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## The importance of genetic testing in adolescent-onset steroid-resistant nephrotic syndrome - Case report

### Importanța testării genetice la adolescenții cu sindrom nefrotic cortico-rezistent – Prezentări de caz

Zsuzsanna Gáll<sup>1\*</sup>, Éva Kiss<sup>2</sup>, Kálmán Tory<sup>3</sup>, Attila Fintha<sup>4</sup>, Carmen Duicu<sup>2</sup>

1. University of Medicine and Pharmacy Tîrgu Mureș, România; 2. Department of II Pediatrics, University of Medicine and Pharmacy Tîrgu Mureș, România; 3. Department of I Pediatrics, Semmelweis University, Budapest, Hungary; 4. Department of II Pathology, Semmelweis University, Budapest, Hungary

#### Abstract

Approximately 10-20% of children and 40% of adults with idiopathic nephrotic syndrome are steroid resistant and progress to end-stage renal disease requiring dialysis or renal transplantation. In these cases, renal histology typically shows focal segmental glomerulosclerosis. Mutations in *NPHS1*, *NPHS2*, *WT1*, *CD2AP* and *ACTN4* genes located on different chromosomes, expressed by glomerular podocytes, have been identified in patients with steroid-resistant nephrotic syndrome.

The authors report two cases of adolescent-onset steroid-resistant nephrotic syndrome. Both cases had similar clinical and histopathological manifestations, with different prognosis and evolution due to different mechanisms leading to proteinuria: an acquired and a genetic form. The first case, a 16 year old girl presented the onset of the disease with massive, generalized edema, secondary hypothyroidism and high blood pressure. Evolution was favorable under cyclosporine therapy. The second case, a 13-years-old adolescent girl, presented an insidious onset of the disease with mild edema. Genetic testing revealed a mutation in the *WT1* gene. The patient developed end-stage kidney failure eight months after the onset of the disease and following kidney transplant had a favorable evolution. Histological examination of the renal biopsy specimen showed focal segmental glomerulosclerosis in both cases.

**Conclusions:** Genetic forms of nephrotic syndrome do not respond to immunosuppressive therapy and may progress to end-stage renal disease, but after kidney transplantation relapse is not expected, in contrast to the immune form. The early genetic diagnosis in steroid-resistant nephrotic syndrome is time-consuming, but is important for proper clinical management of the patients, prognosis and genetic counseling of the families.

**Keywords:** steroid-resistant nephrotic syndrome; adolescent; *WT1*; focal segmental glomerulosclerosis

#### Rezumat

Autorii prezintă două cazuri de sindrom nefrotic cortico-rezistent cu debut în adolescență, cu manifestări clinice și histopatologice similare (scleroză glomerulară focal-segmentală), dar cu mecanisme diferite de

\*Corresponding author: Zsuzsanna Gáll, University of Medicine and Pharmacy Tîrgu Mureș  
Tîrgu Mureș, România, e-mail: nzsuzsanna@yahoo.com

*producere a proteinuriei: o formă dobândită și una genetică, având astfel prognostic și evoluție diferită. Primul caz, o adolescentă de 16 ani prezintă debutul bolii brusc cu edeme masive, generalizate, cu răspuns favorabil la tratamentul cu ciclosporină. A doua pacientă, o adolescentă de 13 ani, a prezentat sindrom nefrotic cortico-rezistent cu debut insidios cu edeme ușoare. În acest caz testarea genetică a evidențiat o mutație în gena WT1, iar evoluția a fost rapidă (în 8 luni) către insuficiență renală terminală, dar cu evoluție și prognostic favorabil după transplant renal. Concluzii: Formele genetice ale sindromului nefrotic nu răspund la tratamentul imunosupresiv și pot evolua către boală renală terminală, dar după transplant recăderea este puțin probabilă, în contrast cu formele imune. Diagnosticul genetic precoce în sindromul nefrotic cortico-rezistent este important pentru managementul optim al pacienților; precizarea prognosticului, respectiv pentru consilierea genetică a familiei.*

**Cuvinte cheie:** sindrom nefrotic cortico-rezistent; adolescent; WT1; FSGS

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## Introduction

Idiopathic nephrotic syndrome (NS) describes the collection of clinical and laboratory findings secondary to glomerular dysfunction, resulting in proteinuria [1, 2]. The annual prevalence is 2-16/100,000 [1, 3]. The diagnostic criteria are massive proteinuria (>50mg/kg/day), generalized edema, hypoalbuminemia and hyperlipidemia [3, 4].

