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BRIEF REPORT

Petras Kaltenis · Valérie Schumacher · Augustina Jankauskienė · Arvydas Laurinavičius · Brigitte Royer-Pokora

Slow progressive FSGS associated with an F392L *WT1* mutation

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Abstract Constitutional missense mutations in the *WT1* gene are usually associated with the Denys-Drash syndrome, characterized by a rapid progressive nephropathy, male pseudohermaphroditism, and an increased risk for Wilms tumor. We report here a patient with scrotal hypospadias and a slow progressive nephropathy due to focal and segmental glomerulosclerosis. *WT1* mutation analysis revealed a constitutional missense mutation in exon 9 resulting in an exchange F392L. This mutation has previously been reported by others in a patient with a similar mild course of nephropathy. In contrast, a mutation in the corresponding codon of exon 8 (F364L) was previously found by us in a patient with a very rapid progression to end-stage renal disease. Whether the position of a mutation may influence the course of the nephropathy must be evaluated in a larger patient cohort. The individual tumor risk for this alteration cannot be given at present because neither of the two patients has shown evidence of a Wilms tumor or a gonadoblastoma to date.

Petras Kaltenis and Valérie Schumacher contributed equally to the work

P. Kaltenis · A. Jankauskienė
Vilnius University Children's Hospital,
Vilnius, Lithuania

V. Schumacher · B. Royer-Pokora
Institute of Human Genetics and Anthropology,
University Hospital, Heinrich Heine University,
Düsseldorf, Germany

A. Laurinavičius
Vilnius University and National Center of Pathology,
Vilnius, Lithuania

V. Schumacher (✉)
Institut für Humangenetik und Anthropologie,
Heinrich-Heine Universität Düsseldorf,
Universitätsstrasse 1, 40225 Düsseldorf, Germany
e-mail: schumacv@uni-duesseldorf.de
Tel.: +49-211-8112356
Fax: +49-211-8112538

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Introduction

The Wilms tumor suppressor gene *WT1* plays an important role in the development of the urogenital system and the gonads. Constitutional missense and splice mutations in this gene cause the two similar clinical conditions Denys-Drash syndrome (DDS) and Frasier syndrome (FS). Both syndromes are characterized by male pseudohermaphroditism, a progressive glomerulopathy, and the development of genitourinary tumors, but specific characteristics apply to each disorder [1, 2, 3, 4]. Accumulating evidence supports the notion that DDS and FS form two ends of a spectrum of disorders with clinical and mutational overlaps [5, 6]. The predominant mutation types in DDS are missense mutations in exon 8 and 9, whereas splice mutations affecting the splice site usage in exon 9, and thus resulting in an abnormal +KTS/-KTS isoform ratio, are usually found in FS [7, 8]. We present here a child with scrotal hypospadias and non-progressive nephrotic syndrome carrying a missense mutation in the *WT1* gene resulting in F392L. The same mutation has been reported in a child with characteristics of FS rather than DDS [9].

Case report

The patient was born from a second uneventful pregnancy with signs of heavy (perineal) hypospadias: small, curved, clitoris-like penis, urogenital sinus, urethra opening at the middle of scrotum; the testes were not palpable, right-sided inguinal hernia and umbilical hernia were noted. Karyotyping showed a normal XY karyotype. Gonadotropin and testosterone levels were slightly elevated. At the age of 3 years the patient underwent surgery because of cryptorchidism. Right orchidopexy was performed and the left gonad was removed. Its pathomorphological examination showed rests of testicular and ovarian tissues. Later two further

surgical procedures were performed to correct the urethra and scrotum.

At the age of 1.5 years proteinuria was detected for the first time, ranging from 0.5 to 5 g/l. Slight hypertension and edema were occasionally noticed. No active treatment was given except for captopril at times of elevated blood pressure. Proteinuria persisted in the range of 1.5–2 g/day. At the age of 4 years 9 months a renal biopsy was performed.

The light microscopy specimens contained renal cortex with 21 glomeruli. No globally sclerosed glomeruli were present. The glomeruli revealed mild expansion of mesangial matrix without hypercellularity, with focal positivity for collagen by van Gieson stain. Seven of the glomeruli (especially those located in the subcapsular area of the kidney) showed extensive mesangial expansion with segmental sclerosis and hyalinosis of the capillaries. Segmental thickening of the glomerular capillary wall was noted in some glomeruli. Tubular epithelial cells showed pronounced degenerative changes. A few tubules showed microcystic dilatation with slightly positive casts with periodic acid-Schiff staining. The interstitium contained very few and small foci of mononuclear cell infiltration and fibrosis. Small arteries were unremarkable. Congo red staining for amyloid was negative. Immunofluorescence on frozen sections using Dako rabbit polyclonal antisera against human IgG, IgA, IgM, and C3 revealed weak-to-moderate staining for IgM in the mesangium and weak segmental pseudolinear IgG in the periphery of the glomerular capillary wall. The changes were summarized as focal and segmental glomerulosclerosis (FSGS) with focal features of mesangiosclerosis and mesangial deposition of IgM.

