ORIGINAL ARTICLE

Autoantibodies Targeting Nephrin in Podocytopathies

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ABSTRACT

BACKGROUND

Minimal change disease and primary focal segmental glomerulosclerosis in adults, along with idiopathic nephrotic syndrome in children, are immune-mediated podocytopathies that lead to nephrotic syndrome. Autoantibodies targeting nephrin have been found in patients with minimal change disease, but their clinical and pathophysiological roles are unclear.

METHODS

We conducted a multicenter study to analyze antinephrin autoantibodies in adults with glomerular diseases, including minimal change disease, focal segmental glomerulosclerosis, membranous nephropathy, IgA nephropathy, antineutrophil cytoplasmic antibody–associated glomerulonephritis, and lupus nephritis, as well as in children with idiopathic nephrotic syndrome and in controls. We also created an experimental mouse model through active immunization with recombinant murine nephrin.

RESULTS

The study included 539 patients (357 adults and 182 children) and 117 controls. Among the adults, antinephrin autoantibodies were found in 46 of the 105 patients (44%) with minimal change disease, 7 of 74 (9%) with primary focal segmental glomerulosclerosis, and only in rare cases among the patients with other conditions. Of the 182 children with idiopathic nephrotic syndrome, 94 (52%) had detectable antinephrin autoantibodies. In the subgroup of patients with active minimal change disease or idiopathic nephrotic syndrome who were not receiving immunosuppressive treatment, the prevalence of antinephrin autoantibodies was as high as 69% and 90%, respectively. At study inclusion and during follow-up, antinephrin autoantibody levels were correlated with disease activity. Experimental immunization induced a nephrotic syndrome, a minimal change disease–like phenotype, IgG localization to the podocyte slit diaphragm, nephrin phosphorylation, and severe cytoskeletal changes in mice.

CONCLUSIONS

In this study, circulating antinephrin autoantibodies were common in patients with minimal change disease or idiopathic nephrotic syndrome and appeared to be markers of disease activity. Their binding at the slit diaphragm induced podocyte dysfunction and nephrotic syndrome, which highlights their pathophysiological significance. (Funded by Deutsche Forschungsgemeinschaft and others.)

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Kidney diseases that lead to nephrotic syndrome are characterized by excessive amounts of protein in urine. This phenomenon is usually caused by kidney damage — specifically, damage to podocytes, which are specialized epithelial cells of the glomerular filtration barrier that regulate the filtration process.[1-3] Podocyte dysfunction has been associated with various factors, including genetic mutations, allergies, infections, lymphoid neoplasms, certain drugs, and autoimmune diseases.[4] Severe podocytopathies that commonly result in nephrotic syndrome include minimal change disease, primary focal segmental glomerulosclerosis (FSGS), and membranous nephropathy, which all are descriptive terms based on histologic patterns. Because children with proteinuria rarely undergo a kidney biopsy, they usually receive a diagnosis of "idiopathic nephrotic syndrome." Although recent advances have clarified the pathophysiological characteristics of membranous nephropathy,[5] the underlying causes of minimal change disease, primary FSGS, and idiopathic nephrotic syndrome are unclear. Given their overlapping histological characteristics, such as extensive effacement of podocyte foot processes, these conditions are often assumed to represent different manifestations within a disease spectrum,[6,7] which highlights the complexity of these kidney diseases and the need for individualized and pathobiology-based approaches to diagnosis and treatment.[8]

In this respect, the recent observation of antibodies against nephrin in some patients with minimal change disease and recurrent FSGS has the potential to improve our understanding, classification, and treatment of minimal change disease and FSGS.[9,10] Nephrin is a key protein of the complex podocyte slit-diaphragm architecture and has extensive signaling functions; severe podocyte injury occurs on genetic mutation or experimental nephrin knockout.[11-13]

In this multicenter study, we developed new methods of detecting antinephrin autoantibodies and examined these autoantibodies in adults with biopsy-confirmed glomerular diseases, children with idiopathic nephrotic syndrome, and controls. We further assessed the relationship between these antibodies and disease activity during clinical follow-up and explored the pathophysiological mode of action of antinephrin autoantibodies on podocytes in an experimental setting.