The majority of patients with NS respond to steroid therapy and have a good clinical prognosis. Unfortunately, approximately 10-20% of children and 40% of adults with idiopathic NS are resistant to steroid treatment (SRNS) and progress to end-stage renal disease (ESRD) requiring dialysis or renal transplantation [2-7]. In these cases, renal histology usually shows focal segmental glomerulosclerosis (FSGS) [3, 6, 7].

Based on etiology, two forms of SRNS are distinguished, an immune and a genetic form, with different treatments, thus a correct clinical diagnosis becomes essential [4, 6, 8]. In genetic forms of SRNS were identified so far mutations in more than 20 genes, most of which encode podocyte proteins. The most frequently mutated genes are *NPHS2*, *WT1*, and *NPHS1* [5, 6, 8-10].

We report two cases of steroid-resistant nephrotic syndrome with FSGS in female adolescents. Both cases had similar clinical and his-

topathologic manifestations, but two different mechanisms leading to proteinuria: an acquired and a genetic form, and therefore a very different prognosis and evolution.

## Patients and observation

The first patient, a 16 year old *WT1*-negative girl, with no significant medical history, was admitted to hospital with a seven week history of edema. Physical examination revealed moderate periorbital and significant lower leg edema. The 24 hour ambulatory blood pressure monitoring showed high blood pressure (above the 95<sup>th</sup> percentile).

Laboratory data (presented in *Table I*) revealed nephrotic-range proteinuria without haematuria, hypoproteinemia and hyperlipidemia. Blood urea nitrogen, creatinine, electrolytes, immunogram and complementemia were normal, but secondary hypothyroidism was observed: FT3 1.04 pg/ml (reference range: 1.4-4.7 pg/ml), FT4 0.45 ng/dl (reference range: 0.8-2.2 ng/dl), and TSH 45 IU/ml (reference range: 0.4-4 IU/ml).

Based on clinical and laboratory data, the diagnosis of nephrotic syndrome was established and prednisone therapy was initiated (60 mg/m<sup>2</sup>). The evolution under steroid treatment was unfavorable with persistent massive proteinuria (2.9-5.8 g/24 hours) (*Figure 1*).

Following corticotherapy our patient presented side effects, showing Cushingoid appearance, hirsutism, striae on the abdomen and thighs, high BP (152/94 mmHg) and obesity (weight 86 kg, BMI: 32.6 kg/m<sup>2</sup>). The diagnosis of SRNS emerged as no remission was observed after 4 weeks of prednisone therapy and three intravenous pulses of methylprednisolone, so corticotherapy was suspended. Cyclophosphamide treatment (3mg/kg, for 8 weeks) was introduced, but no remission was observed, than, subsequently cyclosporine therapy was initiated. In the meantime renal biopsy and genetic testing were performed.

Renal biopsy, performed at 10 months after the diagnosis of the disease, showed focal segmental glomerulosclerosis (FSGS). It reported both focal (6 glomeruli affected) and segmental (only the perihilar portion of the affected glomerulus was sclerotic in 1 juxtamedullary located glomerulus) lesions.

The coding exons and splicing junctions of *NPHS2* and *WT1* were directly (Sanger) sequenced for both patients. Genomic DNA was isolated from peripheral blood by standard methods, after obtaining informed consent from

the patient's parents. Exons 1-8 of *NPHS2* and exons 8 and 9 of *WT1* with their adjacent intronic junctions were analyzed by direct sequencing using the ABI3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) in the genetic laboratory of I<sup>st</sup> Department of Pediatrics, Semmelweis University, Budapest, Hungary. Primers are available upon request. For the sequence analysis, the Sequencher software (Gene Codes, Ann Arbor, MI) was used.

