclerosis	Exons 7, 8 and 9	Steroid-resistant nephrotic syndrome	Diffuse mesangial sclerosis
Isolated urogenital abnormalities	Exons 3, 4 and 7	Testicular dysgenesis or ectopia, hypospadias, ambiguous externa genitalia	–
Isolated Wilms' tumour (12–20% of all Wilms' tumours)	Whole or partial gene deletion, insertion, nonsense and missense	Wilms' tumour (rarely bilateral)	–

complement fractions and, lupus anticoagulant were in the range of normal values. Abdominal ultrasonography showed a very small-sized left kidney with increased cortical echogenicity and no anomalies of the left urinary tract and the bladder. A renal biopsy was not performed. Antihypertensive treatment including an ACE inhibitor (ramipril, 5 mg/day) was started. In the following months, her kidney function decreased further and in November 2008 haemodialytic treatment was begun.

On the basis of the clinical history (Wilms' tumour, chronic progressive proteinuric kidney disease, gonadal abnormalities) we hypothesised DDS.

INVESTIGATIONS

After receipt of informed consent, genomic DNA was extracted from peripheral blood of the patient and her parents, using the NucleonTM BACC2 DNA extraction kit (GE Healthcare, Little Chalfont, UK) according to the producer's protocol.

The WT1 exons 1–9 and their flanking intronic regions were screened for mutations by PCR amplification, purification using ExoSAP-IT (GE Healthcare, Little Chalfont, UK) and directly sequenced using a BigDye terminator kit v.3.1 (Applied Biosystems, Foster City, California, USA). The products of the sequencing reaction were purified using the MontageTM Seq96 Sequencing Reaction Cleanup Kit (Millipore Corporation,

Billerica, Massachusetts, USA) and then run on an ABIPRISM 3130×1 Genetic Analyzer (Applied Biosystems). Primers are reported on table 2.

Primers were designed according to the NCBI WT1 genomic reference sequence NG_009272.1. Variants are referred to the longest isoform D according to the NCBI WT1 mRNA reference sequence NM_000378.3 NM_024426.3 and protein reference sequence NP_000369.3 NP_077744.3.

The presence of c.1012A>T mutation in the general population was searched in a control group of 100 healthy volunteers enrolled among blood donors with no personal or family history of renal disease. Fragments corresponding to exon 6 were amplified by PCR and subsequently screened by denaturing high-performance liquid chromatography (DHPLC; Transgenomic, Omaha, Newark, USA) after the determination of the optimal temperature (63.4°C) for the detection of c.1012A>T mutation.

The karyotype was obtained from peripheral blood lymphoblasts. Chromosome analysis showed a 46,XX karyotype. Molecular analysis of WT1 gene revealed a heterozygous nucleotide substitution in position c.1012A>T in exon 6, resulting in a stop codon at 338 position (p.R338X) of the protein. This sequence variant was not detected in any of her parents. Similarly, it was found in none of the 200 control chromosomes.

Table 2 Primer sequence and PCR conditions used for WT1 sequencing

	Forward	Reverse	Product size (bp)	Ta (°C)
1	CCTACAGCAGCCAGAGCAG	TAAGAGCTGCGGTCAAAAGG	677	61
2	GTGGCTGGTTCAGACCCACT	GTCCTCCTTGCCTAATTGTC	299	62
3	AGGCTCAGGATCTCGTGCTC	GTAGTAGAGTGAGTCGAGGCG	351	62
4	TCCATTGCTTTGAAGAAACAG	GGAAGGAGGAAAGCGTTCTAAT	298	62
5	CAGTGGGACTGGGACTTAG	TCCCATCCACCAATGCTAC	320	62
6	CCATCATTCCTCCTGATTG	AGCCTGCAGTGAAGAAGAGG	304	62
7	ATACTCCAGTGCTCACTCTCC	AAATAACCTGGGTCCTTAGCAG	326	64
8	CCTTAGGCATTTGGGATCT	ACACATGGCTGACTCTCTCATT	300	61
9	GTGAGGCAGATGCAGACATT	TAGCCACGCCTATTCCTTC	366	64

Ta, PCR annealing temperature.

OUTCOME AND FOLLOW-UP

Our patient became dialysis dependent in 2008. After 3 years she received a cadaveric kidney transplant and since then she has been monitored by our ambulatory.

After the transplantation her nephropathy has never relapsed and the patient has normal values of serum creatinine (0.84 mg/dl) and proteinuria (0.08 g/24 h).

DISCUSSION

We report here a WT1-related nephropathy in a 18-year-old girl due to a novel mutation in WT1 gene.

While in the past DDS and FS were described as two well-defined and separated diseases, nowadays it is believed they are two facets of the same disease. Isolated DMS has been reported not only with exon 8 or 9^{7 8 9} but also with intron 9 mutations.⁸ Likewise, isolated FSGS has been reported not only with intron 9 splice donor site mutations^{10 11} but also with exon 8 or 9 mutations.⁹ Patients with DMS and gonadoblastoma carrying exon 9^{8 9} and intron 9 splice donor site mutations⁸ have been described too. Furthermore, it is now clear that WT1 mutations may be associated with clinical intrafamilial variability. Denamour¹⁰ reported a same splice site mutation in intron 9 causing two distinct glomerular diseases among the members of the same family, DDS with renal histological picture of DMS in a XY girl and isolated FSGS in her XX mother. Mucha⁹ reported the transmission of a same exon 9 mutation from a mother who suffered for ESRD to her two daughters, one suffering from nephrotic syndrome by the age of 10 and the other still healthy at the age of 20.