Methods

Patients

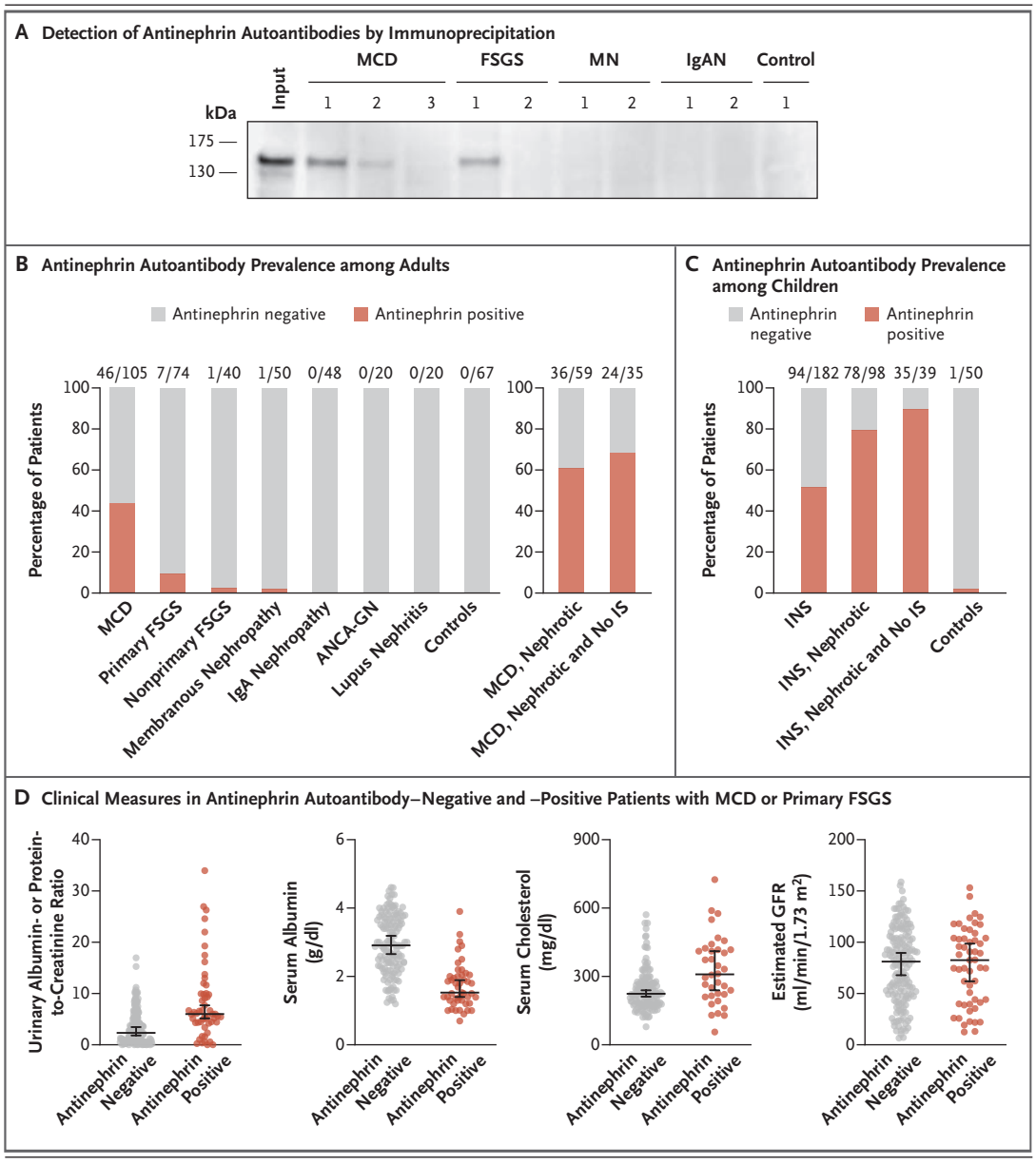
Serum or plasma samples from adults with biopsy-proven glomerular disease were obtained from the Hamburg Glomerulonephritis Registry and the University of Bari Aldo Moro. These cohorts included patients with minimal change disease, FSGS, membranous nephropathy, IgA nephropathy, antineutrophil cytoplasmic antibody (ANCA)–associated glomerulonephritis, or lupus nephritis.

Figure 1 (facing page). Prevalence of Antinephrin Autoantibodies in Patients with Proteinuric Glomerular Diseases and in Controls.

Panel A shows a characteristic Western blot result of immunoprecipitates (recombinant human nephrin ectodomain and human serum) detected with a nephrin-specific antibody. The input lane shows the human nephrin ectodomain used for immunoprecipitation. IgAN denotes IgA nephropathy, FSGS focal segmental glomerulosclerosis, and MCD minimal change disease. Panel B shows the prevalence of antinephrin autoantibodies in the investigated adult cohorts, in patients with MCD and nephrotic-range proteinuria (defined by a urinary albumin-to-creatinine or protein-to-creatinine ratio [with albumin, protein, and creatinine measured in grams] of >3.5 in adults), and in patients with MCD and nephrotic-range proteinuria who were not receiving immunosuppressive treatment (IS) at the time of sample collection. ANCA-GN denotes antineutrophil cytoplasmic antibody–associated glomerulonephritis. Panel C shows the prevalence of antinephrin autoantibodies in the investigated cohorts of children with idiopathic nephrotic syndrome (INS), in the subgroup of children with INS and nephrotic-range proteinuria (defined by a protein-to-creatinine ratio [with protein and creatinine measured in grams] of >2 in children), and in the subgroup of children with INS and nephrotic-range proteinuria who were not receiving IS at the time of sample collection, as well as the prevalence among pediatric controls. Panel D shows the urinary albumin- or protein-to-creatinine ratios, serum albumin levels, serum cholesterol levels, and estimated glomerular filtration rates (GFRs) in antinephrin-negative and antinephrin-positive adult patients with MCD or primary FSGS at the time of study inclusion. Data are shown as scatterplots; long horizontal bars indicate the median, and I bars indicate the 95% confidence interval. The widths of the confidence intervals have not been adjusted for multiplicity and may not be used in place of hypothesis testing.