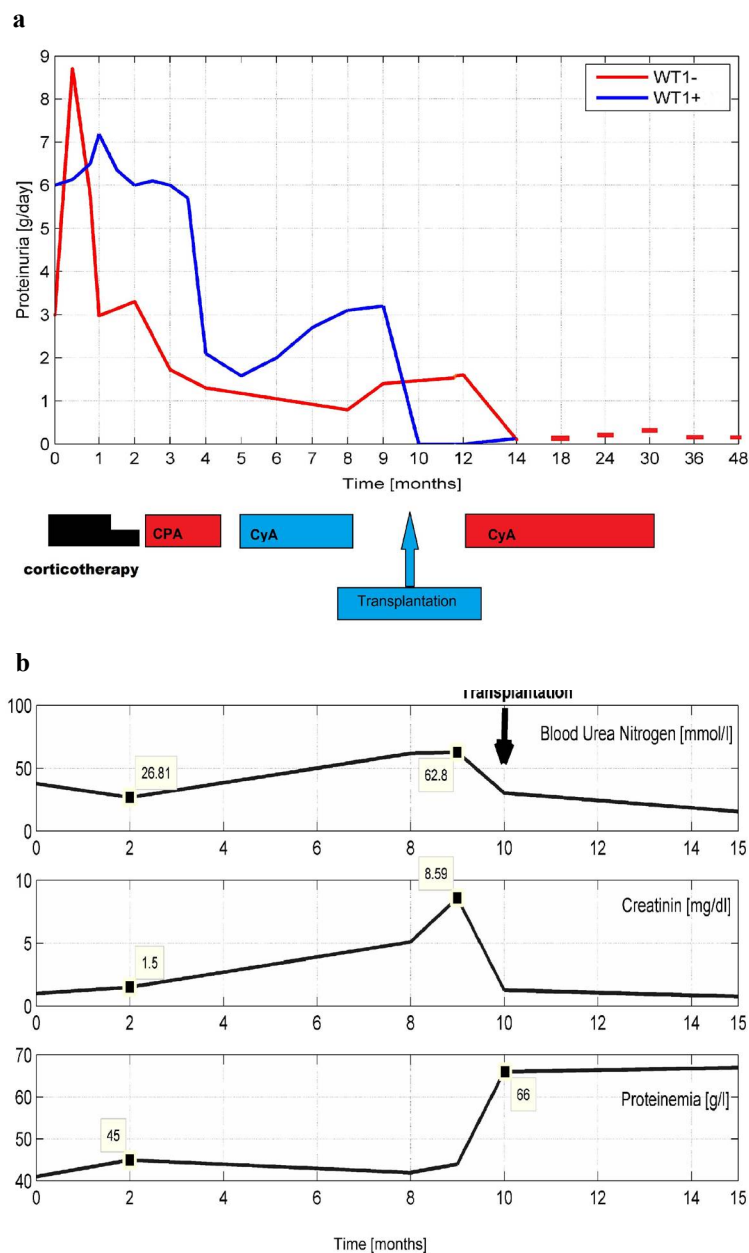
In this case no mutation was found, neither in *NPHS2* and *WT1*, nor in the subsequently sequenced *PAX2* gene.

The patient's evolution under immunosuppressive treatment was favorable, resulting in complete remission (proteinuria <150 mg/day) after 9 weeks of therapy. No relapse has occurred since, the patient being without treatment for the past 22 months.

Our second case, a 13 year old *WT1* positive girl, was admitted to hospital with a four week history of mild eye swelling and facial puffiness. In her medical history we noted an uninvestigated episode of edema syndrome at the age of seven, with spontaneous resolution. Physical examination revealed mild periorbital edema

**Table I. Clinical features and laboratory data at the onset of the disease**

	<i>WT1</i> negative (first patient)	<i>WT1</i> positive (second patient)
<b>Clinical features</b>		
Age (years)	16	13
Weight at presentation (kg)	79	36
Weight at discharge (kg)	69	35
Height (cm)	163	158
Edema		
periorbital edema	moderate	mild
lower leg edema	significant	without
<b>Laboratory parameters</b>		
Proteinuria (g/day)	5.8-8.7	6.35-7.2
Serum protein (g/l)	46	50
Serum cholesterol (mg/dl)	227	345
Serum triglyceride (mg/dl)	181	205



