Anti-nephrin antibodies guide living donor kidney transplantation in a pediatric patient with primary focal segmental glomerular sclerosis

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Abstract

Introduction: Disease recurrence after kidney transplantation (KTx) remains a major challenge in patients with primary focal segmental glomerulosclerosis (pFSGS). Antibodies targeting the slit diaphragm protein nephrin have been identified in patients with early disease recurrence. Here, we describe monitoring and effective pre-transplant elimination of anti-nephrin antibodies in an adolescent with pFSGS prior to living-donor KTx.

Methods: Anti-nephrin antibodies were assessed in pre- and post-transplant serum samples by ELISA, Western blot and immunoprecipitation using three different nephrin proteins.

Results: Pre-transplant treatment including rituximab and repetitive therapeutic plasma exchanges resulted in effective and sustainable reduction of anti-nephrin antibodies. Allograft function has remained excellent without albuminuria over a follow-up of more than one year. Further analysis showed that the antibodies were cross-reactive with NEPH3 (filtrin), another key slit diaphragm protein.

Conclusions: Monitoring and pre-transplant elimination of anti–slit diaphragm antibodies may become a standard, personalized approach in patients with pFSGS requiring KTx.

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Lay Summary

Autoantibodies against the podocyte protein nephrin have been recently identified in pediatric and adult patients with nephrotic syndrome and primary focal and segmental glomerulosclerosis (pFSGS). Further studies retrospectively reported a strong association of anti-nephrin antibodies with focal and segmental glomerulosclerosis recurrence after kidney transplantation. Data on preemptive therapeutic strategies are missing because of the novelty of these findings. Here we describe a pediatric patient with pFSGS and evidence of anti-nephrin antibodies before living-donor kidney transplantation. We monitored and successfully reduced anti-nephrin antibody levels by rituximab and plasma exchange before transplantation. The patient showed an excellent graft function without signs of disease recurrence in the transplanted kidney. Interestingly, we also detected reactivity of antibodies to the podocyte protein nephrin-like protein-3 (NEPH3), which shows similarities to nephrin. Our case report may serve as a starting point to explore treatment protocols for kidney transplantation in patients with antibodies against nephrin and other podocyte proteins.

The understanding of podocytopathies, including minimal change disease and primary focal and segmental glomerulosclerosis (pFSGS), has recently been refined through the identification of anti-nephrin antibodies,[1–10] which supports the concept that minimal change disease and pFSGS are different manifestations of a common molecular pathomechanism[11] and provides immunological evidence for an underlying circulating factor that causes and perpetuates pFSGS.[12,13]