Treatment with prednisolone (40 mg/day) with cyclophosphamide (50 mg/day) and enalapril was introduced. One month later no substantial decrease of proteinuria was observed. The treatment was changed to three intravenous pulses of methylprednisolone followed by alternate-day prednisolone, cyclophosphamide, and enalapril. However, this produced no obvious benefit. For this reason cyclophosphamide was replaced by cyclosporine at doses of 100–200 mg/day depending on its serum concentration. These changes of treatment resulted in a marked decrease in proteinuria, enabling us to gradually taper doses of prednisolone and finally discontinue the steroid therapy. Proteinuria decreased to approximately 0.1 g/day, blood pressure and blood chemistry normalized. The general status of the patient was good at all times. No new complaints appeared, except for a mild gingival hyperplasia. Proteinuria was minimal, renal function remained stable, and kidney ultrasonography normal. After approximately 2.5 years of cyclosporine therapy, a second kidney biopsy was performed to judge the necessity of further therapy.

The specimen submitted for light microscopy contained renal cortex with 15 glomeruli. There were no globally sclerosed glomeruli present. A few glomeruli revealed mild expansion of mesangial matrix without hypercellularity. Three of the glomeruli revealed segmental thickening and reduplication of glomerular capillary wall. No evidence of FSGS was found. Tubular epithelial cells were unremarkable. Interstitium was not expanded. Small arteries were unremarkable. Congo red staining for amyloid was not performed. Immunofluorescence was performed on frozen sections using Dako rabbit polyclonal antisera against human IgG, IgA, IgM, and C3. Six glomeruli were present in the sections and revealed segmental weak pseudolinear and slightly granular fluorescence for IgM and IgG and equivocal staining for IgG and C3 in the periphery of the glomerular capillary wall. Electron microscopy was not performed. The changes were summarized as focal mesangial and membranoproliferative glomerular changes with weak segmental deposition of IgM and IgG in the periphery of the glomerular capillary wall. Immunosuppressive treatment was discontinued and the patient received only enalapril.

Several months later proteinuria increased to approximately 1.5 g/day and a transitory rash was observed that was considered due to enalapril. Cyclosporine therapy was reintroduced and enalapril replaced by lacidipine.

Several mild adverse effects, possibly related to immunosuppressive therapy, were noted during the next 6 months, namely, transient aphthous eruptions in the mouth, upper respiratory tract infection with radiological signs of sinusitis, and desquamation of fingers, toes, and soles, considered by a dermatologist as a fungal infection. Cyclosporine therapy was discontinued.

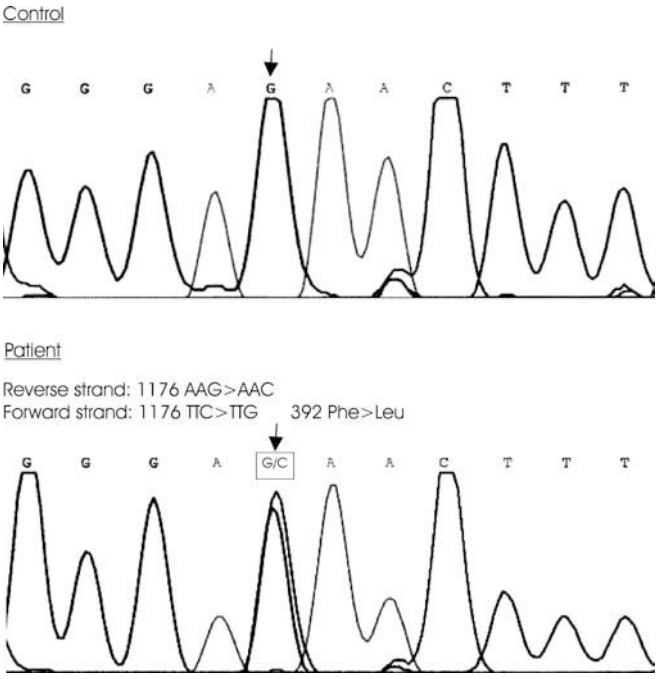

Fig. 1 Sequencing chromatogram of the reverse strand from exon 9. The nucleotide exchange is marked by an *arrow* and the mutated triplet and corresponding amino acid change are shown

The last examination at the age of 9 years revealed some elevation of blood pressure (128/98 mmHg); peripheral blood analysis showed a slightly elevated erythrocyte sedimentation rate (24 mm/h by Westergren). The serum creatinine level was 79 µmol/l. The glomerular filtration rate by the Schwartz formula was 94 ml/min per 1.73 m^2. Kidney ultrasound examination remained without noticeable changes. Proteinuria reached 3.6 g/day. The parents refused the reintroduction of cyclosporine therapy and the patient remained on enalapril only.