**Figure 1. Laboratory test results over time. a. Evolution of the urinary protein excretion – stationary in both cases under corticosteroid treatment. Disappearance of proteinuria in *WT1* negative case under immunosuppressive therapy and in the *WT1* positive case after transplantation. b. Laboratory investigations in dynamics in the second *WT1* positive case. (CPA – cyclophosphamid, CyA – cyclosporine A)**

without any extension to the legs, otherwise normal status with normal heart rate and blood pressure and a normal female phenotype. The main laboratory results are presented in *table I*. Based on these data the diagnosis of NS was made and corticotherapy was started, but no response was observed after 4 weeks. The side effects of corticotherapy were also noticed in this girl: moon face, hirsutism, striae, arterial hypertension 140/90 mmHg with epistaxis and transient hyperglycemia (179 mg%). Therefore, steroid treatment was discontinued and cyclosporine treatment was started. Complementary investigations (kidney biopsy, genetic test) were performed in this case too.

The renal biopsy, performed at 6 months after the diagnosis of the disease, also reported both focal (9 glomeruli affected) and segmental (perihilar portion of affected glomerulus sclerotic in 3 juxtamedullary located glomeruli) lesions.

In both cases the interstitium was widened and tubular atrophy was observed only in the vicinity of the affected glomeruli. Small and medium-sized arteries were showing specific changes for hypertension. Direct immunofluorescent staining revealed no immune deposits: negative for IgA, IgG, IgM immunoglobulins, kappa and lambda light chains, and Complement C4 and C1q proteins. The anti-C3 antibody test was weakly positive in arteries and glomeru-

lar arterioles. Electron microscopy examination showed diffuse effacement of the epithelial cell foot (podocyte) processes (*Figure 2*).

In case of this patient genetic testing revealed a heterozygous mutation in the *WT1* gene (c.1228+5 G>A) (*Figure 3*). As frequently observed, the mutation is likely to be de novo, as the father does not carry the mutation, though no

DNA sample was available from the asymptomatic mother. To rule out the diagnosis of Frasier syndrome, generated by the mutation in the *WT1* gene, a karyotype was performed, that indicated a female 46,XX chromosomal formula.

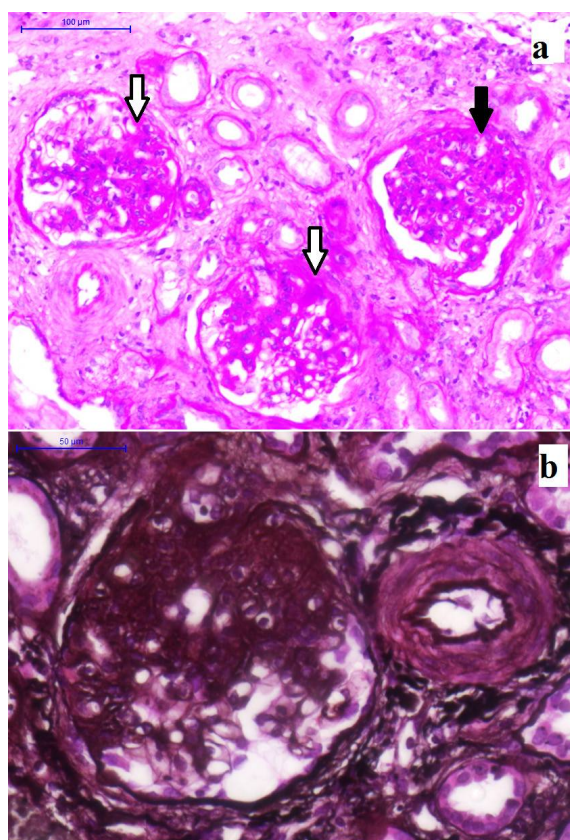
In this *WT1* positive patient, no changes in the degree of proteinuria were seen after 3 months of cyclosporine therapy, therefore treatment was discontinued.