Samples from 67 healthy blood donors were included as additional controls. Of the 115 patients with FSGS, 75 had primary FSGS. Samples from children with idiopathic nephrotic syndrome were obtained from the Division of Nephrology at Bambino Gesù Children's Hospital, the University of Bari Aldo Moro, and the NEPHROVIR cohort, which includes patients from pediatric centers in the Paris area and is coordinated at the Division of Pediatric Nephrology at Robert Debré University Hospital, Assistance Publique Hôpitaux de Paris. We included serum samples from 50 children from the Prenatal Identification of Children's Health (PRINCE) study as pediatric controls.[14]

All the patients or their legal guardians gave written informed consent, and the study was conducted in accordance with federal, state, and institutional guidelines and approved by the local ethics committee of the Chamber of Physicians in Hamburg, the Policlinic of Bari Hospital, the ethics committee of Saint Louis Ile de France IV,



and the Bambino Gesù Children's Hospital Ethical Committee in Rome. Further details of the patients are provided in the Supplementary Appendix and the protocol, available with the full text of this article at NEJM.org. The authors vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

IMMUNOPRECIPITATION

Antinephrin autoantibody status was determined by immunoprecipitation[9] with the use of the recombinant human nephrin ectodomain (amino acids A36 through L1052). The protein was incubated overnight with human serum or plasma before protein G was added. Immunoprecipitates were collected, electrophoresed, blotted, and analyzed for nephrin with an antinephrin antibody (AF4269; R&D Systems). Samples were analyzed in a blinded fashion, with unblinding of disease diagnosis and clinical data occurring after the determination of antinephrin autoantibody status. For quantitative readout of antinephrin autoantibodies, a hybrid assay involving immunoprecipitation followed by quantification of immunoprecipitated nephrin was established. Further details are provided in the Supplementary Appendix.

EXPERIMENTS IN ANIMALS

All experiments complied with national and institutional ethical standards and animal care guidelines and were approved by the Veterinarian Agency of Hamburg and the local committee for animal care. Male wild-type BALB/c mice were immunized subcutaneously with recombinant murine nephrin and complete Freund's adjuvant. Control animals received complete Freund's adjuvant mixed with phosphate-buffered saline. Further details of the experiments in animals are provided in the Supplementary Appendix.

STATISTICAL ANALYSIS

Because of the exploratory nature of the analyses in patients, no P values are provided and clinical data are shown as scatterplots with medians and 95% confidence intervals. The widths of confidence intervals have not been adjusted for multiplicity and may not be used in place of hypothesis testing. To quantify the association between antinephrin autoantibody status (positive or negative, as measured by immunoprecipitation) and disease activity (measured as the urinary albumin-to-creatinine or protein-to-creatinine ratio), linear repeated-measures models were fitted to natural log–transformed urine protein data for adults and children who tested positive for antinephrin autoantibodies and had available follow-up samples and data. Further details are provided in the Supplementary Appendix.

RESULTS

PREVALENCE OF ANTINEPHRIN AUTOANTIBODIES AMONG PATIENTS WITH PROTEINURIC GLOMERULAR DISEASES

We initially examined serum or plasma samples from 357 adult patients with biopsy-proven glomerular diseases (Table S1 in the Supplementary Appendix). Because we were not able to detect circulating antinephrin autoantibodies using direct Western blotting or enzyme-linked immunosorbent assay (ELISA) (Fig. S1), we optimized the assay for the detection of antinephrin autoantibodies using immunoprecipitation (Fig. 1A and Fig. S2). We found that 46 of 105 patients (44%) with minimal change disease, 7 of 74 (9%) with primary FSGS, 1 of 40 (2%) with nonprimary FSGS, and 1 of 50 (2%) with membranous nephropathy tested positive for circulating antinephrin autoantibodies, whereas all 48 patients with IgA nephropathy, all 20 patients with ANCA-associated glomerulonephritis, all 20 patients with lupus nephritis, and all 67 healthy controls remained negative (Fig. 1B, left panel). Antinephrin autoantibodies were detected in 36 of 59 patients (61%) with minimal change disease who had nephrotic-range proteinuria (defined by an albumin-to-creatinine or protein-to-creatinine ratio [with albumin, protein, and creatinine measured in grams] of >3.5 in adults and, in accordance with the 2022 Pediatric Nephrology Association Guidelines, >2 in children), as well as in 24 of 35 patients (69%) with minimal change disease who had nephrotic-range proteinuria and were not receiving immunosuppressive treatment at the time of sample collection (Fig. 1B, right panel).