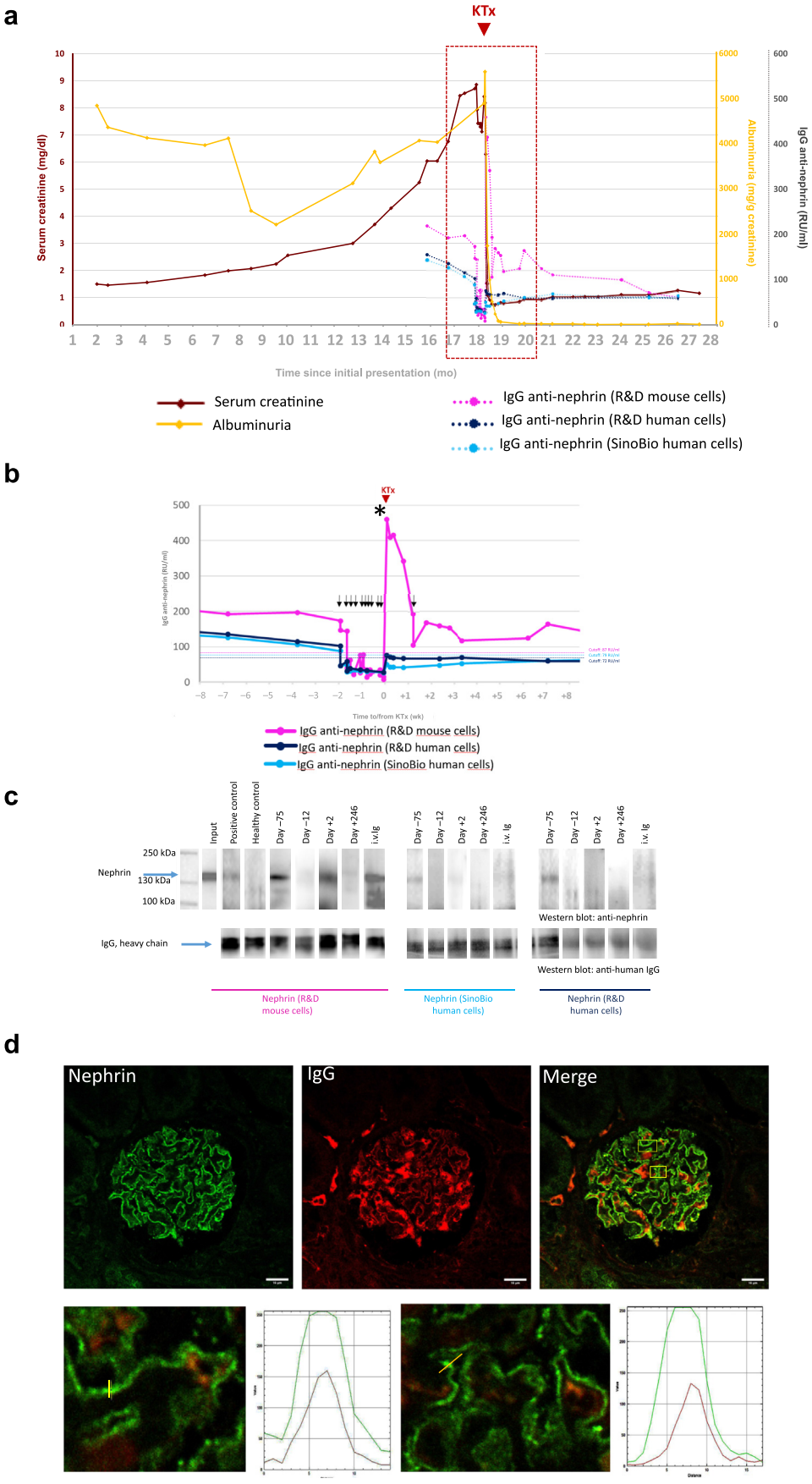

Figure 1 | Time course of serum creatinine, albuminuria, and anti-nephrin antibody levels and kidney biopsy staining. (**a,b**) Course of serum creatinine, serum anti-nephrin antibody levels as determined by 3 different enzyme-linked immunosorbent assays (continued)

Although several immunosuppressive regimens have been proposed to slow disease progression, ~50% of patients with pFSGS still progress to kidney failure within a few years.[12] Rapid disease recurrence after kidney transplantation (KTx) occurs in ~30% (9%–55%)[12] and is associated with a high risk of graft loss. In 2 recent retrospective studies, FSGS recurrence was associated with anti-nephrin antibodies.[4,6]

Here, we describe an adolescent with pFSGS presenting with high levels of anti-nephrin antibodies. We reasoned that therapeutic reduction in anti-nephrin antibodies, assessed by real-time antibody monitoring, could prevent recurrent disease after KTx.

METHODS

For measurements of anti-nephrin antibodies by enzyme-linked immunosorbent assay (ELISA), NuncMaxiSorp ELISA plates (Thermo Fisher Scientific) were coated with 100 ng/well of 3 different recombinant extracellular domains of the human nephrin protein. Details are given in Supplementary Methods.

Immunoprecipitation, Western blotting, and immunofluorescence were performed with standard techniques. Cross-reactivity was tested in cross-inhibition experiments assessing patient's antibody binding after serum preincubation with or without nephrin or kirre like nephrin family adhesion molecule 2 (Kirrel2)/nephrin-like protein-3 (NEPH3). Details are given in Supplementary Methods.