The evolution under symptomatic therapy was unfavorable with high BP (180/115 mmHg) unresponsive to antihypertensive treatment and reduction of glomerular filtration rate (*Figure 1*), progressing to ESRD at 8 months after the onset of the disease. She underwent transplantation two months later. There was no recurrence during the follow-up period of 14 months.

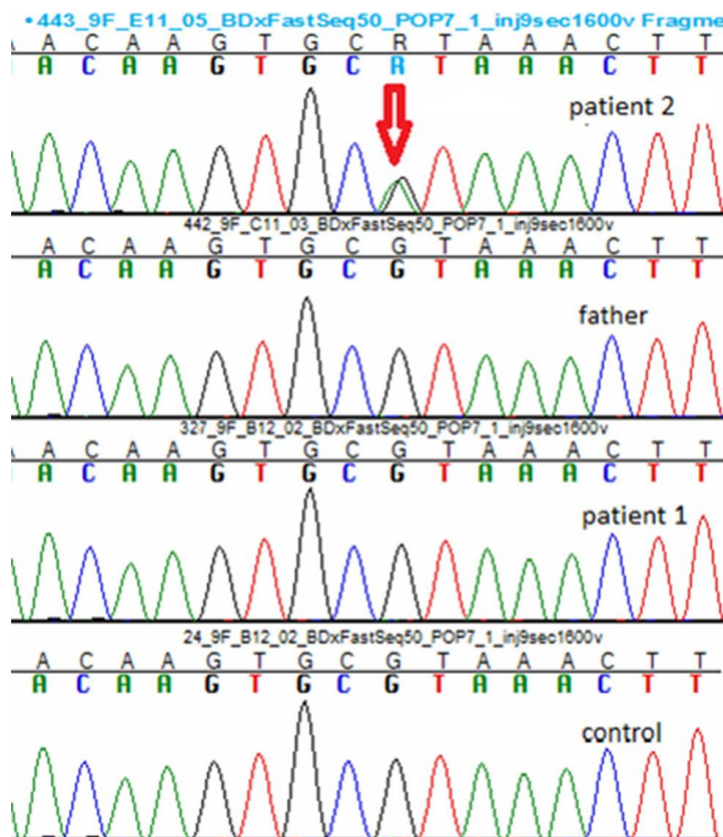
Based on clinical and laboratory data, the diagnosis of steroid-resistant nephrotic syndrome was established in both cases; in the first case, an immune form, with favorable evolution under immunosuppressive therapy, while in the second case, a genetic form, with a pathogenic mutation in the *WT1* gene, with favorable evolution after renal transplantation.

## Discussion

In all children with SRNS, treatment is problematic and is directed against immunological abnormalities. In all cases it is recommended to perform a renal biopsy before immunosuppressive therapy. As a second-line drug, two options are considered: alkylating agents and calcineurin inhibitors. [1] The randomized controlled trials in children with SRNS have demonstrated that calcineurin inhibitors are more effective than placebo or no specific therapy and also alkylating agents (30-33% of patients treated with cyclosporin obtained complete remission and 70% partial remission, while with cyclophosphamide favorable response was observed in 17-28%). [1, 11]



**Figure 2.** Light microscopy image of renal biopsy specimen from the *WT1* positive patient (with similar modifications in the first patient): a. one glomerulus with global sclerosis (black arrow) and two glomeruli with perihilar segmental sclerosis (white arrow) (PAS stain, x200). b. A glomerulus with perihilar segmental sclerosis (Jones-stain, x400)



**Figure 3. Sequencing chromatogram of the *WT1* gene in the donor splice-site of intron 9: c.1228+5 G>A mutation in heterozygous state in the second patient**

In this report two cases of adolescent onset SRNS are presented, with two different mechanisms leading to proteinuria and therefore with very different evolutions. Both cases presented primary resistance to steroid therapy. The first adolescent presented an acquired form of SRNS and FSGS, with favorable response to immunosuppressive therapy with a calcineurin inhibitor. In contrast, the second case presented an adolescent onset congenital form of SRNS and FSGS, with no response to the corticosteroid and immunosuppressive treatment and rapid progression towards ESRD.