Identifying biomarkers in children with nephrotic syndrome is particularly important because these children often do not undergo a kidney biopsy. We therefore assessed whether antinephrin autoantibodies can also be found in children with idiopathic nephrotic syndrome (Table S2). We performed immunoprecipitation analyses using samples from 182 children with

idiopathic nephrotic syndrome and found that 94 (52%) had detectable antinephrin autoantibodies at the time of sample collection (Fig. 1C). Antinephrin autoantibodies were detected in 78 of 98 patients (80%) with idiopathic nephrotic syndrome who had nephrotic-range proteinuria and in 35 of 39 patients (90%) with idiopathic nephrotic syndrome who had nephrotic-range proteinuria and were not receiving immunosuppressive treatment at the time of sample collection (Fig. 1C). Of the 50 control children, 1 was positive for antinephrin autoantibodies by immunoprecipitation (Fig. 1C).

Overall, the cohorts of patients with minimal change disease, FSGS, and idiopathic nephrotic syndrome had the expected ratio of male to female patients and spanned a broad age distribution similar to that in earlier studies. Adult non-White patients were underrepresented in this study because of limited sample availability in the investigated European patient cohorts (Table S3).

At study inclusion, patients with antinephrin-associated minimal change disease or primary FSGS had a more severe nephrotic syndrome than patients with these conditions who were antinephrin-negative, although kidney function was

