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BRIEF REPORT

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NPHS2 mutation associated with recurrence of proteinuria after transplantation

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Abstract Mutations in the *NPHS2* gene encoding podocin are associated with steroid-resistant nephrotic syndrome (SRNS) in childhood. Patients usually present with focal segmental glomerulosclerosis (FSGS). It is unclear to what extent SRNS due to *NPHS2* mutations predisposes to recurrence of proteinuria/FSGS after renal transplantation (RTx). A 4-year-old girl with infantile SRNS was started on peritoneal dialysis because of end-stage renal disease due to FSGS. Mutational screening of the patient and her parents revealed a novel single nucleotide deletion in exon 8 of the *NHPS2* gene (948delT), for which the patient was homozygous and her parents confirmed heterozygous asymptomatic carriers. At the age of 4.5 years the patient received a renal graft from her mother. On day 7 after RTx, the patient developed progressive proteinuria (urine protein/creatinine ratio 2.4 g/g), which responded within 1 week to prednisone pulse therapy, an increased cyclosporin A dosage, and ramipril therapy. The patient has maintained stable graft function and no further recurrence of proteinuria has been observed. In conclusion, patients with SRNS due to *NPHS2* mutations are not protected from recurrence of proteinuria after RTx. The quick response to increased immunosuppression in our patient suggests an immune-mediated pathomechanism for recurrence of proteinuria.

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Introduction

Focal segmental glomerulosclerosis (FSGS) is one of the most common histological findings in childhood steroid-resistant nephrotic syndrome (SRNS). FSGS represents a pool of clinically and genetically distinct disorders. Recently, several genes and their corresponding gene products, such as nephrin and alpha-actinin-4, have been demonstrated to be involved in the pathogenesis of inherited forms of SRNS and FSGS [1]. Mutations in the *NPHS2* gene encoding the membrane-bound protein podocin were identified as one of the most common causes of SRNS. Homozygous or compound heterozygous mutations in the *NPHS2* gene have been demonstrated in up to 30% of patients with sporadic or familial SRNS and FSGS presenting in childhood [2, 3]. Moreover, it has been proposed that patients with SRNS due to *NPHS2* mutations will be protected from recurrence of proteinuria/FSGS after renal transplantation (RTx) [3]. This would have important consequences for graft survival and overall prognosis of these patients. At variance with this hypothesis, we report on a 4-year-old girl presenting with SRNS due to FSGS caused by a novel homozygous mutation of the *NPHS2* gene who developed heavy proteinuria early after living-related RTx.

Case report

A 4-year-old girl had been suffering from recurrent upper airway tract infections from early infancy. At the initial presentation at the age of 6 months, clinical work-up revealed heavy proteinuria (4.4 g/l), hematuria (60/μl), and hypogammaglobulinemia (IgG, 0.5 g/l). At this time, blood pressure and serum creatinine levels were normal. Since the patient was resistant to steroid treatment (pred-nisone 2 mg/kg per day for 4 weeks followed by three methylprednisolone pulses 1 g/m^2 per day), a renal biopsy was performed at the age of 15 months, revealing FSGS (Fig. 1). Despite treatment with cyclosporin A (blood trough levels 80–