Identification of a pathogenic mutation in a patient with SRNS has direct clinical consequences, as it makes further immunosuppressive

treatment unnecessary and the recurrence of NS in a kidney graft unlikely [2, 4, 9, 12-15].

In patients with SRNS, *WT1* mutations can lead to three distinct clinical entities [2, 9, 13-15]. 1. Denys-Drash syndrome, typically characterized by the triad of infantile SRNS with male pseudo-hermaphroditism, diffuse mesangial sclerosis and Wilms tumor; this was ruled out by the clinical manifestations (adolescent onset) and the histologically evidenced FSGS. 2. Frasier syndrome, characterized by association of SRNS with male pseudohermaphroditism, excluded by normal female karyotype (46, XX). 3. isolated NS – as we found in our case.

The identified *WT1* mutation is a frequent mutation in Frasier syndrome, which affects the



cleavage of exon 9 [13,14]. This form of NS does not respond to immunosuppressive therapy and patients should receive symptomatic treatment only. This mutation leads to several pathological conditions, including renal failure in the second or third decade of life. In case of our second patient the disease evolved rapidly to ESRD, within 8 months and as expected, no recurrence was seen after kidney transplantation.

The autosomal dominant transmission of this mutation means that the patient's daughters will have a 50% risk of inheriting the mutation. While male descendants will have the same risk of inheritance, they will also show associated genital disorders (intersex genitalia). If these children then develop FSGS, their disease will be more severe than that of their affected parent. The newborn child of a woman showing a mutated *WT1* gene associated with isolated FSGS (like our patient) can develop a more severe phenotype, like Denysh-Drash Syndrome or Frasier Syndrome [17]. If the mutation is known, targeted molecular prenatal diagnosis is possible and genetic counseling is highly recommended. Although the *WT1* gene mutations predispose to Wilms tumor and gonadoblastoma, they rarely develop when the mutation affects the splice site regions [9, 13, 16, 18]. According to literature, the risk of nephroblastoma in our patient is 5% and germ cell tumors occur only in patients with sex reversal [18].

Because detecting a mutation in patients with NS decisively influences the therapeutic options and reduces the possibility of post transplant relapses, screening for mutations in these genes is justified in clinical practice. If mutations in the *WT1* gene are found in a patient with a female phenotype, the gender genotype must be investigated (thus excluding the XY genotype with pseudohermaphroditism) [17].

There is an urgent need to distinguish between patients who may benefit from prolonged immunosuppressive treatment and those who

do not, nevertheless suffering from its side effects. In clinical practice, steroid therapy is initiated in all patients and genetic investigations are required only if steroid resistance is being observed. This initial steroid treatment can be avoided in patients with a family history of SRNS or in those who have a known causative mutation. In many cases, there is a tremendous delay between diagnosis and the request for genetic testing, although the genetic forms of SRNS should receive only symptomatic treatment [11].

## Conclusion

Steroid-resistant nephrotic syndrome is not a single entity, therefore it is important to determine and understand the underlying mechanisms and diseases. The genetic form of NS does not respond to immunosuppressive therapy and may progress to ESRD, but after kidney transplantation relapse is not expected, in contrast to the immune form. As *WT1* mutation is frequently found in young patients with NS, genetic screening is important for tumor prophylaxis, these patients having an increased risk to develop Wilms tumor or gonadoblastoma. These cases highlight the importance of early genetic diagnosis in SRNS. Although genetic testing is time-consuming, it is important for proper clinical management of the patients, prognosis and genetic counseling of the families.

## Conflicts of interest

The authors declare that they have no conflict of interest.

## Abbreviations

BMI -body mass index

BP - blood pressure

C - complement

ESRD - end-stage renal disease

FSGS - focal segmental glomerulosclerosis  
 FT3 - free triiodothyronine  
 FT4 - free thyroxine  
 NS - nephrotic syndrome  
 NPHS2 - nephrosis 2, idiopathic, steroid-resistant (podocin) gene  
 NPHS1 - nephrosis 1, congenital, Finnish type (nephrin) gene  
 SRNS - steroid resistant nephrotic syndrome  
 TSH - Thyroid-stimulating hormone  
 WT1 - Wilms tumor 1 gene

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