RESULTS

A 14-year-old girl of European descent with an uneventful medical history was referred for nephrotic-range proteinuria detected in routine workup. She was in excellent clinical conditions without edema. Laboratory workup classified her into categories G3A3 (estimated glomerular filtration rate 42.6 ml/min per 1.73 m^2 [creatinine-based Schwartz formula[14]]; urine albumin-creatinine ratio [ACR] of 547.94 mg/mmol creatinine [4849 mg/g creatinine]). A kidney biopsy revealed advanced FSGS with marked interstitial fibrosis and tubular atrophy. Standard biochemical and immunological workup was unremarkable as was a next-generation sequencing panel analysis for FSGS-associated genes. Subsequent trio exome analysis of the patient and her non-consanguineous healthy parents did not reveal FSGS-associated genetic variants (Supplementary Methods).

Therapy with ramipril resulted in good blood pressure control and a mild decrease in albuminuria. Tacrolimus over 3 months (trough levels 6–8 μg/l) failed to induce remission. Because of rapid disease progression, the girl was prepared for living-donor KTx only 10 months after the initial onset of the disease.

To better assess the risk of disease recurrence, we included analysis of anti-nephrin antibodies in our pretransplant workup. The patient tested positive for anti-nephrin antibodies by different methods (ELISA, immunoprecipitation, and Western blotting) using human nephrin produced in murine cells. These initial data were later confirmed using 2 nephrin proteins produced in human cells (Figures 1a–c and 2a and b; Supplementary Tables S1 and S2 and Supplementary Figures S1 and S2).

We therefore updated our transplantation protocol to include rapid, nearly real-time, antibody assessments to ensure optimal KTx scheduling (Table 1). Levels of anti-nephrin antibodies remained above the cutoff 3 weeks after 2 doses of rituximab, despite complete, sustained suppression of CD19+ B lymphocytes. Subsequent removal of antibodies by therapeutic plasma exchange (TPE) was effective (Figures 1a–c and 2b; Supplementary Table S2 and Supplementary Figure S2). Preemptive living-donor KTx was then performed with immediate graft function and without complications. As suggested by the International Pediatric Nephrology Association (IPNA) recommendations for steroid-resistant nephrotic syndrome, the ipsilateral native kidney was removed to better screen for *de novo* proteinuria deriving from the transplanted kidney post-KTx.[15] The initial biopsy and nephrectomy specimens were analyzed for nephrin and IgG. In the best-preserved glomeruli, discontinuous linear staining of nephrin colocalized with IgG was observed, albeit with less intensity than in the native kidney biopsy (Figure 1d; Supplementary Figures S3 and S4).

Until current follow-up more than a year after KTx, graft function has been excellent without albuminuria and with persistingly low levels of antibodies below the cutoff determined by the 3 assays (Figure 1a; Supplementary Table S2).

Cross-reactivity with NEPH3

We additionally looked for antibodies against the slit diaphragm protein NEPH3. The *KIRREL2* locus encoding NEPH3 is contiguous to *NPHS1* encoding nephrin, and single-nucleotide polymorphisms in both loci are strongly

Figure 1 | (continued) (ELISAs), and albuminuria over 2 years as well as around transplantation in higher resolution. The arrows indicate therapeutic plasma exchange, and the asterisk denotes infusion of 1 g/kg of i.v. Ig. The dashed lines in (**b**) indicate the cutoff for anti-nephrin antibody levels determined by the applied ELISA assays. Note that levels of IgGs detecting nephrin in murine cells decreased during further follow-up, falling below the cutoff, as shown in (**a**) and Supplementary Table S2. (**c**) Time course of anti-nephrin antibodies analyzed by immunoprecipitation (IP) using 3 different nephrin proteins. IP followed by Western blotting revealed with either a sheep anti-human nephrin antibody or a horseradish peroxidase (HRP)–conjugated donkey anti-sheep IgG antibody (upper blot) or a HRP-conjugated donkey anti-human IgG (lower blot). All experiments were conducted at the same time with the same reagents and were blotted in the same experiment with equal exposure times. (**d**) Glomeruli from the initial biopsy double labeled with anti-nephrin (green) and anti-human IgG (red). The right panel shows the merged image. The lower panels show the enlarged images of the boxed areas in the upper panels. The graphs show quantitative analyses of the fluorescence recorded across sections (yellow) of a representative capillary loop. Note the superimposition of the 2 signals, which indicates colocalization of nephrin (green) and IgG (red). KTx, kidney transplantation; RU, reference units. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