At the age of 8 years a genetic analysis of the *WT1* gene was performed. Using polymerase chain reaction/single-strand conformation polymorphism analysis as described previously [10], an altered banding pattern was found in exon 9 of the *WT1* gene. Direct sequencing revealed a transversion from TTC>TTG at nucleotide position 1176 (Fig. 1). This results in an exchange from Phe>Leu at codon 392. The same mutation has been found previously in a patient described as having FS [9]. The main characteristics of that patient were hypospadias, cryptorchidism, and proteinuria noted at 5 years, with a slow progression.

Discussion

This patient is interesting in several aspects. Firstly, one of the undescended testes contained both testicular and ovarian elements with fimbriae. Secondly, the nephropathy was morphologically characterized as FSGS rather than diffuse mesangial sclerosis. To date it is nonprogressive and partially responsive to immunosuppressive therapy. Under such therapy the nephropathy even improved, as was shown by the second kidney biopsy. Such a course and gonadal dysgenesis are more charac-

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P49953!WT1_SMIMA 162 RHQRRHTGVKPFQCKTCQRK F SRSDHLKTHTRTHTGKTSEK 202
P49952!WT1_RAT   371 RHQRRHTGVKPFQCKTCQRK F SRSDHLKTHTRTHTGKTSEK 411
O62651!WT1_PIG   372 RHQRRHTGVKPFQCKTCQRK F SRSDHLKTHTRTHTGKTSEK 412
P22561!WT1_MOUSE 372 RHQRRHTGVKPFQCKTCQRK F SRSDHLKTHTRTHTGKTSEK 412
P19544!WT1_HUMAN 372 RHQRRHTGVKPFQCKTCQRK F SRSDHLKTHTRTHTGKTSEK 412
P50902!WT1_ALLMI 211 RHQRRHTGVKPFQCKTCQRK F SRSDHLKTHTRTHTGKTSEK 251
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Fig. 2 Comparison of a part of the *WT1* sequence from different species demonstrating conserved amino acids. The affected phenylalanine (*F*) is highlighted by a *box* and the two cysteines (*C*) and two histidines (*H*) of the zinc finger are printed in *bold* (*SMIMA Sminthopsis macroura*, *ALLMI Alligator mississippiensis*)