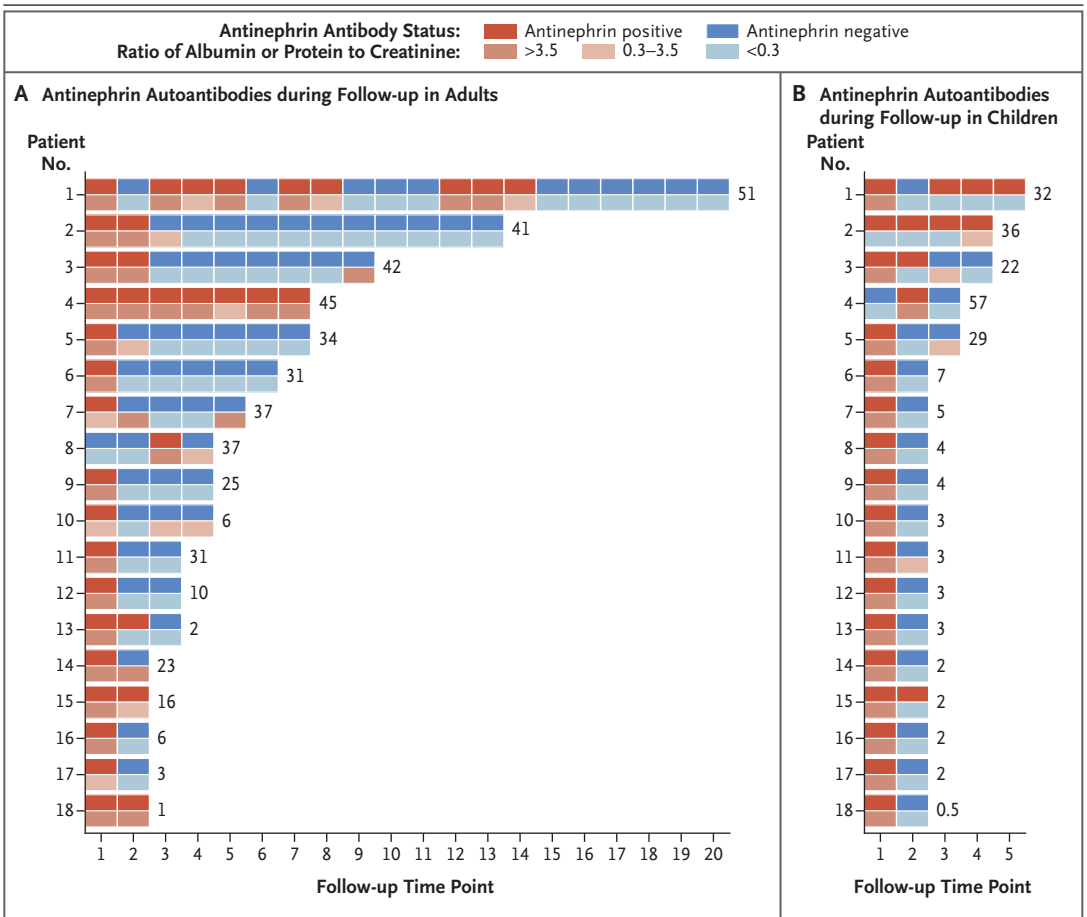
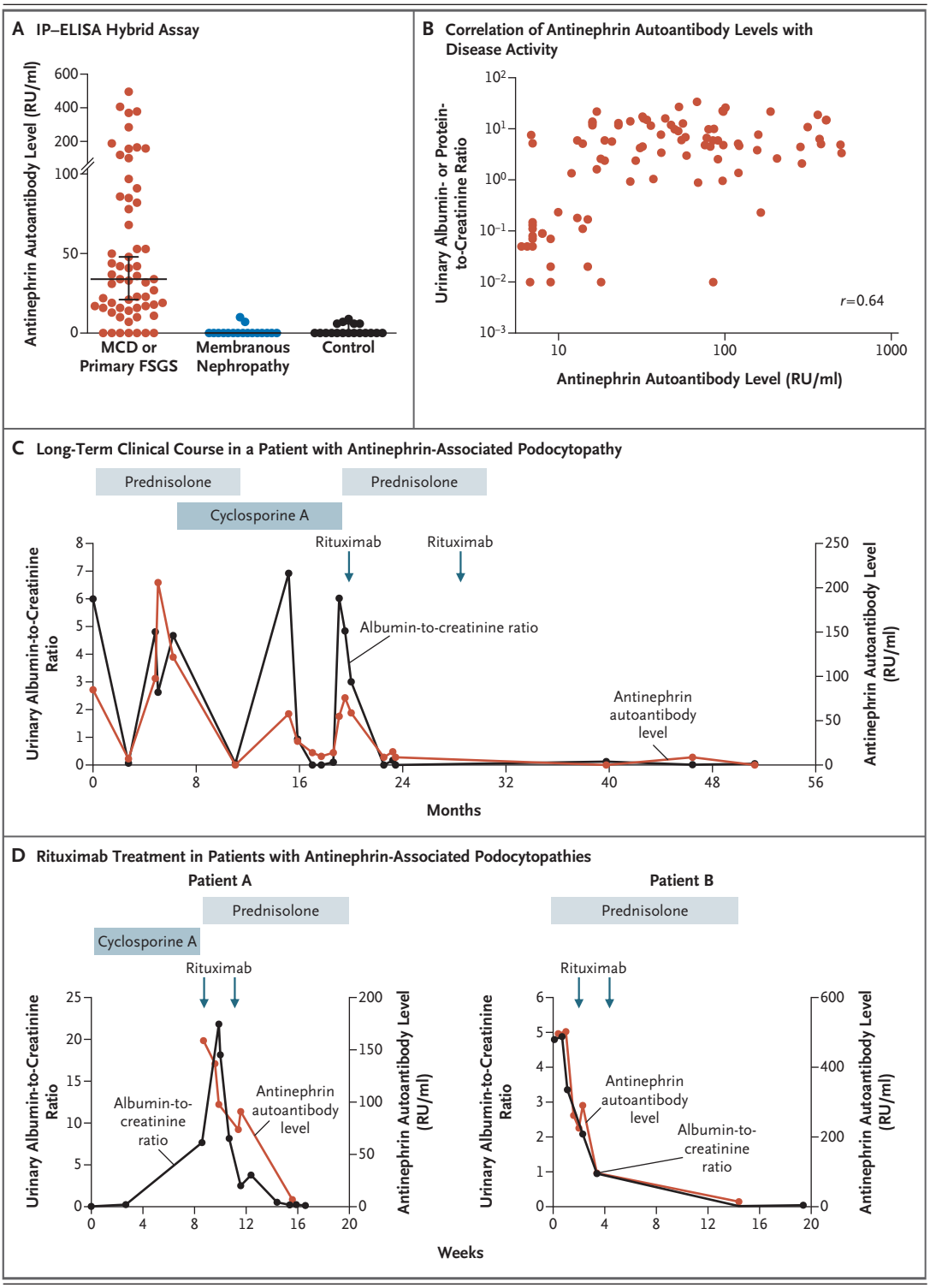


Figure 2. Correlation of Antinephrin Autoantibodies with Longitudinal Disease Course of Remission and Relapse.