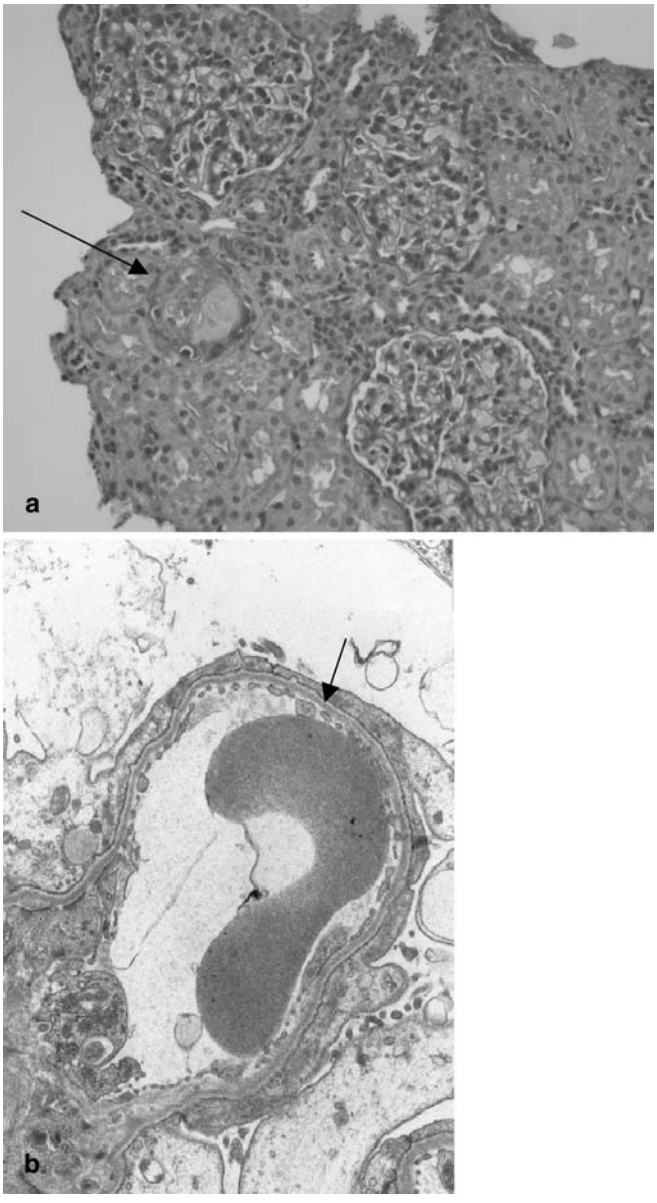

Fig. 1 a Light microscopy of the kidney biopsy revealed global glomerulosclerosis (*arrow*) in 5 of 12 glomeruli (periodic acid-Schiff, x200). Immunofluorescence microscopy revealed discrete mesangial IgM deposits and negative results for C3, IgA, and IgG (not given). Taken together the histological findings suggest advanced focal segmental glomerulosclerosis already leading to global sclerosis in some affected glomeruli. **b** Electron microscopy showing normal glomerular basement membrane, effacement of the visceral epithelial cell foot processes (*arrow*)

120 ng/ml) for 2 years, seven pulses of cyclophosphamide (0.5 g/m^2 per month) and methylprednisolone (1 g/m^2 for 3 days), and angiotensin converting enzyme inhibitors, the patient showed persistent nephrotic syndrome and progressive renal failure. At the age of 4 years the patient was started on peritoneal dialysis because of end-stage renal disease, and was referred to our hospital.

Mutational screening of the patient and her parents was performed by semi-automatic sequencing after DNA isolation from peripheral leukocytes as reported previously [3]. This revealed a novel single nucleotide deletion in exon 8 of the *NPHS2* gene (948delT) (Fig. 2). The patient was found to be homozygous and,

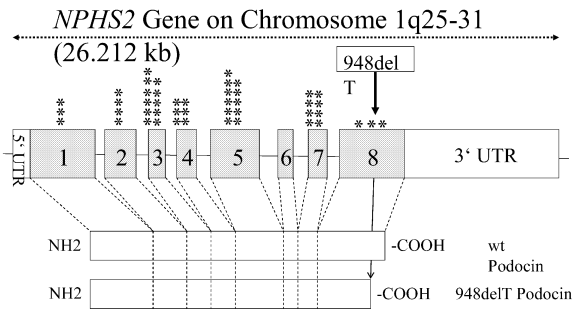

Fig. 2 Genomic organization of the *NPHS2* gene. The gene structure is displayed with its intron-exon boundaries and its gene product, podocin. The encountered mutation in our patient (948delT) is marked and the gene product, a prematurely truncated protein, is shown schematically. *Asterisks* mark the localization of the *NPHS2* mutations reported to date. Exons and untranslated regions (*UTRs*) are proportionally displayed according to their length, introns are not

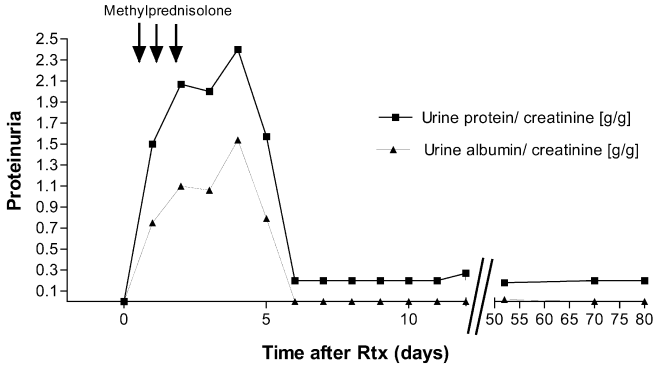

Fig. 3 Course of proteinuria after renal transplantation (RTx). The marked proteinuria that was first noted on day 7 post RTx responded within 1 week to methylprednisolone pulse therapy and increased cyclosporin A dosage

accordingly, her parents were both heterozygous for this mutation. As a formal consequence, this frame shift abolishes in-frame reading of the last regular 204 nucleotides and instead introduces a premature stop 93 bases after the single nucleotide deletion encoding a prematurely truncated protein (L347X).

At the age of 4.5 years the patient received a renal graft from her mother who had been confirmed to be heterozygous for the same mutation, but had no evidence of renal disease. The patient was anuric at the time of RTx. RTx was performed without technical problems, and graft function was excellent, with a daily urine output ranging from 1,200 ml to 1,600 ml. Post-transplant immunosuppression consisted of cyclosporin A (blood trough levels 180–210 ng/ml), mycophenolate mofetil (blood trough levels 1–3 µg/ml), and prednisolone. After RTx, daily assessment for proteinuria was performed. On day 7 after RTx, the patient developed progressive proteinuria (urine protein/creatinine ratio 2.4 g/g, urine albumin/creatinine ratio 1.1 g/g) (Fig. 3). The patient showed no edema, hematuria, or hypertension, the serum creatinine was 40 µmol/l, and the serum albumin was 3.0 g/dl. Patency of renal vessels was shown by Doppler ultrasonography.