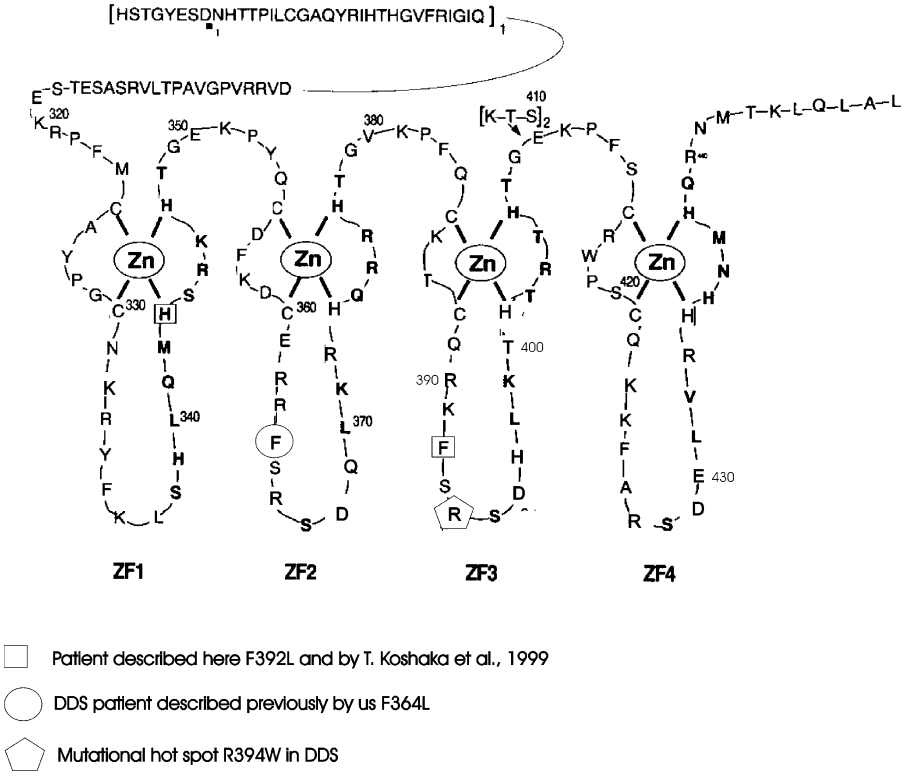

Fig. 3 Schematic drawing of the zinc finger domain of the WT1 protein. *Highlighted* is the mutation described here (F392L), a previously reported mutation (F364L) and the Denys-Drash syndrome hot spot mutation (R394 W)

teristic of FS [11]. However, the mutation found in this patient is not a splice site mutation, characteristic for FS, but a missense mutation. Missense mutations are usually associated with DDS.

The same mutation has been previously described in a patient with FS [9]. Interestingly, the clinical course was very similar to that of our patient. Proteinuria was noted at the age of 5 years with minimal change on renal biopsy. At the age of 8 years, proteinuria was aggravated, and at the age of 13 years renal biopsy showed a progressive stage of glomerulosclerosis. Hemodialysis was started at that time. He had a 46,XY karyotype with hypospadias and cryptorchidism. Pathological findings at the time of gonadectomy revealed gonadal dysgenesis or streak gonad. He did not develop Wilms tumor. Missense mutations commonly associated with DDS have not been reported in FS patients to date. For this reason Kohsaka et al. [9] analyzed whether the nucleotide change affects the splicing of the +KTS formation. This was not the case.

Most missense mutations found in DDS patients involve amino acids either important for DNA binding (exon 8 and 9, arginines and aspartic acids) or critical for zinc finger structure (exon 8 and 9, histidines and cysteines) by complexing zinc ions [12]. To assess the effect of DDS mutations affecting the arginine at position 394 and the aspartic acid at position 396, Pelletier et al. [7] performed gel shift experiments with an oligonucleotide containing the EGR consensus sequence. Neither of the two mutant WT1 proteins was capable of binding this sequence. The mutation at codon 392 Phe>Leu presented here does not fall into the two categories described but affects an amino acid critical for the zinc finger structure, and may therefore lead to a protein with defective DNA binding. The WT1 protein belongs to the category of C2H2 zinc fingers where two cysteines and histidines coordinate a zinc ion. The following pattern describes this zinc finger. #-X-C-X(1–5)-C-X3-#-X5-#-X2-H-X(3–6)-[H/C], where X can be any amino acid, and numbers in parentheses indicate the number of residues. The posi-

tions marked # are those that are important for the stable fold of the zinc finger. The final position can be either His or Cys. The C2H2 zinc finger is composed of two short beta strands followed by an alpha helix, and binds to the major groove of the DNA double helix (accession number PF00096). In the case of zinc finger 3 of WT1 the sequence is as follows: FQCKTCQRKFSRSDHLK-THTRTH. Underlined is the affected amino acid phenylalanine, corresponding to one of the amino acids marked with # in the sequence above. This phenylalanine is conserved in WT1 zinc fingers 2, 3, and 4 and between different species (Fig. 2). Previously, we have found a mutation in exon 8 at codon 364 changing a phenylalanine to leucine at the corresponding position in zinc finger 2 (Fig. 3, Schumacher et al. [13]). The patient had DDS with male pseudohermaphroditism and an early onset of nephropathy (6 months) progressing to end-stage renal disease within 3 months. Renal biopsy revealed the classical diffuse mesangial sclerosis as described by Habib et al. [14]. This rapid progression is in contrast to the slow progression in the patient presented here and reported by Kohsaka et al. [9], suggesting a positional effect of the mutations. Interestingly, Jeanpierre et al. [15] found a similar positional effect of mutations on the phenotype. Depending on which arginine was affected, R394 in exon 9 or the corresponding R366 in exon 8, the external genitalia were ambiguous or female in 46,XY patients. They concluded that exon 8 and 9 mutations may have different roles in urogenital development. Whether the F392L mutation is typically associated with a milder form of nephropathy or may present phenotypically as variable as the R362X [16] needs further investigation. In conclusion, *WT1* genotype-phenotype correlation studies may be useful for estimation of the prognosis in children with a nephropathy. As none of the two patients with the F392L mutation has either developed a Wilms tumor or a gonadoblastoma, the individual tumor risk cannot be given at present.

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