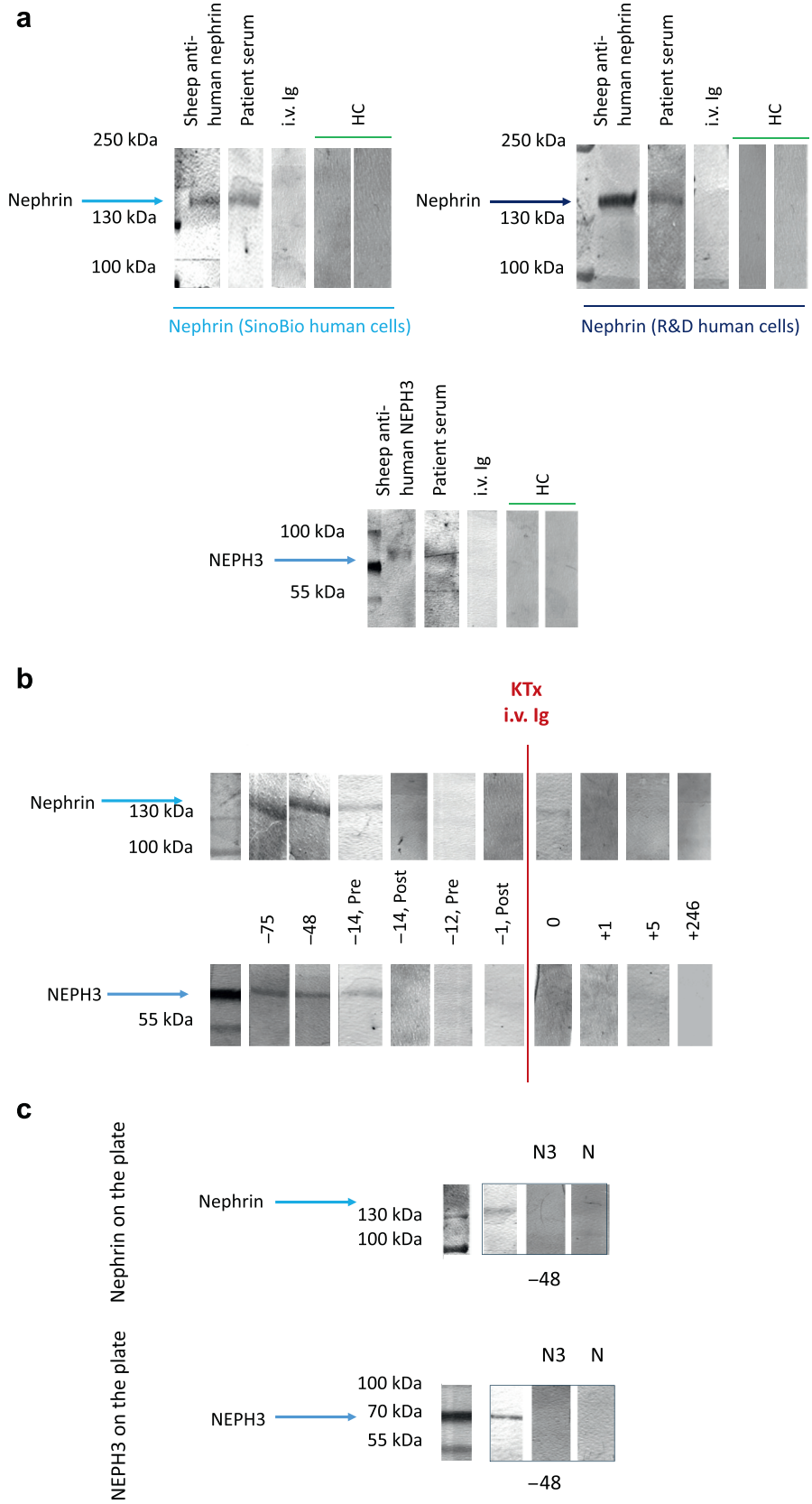

Figure 2 | Analysis of anti-nephrin and anti–nephrin-like protein-3 (NEPH3) antibodies using proteins from human cells. (**a**) Detection of circulating anti-nephrin and anti-NEPH3 antibodies. Recombinant human nephrin was detected as a band at ∼140 kDa using a sheep anti-human nephrin antibody. The same band was detected in patient sera but not in i.v. Ig. or sera from healthy (continued)