Panel A shows a heat map indicating antinephrin antibody status (upper half of each square) and urinary albumin- or protein-to-creatinine ratio (lower half of each square) at study inclusion and during follow-up in adult patients with minimal change disease or primary FSGS who were positive for antinephrin autoantibodies in at least one serum sample. Patients 2, 12, 15, 16, and 18 had primary FSGS; the other patients shown had minimal change disease. Each row represents one patient, and each column represents one follow-up time point. The numbers on the right indicate the total follow-up time in months. Panel B shows the heat map indicating antinephrin antibody status and urinary protein-to-creatinine ratio at study inclusion and during follow-up in children with idiopathic nephrotic syndrome who were positive for antinephrin autoantibodies in at least one serum sample.



similar in the two groups (Fig. 1D). Findings were similar among the children with idiopathic nephrotic syndrome (Fig. S3). Moreover, none of the patients with minimal change disease, primary FSGS, or idiopathic nephrotic syndrome had type 1 diabetes, which argues against a major role of these autoantibodies in the development of pancreatic autoimmunity, as has been suggested previously.[15]

Together, these analyses corroborate the pres-

Figure 3 (facing page). Quantitative Measurement of Antinephrin Autoantibodies.

Panel A shows antinephrin autoantibody levels in adult patients with MCD or primary FSGS, patients with membranous nephropathy, and in controls at study inclusion, measured with the use of an immunoprecipitation (IP)–enzyme-linked immunosorbent assay (ELISA) hybrid assay. Data are shown as scatterplots; long horizontal bars indicate the median, and I bars indicate the confidence interval. The widths of confidence intervals have not been adjusted for multiplicity and may not be used in place of hypothesis testing. RU denotes relative units. Panel B shows the Spearman correlation between antinephrin autoantibody levels and the urinary albumin- or protein-to-creatinine ratio among patients with antinephrin-associated MCD or primary FSGS. Panel C shows the longitudinal disease course in one patient with antinephrin-associated MCD (Patient 1). Panel D shows two patients, one with antinephrin-associated MCD (Patient A, left) and one with antinephrin-associated primary FSGS (Patient B, right), who were prospectively treated with rituximab because of antinephrin autoantibody positivity during disease relapse.

ence of circulating antinephrin autoantibodies in a substantial proportion of adult patients with minimal change disease or primary FSGS as well as in children with a diagnosis of idiopathic nephrotic syndrome, indicating a prevalence of approximately 70% among patients with minimal change disease and 90% among those with idiopathic nephrotic syndrome during active disease before treatment.

ANTINEPHRIN AUTOANTIBODIES AND LONGITUDINAL DISEASE COURSE OF REMISSION AND RELAPSE

We next examined the utility of longitudinal measurement of antinephrin autoantibodies in patients with antinephrin-associated podocytopathies. We examined 163 follow-up serum samples from 38 adult patients with minimal change disease or primary FSGS for circulating antinephrin autoantibodies by immunoprecipitation in available samples, which were obtained without a prespecified follow-up time. Of these patients, 18 were positive for antinephrin autoantibodies at one or more points, 13 of whom had minimal change disease and 5 of whom had primary FSGS (Fig. 2A and Fig. S4A). The presence and absence of antinephrin autoantibodies were associated with active disease and remission, respectively, in these patients (Fig. 2A), and linear repeated-measures models revealed a strong association between antinephrin autoantibody positivity and protein levels in urine (Table S4).

We also investigated 33 available follow-up samples from 22 children with idiopathic nephrotic syndrome, 18 of whom were positive for antinephrin autoantibodies at one or more time points (Fig. 2B and Fig. S4B). Of the 13 children with antinephrin-associated idiopathic nephrotic syndrome for whom we had longitudinal paired samples at disease onset before immunosuppressive treatment and after a few weeks in remission, all 13 (100%) were antinephrin-positive at onset and 12 of 13 (92%) were antinephrin-negative in remission (Patients 6 through 18 in Fig. 2B).

In addition to immunoprecipitation with subsequent Western blotting, we developed a standardized immunoprecipitation–ELISA hybrid assay to quantify antinephrin autoantibody levels. At study inclusion, this assay reliably detected circulating antinephrin autoantibodies in 59 adult patients with minimal change disease or primary FSGS, whereas 17 patients with membranous nephropathy and 18 healthy controls remained negative (Fig. 3A and Fig. S5 and Table S5). Using this assay, we measured antinephrin autoantibody levels in follow-up samples from the 18 adults with antinephrin-associated minimal change disease or primary FSGS for whom such samples were available (Fig. S6 and Table S6). This analysis revealed a strong correlation between all measured antinephrin autoantibody levels and the urinary albumin-to-creatinine ratio ($r=0.64$) (Fig. 3B).

The clinical utility of antinephrin autoantibody measurements is exemplified by the clinical course in one patient with antinephrin-associated minimal change disease (Patient 1 in Fig. 2A). This patient had three episodes of relapsing nephrotic syndrome over an observation period of 51 months. Both antinephrin autoantibody positivity measured by immunoprecipitation and quantitative antinephrin autoantibody levels measured by combined immunoprecipitation–ELISA were closely correlated with the urinary albumin-to-creatinine ratio (Fig. 3C). During follow-up, immunosuppressive treatment with glucocorticoids and cyclosporine A led to a transient remission, whereas CD20-mediated B-cell depletion with rituximab induced complete and sustained remission, as indicated by undetectable antinephrin autoantibodies and no proteinuria (albumin-to-creatinine or albumin-to-protein ratio, <0.3).