The proteinuria responded within 1 week to methylprednisolone pulse therapy (500 mg on 3 consecutive days), an increased cyclosporin A dosage (blood trough levels 200–250 ng/ml), and ramipril therapy (3.75 mg/day) (Fig. 3). Because of the rapid response of proteinuria to treatment, renal biopsy was not performed. Histological examination of a protocol biopsy performed

6 months post RTx (light microscopy) showed no glomerular or tubular abnormalities, and negative immunofluorescence with antibodies to IgG, IgM, IgA, fibrinogen, C3, C4d, and C5b-9. Thereafter, the cyclosporin A dosage was reduced in order to achieve blood trough levels of 100–150 ng/ml. During further follow-up for 9 months her graft function has remained excellent (creatinine clearance 98 ml/min per 1.73 m^2) and no further recurrence of proteinuria has been observed. The transplant donor did not show any signs of renal disease before or after RTx.

Discussion

Recurrence of proteinuria after RTx has been described in 30% of pediatric patients suffering from SRNS due to FSGS. This is of essential therapeutic and socioeconomic importance because, despite aggressive treatment protocols (including steroid pulses, cyclosporin A, cyclophosphamide, plasma exchange, and immunadsorption), more than 50% of grafts are lost [4]. The almost immediate recurrence of proteinuria in children, which is often observed within the first weeks after RTx, strongly suggests the presence of a circulating factor producing proteinuria by a hitherto unknown mechanism. Other diseases causing recurrence of proteinuria after RTx include de novo glomerulonephritis and the formation of antibodies to podocyte proteins, as has been shown in patients with congenital nephrotic syndrome of the Finnish type [5].

Recently, a study involving a large cohort of patients with FSGS due to mutations in the *NHSP2* gene concluded that these patients will not develop recurrence of proteinuria after RTx [3]. In contrast, our patient developed heavy proteinuria early after RTx. In addition, five other patients with SRNS with homozygous or heterozygous *NPHS2* mutations showing early recurrence of proteinuria after RTx have recently been reported [6, 7, 8]. This phenomenon remains without explanation, especially since proteinuria in all except one patient responded well to increased immunosuppression, whereas their initial SRNS did not. Therefore, it might not be appropriate to speak of recurrence of the original disease. The origin of post-transplant proteinuria is currently unknown, but the *early* occurrence after RTx strongly argues against a de novo antibody mediated mechanism. In patients with the congenital syndrome of the Finnish type, post-transplant proteinuria occurred several months after RTx and was associated with antibodies to the podocyte protein nephrin. No anti-podocin antibodies could be detected by immunoblotting in three SRNS patients showing podocin mutations and early recurrence of proteinuria after RTx and no immunoglobin deposits could be detected by renal histology [8], making an antibody mediated mechanism rather unlikely.

The underlying mutation (L347X) in our patient was located close to the C-terminal end of the protein, suggesting that this area is of major importance for proper protein routing and/or function (e.g., nephrin/podocin interaction). Beside this mutation, two additional mutations in exon 8 of the *NPHS2* gene have been encountered to date (L327F, 923/2delAA) [9]. All these mutations are of special interest, since their particular location will serve to identify important regulatory elements located downstream of the first changed amino acid of the altered protein. The NCBI conserved domain search and CDART program (http://www.ncbi.nlm.nih.gov/Structure) were used to determine the possibility that this frame shift generated novel amino acid domains in the resulting protein. However, no known protein motif was identified. This was also the case for the wild type podocin amino acid sequence, which was subjected to the same database search. Recently, it has been shown that podocin co-localizes with several other podocyte proteins, such as nephrin, so that it is likely that the C-terminal part harbors essential regions for protein sorting, membrane targeting, and protein/protein interaction that remain to be determined [10].

In two other patients with SRNS due to a homozygous *NPHS2* mutation showing heavy proteinuria early after RTx, recurrence of FSGS was proven by renal biopsy. Both children displayed the same missense mutation (R138Q) located in exon 3 [6, 7, 8]. From this information, it can be hypothesized that recurrence of proteinuria is not dependent on the localization of the mutation within the gene. SRNS due to *NPHS2* mutations is thought to be a recessive disease requiring a mutation on both alleles coding for podocin. Therefore, mutations involving one or more as yet uncharacterized genes of the podocyte producing an additive effect must be postulated in these patients. Living-related RTx from donors bearing a heterozygous *NPHS2* mutation seems not to be per se associated with an increased risk of recurrence of proteinuria after RTx in this patient population, although to our knowledge this case report is the first living-donor related recurrence of proteinuria in *NPHS2* patients. All other reported patients have undergone cadaveric RTx [6, 7, 8]. Careful clinical work-up of potential transplant donors who are heterozygous for a *NPHS2* mutation, as in our case, should be performed before and after RTx in order to exclude glomerular disease leading to progressive renal failure in the donor after unilateral nephrectomy.

In conclusion, patients with SRNS due to *NPHS2* mutations are not protected from recurrence of proteinuria after RTx. Although the quick response to increased immunosuppression in our patient suggests an immune-mediated glomerular disease, the exact mechanism remains to be clarified.

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