So far, WT1-related nephropathies have almost exclusively associated to changes in intron 9 or exon 8 or 9. Reports suggesting the involvement of other exons of the gene are very scarce. A WT1 mutation in exon 7 was detected in a 8-year-old XY female with abnormal internal sexual development, early progressive renal failure, small foci of gonadoblastoma and no Wilms' tumour.¹² An exon 6 truncation mutation was observed in a 46,XY child with ambiguous genitalia who underwent partial bilateral nephrectomy for bilateral Wilms' tumour but his renal function was normal and the glomeruli did not exhibit mesangial sclerosis on histology.¹³ A significant association of a single-nucleotide polymorphism (SNP; rs2234590) in WT1 exon 6 region with isolated focal segmental glomerulosclerosis was reported by haplotype analysis in an African American population.¹⁴ However, this polymorphism resulted in a synonymous change, so the AA hypothesised this SNP was in linking disequilibrium with point mutations elsewhere in the gene. Other AA found a WT1 exon 6 mutation (a single base pair insertion at nucleotide position 821) resulting in the generation of a premature stop codon in a child affected from DDS but the same patient showed also a missense mutation in exon 8.¹⁵

Our patient had been affected from Wilms' tumour in infancy, showed a female phenotype with a 46,XX karyotype and only minor gonadal abnormalities (right ovarian hypertrophy with menstrual cycles irregular in length) associated to a WT1 gene exon variant. These clinical features could be consistent with the classical cases of XX patients affected from DDS. Nevertheless, at variance with 'classical' DDS, her nephropathy had a later onset and a slower progressive course. To the best of our knowledge, this is the first reported case of incomplete DDS associated with a c.1012A>T mutation. The same variant could not be found in any of her parents, proving itself a 'de novo' mutation, as it occurs in the most cases of WT1-related diseases.

This mutation should create a stop codon, indeed leading to the production of a truncated protein lacking the domains which normally bind to the DNA. Because of these possible effects and the observed clinical picture, we think the variant c.1012A>T (p.R338X) had a pathogenic role in the nephropathy of our patient, although such a variant has never described so far.

We cannot completely exclude other causes for her chronic nephropathy. A nephrotic syndrome (especially if steroid-resistant) occurring in children is more often due to a reduced working renal mass (as in children with low birthweight or affected from renal dysplasia) or genetic mutations in genes encoding for specific proteins (eg, nephrin, podocin, phospholipase C ϵ 1, WT1 and laminin β 2). In our case the past finding of dysplastic tissue in the surviving kidney might suggest that also the left kidney was abnormal. Nephron reduction, due to the association of uninephrectomy with abnormal contralateral kidney, could have caused secondary glomerulosclerosis and chronic progressive proteinuric nephropathy. However, dysplasia was found in an area of the kidney that was also radiologically abnormal while the remaining tissue appeared normal. Moreover, when congenital nephropathies are a part of complex syndromes, the diagnosis is led by the clinical presentation. In our case, the association of Wilms' tumour, minor gonadal changes and progressive proteinuric nephropathy strongly suggested a WT1-related nephropathy. Because of the advanced stage of renal failure we did not perform kidney biopsy as we felt it would have hardly changed our clinical behaviour while carrying a high risk for the patient.

Indeed, in our opinion this case reinforces the hypothesis that WT1-related nephropathies can occur with a different grade of severity and be variably associated with other clinical manifestations (gonadal abnormalities, Wilms' tumour or gonadoblastoma). This variability could occur also independently of the gene variant interested in the single case, as suggested by reported cases of a same mutation causing different clinical pictures among the members of a same family.^{9 10}

Our case may be relevant from a clinical point of view, too. As WT1-related nephropathies can present themselves not associated with gonadal abnormalities (or with only minor changes) and/or congenital neoplasia and may run a relative relentless course, they should be considered in every patient with a proteinuric nephropathy not associated to either immunological changes or clear evidence of secondary nephropathies. Age is no longer exclusion criterion as recently Benetti *et al*¹¹ described three cases of

Learning points

- ▶ The extreme clinical expressions of WT1 mutations are Denys-Drash syndrome (DDS) and Frasier syndrome. Among them a series of different clinical pictures are possible, as our patient shows.
- ▶ WT1 mutation analysis should be routinely done in children and adolescents with steroid-resistant nephrotic syndrome. When increased urinary protein excretion in a young patient is associated to even minor (ie, atypical) gonadal changes, WT1-nephropathy is a likely diagnosis and genetic analysis must be done.
- ▶ Our case differs from those previously described because we report a novel sequence variant (c.1012A>T) of the WT1 gene in exon 6, responsible for a form of incomplete DDS. This atypical association has never been described.

WT1-related nephropathy discovered in adult age in the setting of a familiar transmission of a sequence variant of the gene. The probability of a WT1-related nephropathy becomes higher if renal biopsy shows a compatible histological picture (DS, FSGS) with immunofluorescence staining negative for immunoglobulins and complement proteins. Finally, if a congenital neoplasia (Wilms' tumour or gonadoblastoma) occurred in the past, the diagnosis is almost certain also in absence of gonadal anomalies (ie, the rule for patients with XX karyotype). In all these cases, the nephrologist should send blood samples to genetic analysis.

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Competing interests None.

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