associated with minimal change disease in the South Asian population.[16] We indeed detected antibodies reactive to NEPH3, which were also reduced by our preemptive therapeutic regimen (Figure 2a and b; Supplementary Figure S2). Western blotting cross-inhibition studies suggested that the same population of antibodies was reactive with both antigens (Figure 2c).

Follow-up after KTx

We continued to assess antibody levels after transplantation (Figures 1a–c and 2b; Supplementary Table S2 and Supplementary Figure S2). i.v. Ig was given on the day before KTx, as overall Ig levels in the patient were low after the repetitive TPE sessions. After infusion of i.v. Ig, the assays using nephrin and NEPH3 produced in murine cells revealed an increase in antibody levels and several i.v. Ig batches tested positive for anti-nephrin antibodies (Figure 1a and b; Supplementary Figure S2). Surprisingly, we did not observe any clinical correlation with this finding and therefore decided to retrospectively reanalyze the sera using 2 additional nephrin proteins expressed in human cells. Although the pretransplant reactivity to nephrin and NEPH3 was confirmed, the reactivity of i.v. Ig and the associated course of anti-nephrin levels post-KTx were only faintly detected (Figures 1a and 2b; Supplementary Table S2).

DISCUSSION

Here, we present the first case of successful living-donor KTx that was guided by prospective monitoring and effective pretransplant elimination of anti-nephrin antibodies assessed by ELISA, Western blotting, and immunoprecipitation. This case is a model of individualized precision medicine for a disease in which recurrence rates are high and targeted therapy lacking. We identify additional reactivity of antibodies against NEPH3, suggesting more effects on podocyte integrity.

Overall, 21 patients with post-transplant recurrence of pFSGS and evidence of anti-nephrin antibodies have been reported.[2,4,6,17] In a Japanese cohort of 14 pediatric patients with kidney transplant and pFSGS, 11 had recurrence, and all retrospectively had anti-nephrin antibodies.[4] In a retrospective study of 39 European and North American patients with diffuse podocytopathies, 21 had recurrence, and 8 of these patients had anti-nephrin antibodies.[6] All patients with anti-nephrin antibodies recurred. As disease recurrence occurred at a median of 1 day,[2,4,17] the absence of disease recurrence in our patient more than a year is reassuring. Longitudinal data on individual monitoring in patients with nephrin antibodies remain sparse. Reduced levels were observed retrospectively in 6 patients after achieving remission, but analyses were performed at a single time point more than a year after KTx in most patients.[2,4] Protocols to prevent disease recurrence, including the optimal number of TPE sessions, have not been clearly defined. Our case report and experiments add novel information, for example, on prospective personalized monitoring, responses to individual TPE sessions, and post-KTx monitoring. A recent report described a comparable approach in a patient with steroid-resistant nephrotic syndrome and anti-nephrin antibodies.[18]

NEPH3/KIRREL2/filtrin is a member of the nephrin-like proteins of the Ig superfamily located at the slit diaphragm, which participates in the maintenance of the glomerular filtration barrier.[19] Like nephrin, immunostaining showed changes in the expression pattern of NEPH3 in proteinuric diseases.[20] Here, we show that anti-nephrin and anti-NEPH3 antibodies are simultaneously detected in the same patient and disappear after intensive treatment, a finding explained by cross-reactivity, although the presence of 2 populations of antibodies cannot be definitely excluded. The hypothesis that the dual reactivity of the antibodies may cause greater damage to the podocyte needs