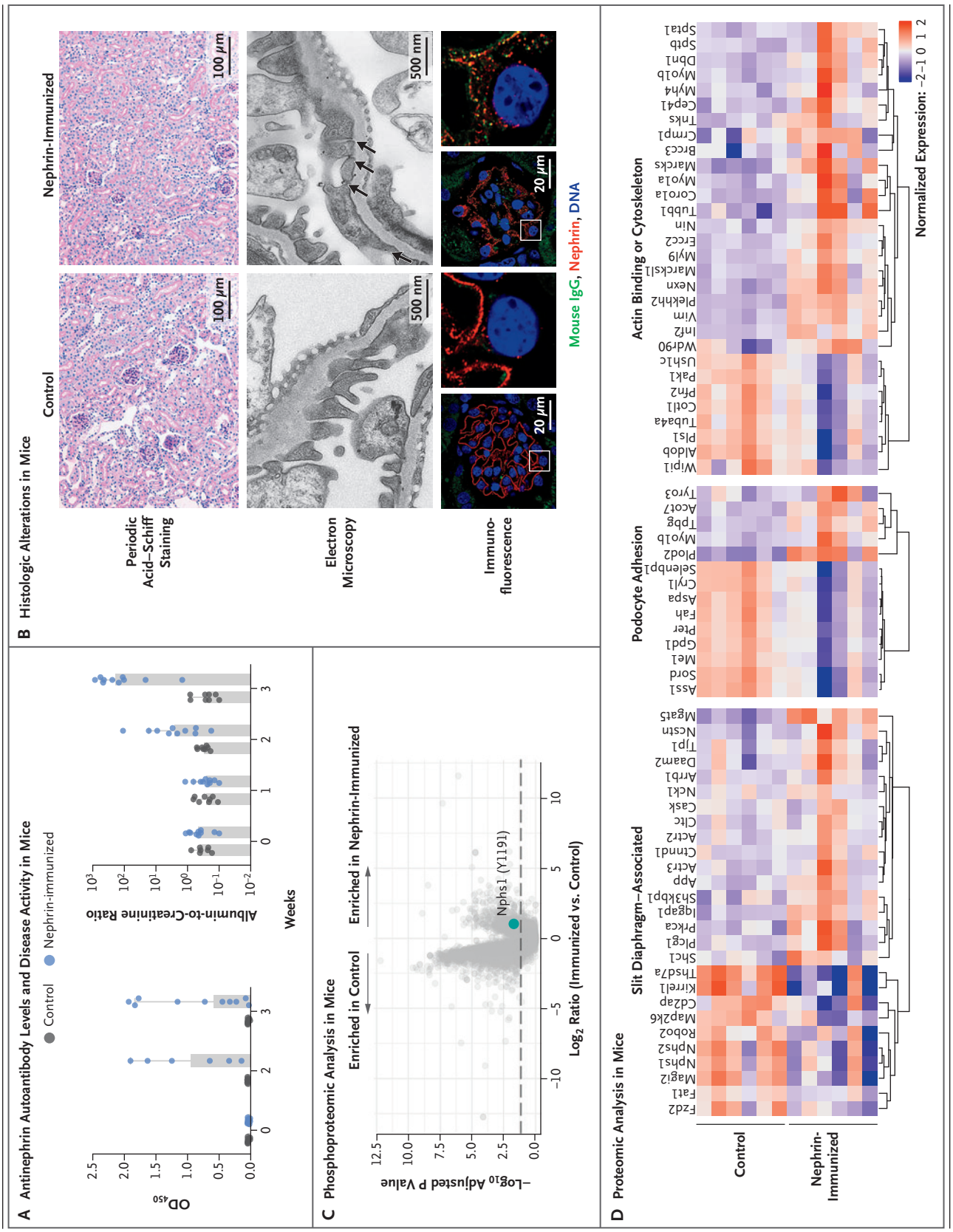


Figure 4 (facing page). Induction of an MCD-like Phenotype and Rapid-Onset Nephrotic Syndrome in Mice.