Table 1 | Treatment approach for living-donor KTx in our patient with pFSGS and evidence of anti-nephrin antibodies

1. Two doses of rituximab (375 mg/m^2) in weekly intervals, starting 5 wk before planned KTx
2. Initiation of TPE of 1.5-fold the estimated plasma volume using albumin as a substitute, starting 14 d after the second RTX infusion
3. Real-time analysis of anti-nephrin antibodies before and after each TPE session
4. TPE sessions/immunoadsorptions (daily or with 1- to 2-d intervals to assess inter-treatment rebound) with continuous anti-nephrin antibody level monitoring until antibody levels were persistently below the cutoff
5. Infusion of 1 g/kg body weight of i.v. Ig after the last TPE session on the day before KTx
6. KTx with basiliximab as induction therapy followed by standard triple immunosuppressive therapy (tacrolimus, mycophenolic acid, and steroids)
7. Daily assessment of albuminuria after KTx for the early detection of disease recurrence
8. Reinitiation of 5 TPE sessions in case of disease recurrence and only 1 additional TPE session 7 days after KTx in the absence of evidence of disease recurrence

KTx, kidney transplantation; pFSGS, primary focal segmental glomerulosclerosis; TPE, therapeutic plasma exchange.

Figure 2 | (continued) controls (HCs). Recombinant human NEPH3 was detected as a band at ~70 kDa using a sheep anti-human NEPH3 antibody. The same band was detected in patient sera but not in i.v. Ig or sera from HCs. (**b**) Western blotting reactivity under reducing conditions of patient sera against nephrin and NEPH3. Sera were sampled at different time points before and after kidney transplantation (KTx). Days before and after KTx are indicated. Pre and post indicate before and after therapeutic plasma exchange. All samples were blotted in the same experiment for nephrin and NEPH3, respectively. (**c**) Cross-inhibition under reducing conditions of patient sera binding to recombinant nephrin (upper panels) or NEPH3 (lower panels) after serum preincubation in the absence or presence of nephrin (N) or NEPH3 (N3).

to be explored further. A recent publication suggested the presence of additional anti–slit diaphragm antibodies beyond anti-nephrin antibodies.[21,22]

The initially detected reactivity to nephrin in i.v. Ig, prepared from pooled serum, was surprising. It is well known that i.v. Ig can perturb autoantibody testing,[23,24] and there were no signs of clinically relevant autoantibody activity. Our follow-up data suggest that the generation of human nephrin in murine cells affected the reactivity of i.v. Ig through different IgG subclasses (Supplementary Figure S5). Variability in reactivity to nephrin from different sources has recently been discussed.[8,25] Importantly, our multilayered experiments on the initial presence of anti-nephrin antibodies, the reduction by rituximab and TPE, and the cross-reactivity between nephrin and NEPH3 showed consistent results with all 3 types of nephrin, although quantitative differences were observed.

This study has multiple limitations. It reports a single case with an association of a biomarker finding, which needs to be confirmed in other patients and ultimately in prospective multicenter studies. We could not retrospectively identify a specific date on which anti-nephrin antibodies appeared and whether additional factors may have contributed to the initial disease. We also cannot provide results of a post-transplant kidney biopsy, as we did not yet perform any post-transplant biopsy, given the excellent clinical course. We have not identified the epitopes recognized by the patient's antibodies and by natural autoantibodies, which will need further investigation. Importantly, it remains speculative what the disease course would have been without the intensive prophylactic measures, including rituximab and repeated TPE sessions, and what the required number of TPE sessions should be. Specifications are required to adapt protocols for deceased-donor KTx.

The in-depth clinical, genetic, and immunological workup allowed establishment of a highly personalized, successful treatment approach for this specific patient. Independent confirmation is required, and the data highlight the complexity of diagnostic assays in the field of anti-nephrin autoantibodies. Still, these findings seem relevant for treatment algorithms in the care of patients with pFSGS requiring KTx.

DISCLOSURE

All the authors declared no competing interests.

DATA STATEMENT

All data are shown in the manuscript and its supplement. Genetic data are available on request.

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Supplementary material is available online at www.kidney-international.org.

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