Panel A shows antinephrin autoantibody levels measured by ELISA and albumin-to-creatinine ratios in control and nephrin-immunized mice. Data are shown as scatterplots; long horizontal bars indicate the median, and I bars indicate the 95% confidence interval. The widths of confidence intervals have not been adjusted for multiplicity and may not be used in place of hypothesis testing. OD_{450} denotes optical density at a wavelength of 450 nm. Panel B shows representative periodic acid–Schiff staining, electron microscopy images, and immunofluorescence staining for mouse IgG in colocalization with nephrin in control and nephrin-immunized mice. Electron microscopy was performed with gold labeling for mouse IgG (black arrows). For immunofluorescence, panels on the right are enlargements of the boxed areas in the panels on the left. Panel C shows a volcano plot of differential phosphorylation (log_2 ratio of expression) in mice immunized with nephrin as compared with controls. Protein phosphosites are represented by gray dots, and the dashed line indicates the cutoff for significance (false-discovery rate–adjusted $P \leq 0.05$ by two-sided t-test). The blue dot indicates nephrin (Nphs1) phosphorylated at tyrosine residue Y1191, levels of which were increased in nephrin-immunized mice (log_2 ratio [nephrin-immunized vs. control], 1.053; adjusted $P=0.02$; 6 animals per experimental group). Panel D shows heat maps (maximum distance) of protein expression in controls and nephrin-immunized animals. The color scale of the heat map indicates protein expression levels relative to the mean expression; blue denotes protein expression below the mean, and red denotes protein expression above the mean.

We further prospectively treated two patients with rituximab (one with relapsing minimal change disease and one with relapsing primary FSGS), both of whom were positive for antinephrin autoantibodies. Both patients (Fig. 3D) went into immunologic and clinical remission after rituximab treatment.

Thus, antinephrin autoantibodies served as indicators of disease activity in patients with antinephrin-associated podocytopathy. In addition, in two prospectively followed patients, B-cell depletion with rituximab appeared to induce the reduction of antinephrin autoantibodies and lead to clinical remission.

ANTINEPHRIN AUTOANTIBODY INDUCTION AND MINIMAL CHANGE DISEASE–LIKE PHENOTYPE IN MICE

We next examined whether antinephrin autoantibodies could induce a podocytopathic phenotype in an experimental model. Active immunization of mice with the ectodomain of murine nephrin induced antinephrin autoantibody formation, which was followed by the rapid development of a nephrotic syndrome with high albumin-to-creatinine ratios, reduced serum albumin levels, and increased cholesterol levels 3 weeks after immunization (Fig. 4A and Fig. S7A through S7E). Periodic acid–Schiff staining did not reveal major glomerular differences between control and nephrin-immunized mice, and electron microscopy showed diffuse effacement of podocyte foot processes in the absence of electron-dense deposits in nephrin-immunized mice but not in control mice (Fig. 4B), findings consistent with histologic minimal change disease. Immunogold labeling of mouse IgG in nephrin-immunized mice revealed its localization at the slit diaphragm specifically in areas between effaced podocyte foot processes (Fig. 4B, black arrows, and Fig. S7F). Furthermore, nephrin-immunized mice showed nephrin redistribution from the membrane to the cytosol and a fine punctate pattern of mouse IgG positivity partially colocalizing with nephrin in the absence of immune-cell infiltrates, reduced podocyte number, or complement deposition (Fig. 4B and Fig. S8).

ANTINEPHRIN AUTOANTIBODIES, NEPHRIN PHOSPHORYLATION, AND PODOCYTE INJURY SIGNALING

To test whether antinephrin autoantibodies exert direct effects on nephrin signaling and downstream pathways, we performed proteomic and phosphoproteomic analyses of glomeruli isolated from mice 3 weeks after immunization. We quantified 10,545 phosphosites, 5696 of which differed significantly between control and nephrin-immunized animals, and we found that phosphorylation of nephrin was increased at tyrosine residue Y1191 (log_2 ratio [nephrin-immunized vs. control], 1.053; adjusted $P=0.02$), a conserved tyrosine residue corresponding to Y1176 in human nephrin (Fig. 4C and Fig. S9).[16-19] This phosphorylation site is known for its involvement in nephrin signaling that leads to actin assembly, cytoskeletal reorganization, and nephrin endocytosis.[20,21] Proteomic analyses of mouse glomeruli 3 weeks after immunization revealed that in nephrin-immunized mice, levels of adaptor proteins involved in endocytotic nephrin trafficking, such as ShcA (Shc1) and clathrin (Cltc),

were increased, whereas core slit-diaphragm–associated proteins were strongly down-regulated (Fig. 4D); these findings indicate the loss of a functional slit diaphragm in nephrin-immunized mice and are consistent with the previously described consequences of nephrin tyrosine phosphorylation. In addition, downstream effects of altered nephrin signaling were reflected by changes in key podocyte adhesion proteins and actin-binding or cytoskeletal proteins (Fig. 4D, middle and right panels). Thus, antinephrin autoantibodies induced nephrotic syndrome, nephrin phosphorylation, and cellular rearrangement on the proteomic scale, leading to ultrastructural changes that are typical of minimal change disease.

DISCUSSION

In this multicenter study, we systematically investigated the prevalence and clinical significance of antinephrin autoantibodies in patients with adult or childhood-onset glomerulopathies. We found antinephrin autoantibodies in 44% of adult patients with minimal change disease and in 9% of those with primary FSGS, as well as in 52% of children with idiopathic nephrotic syndrome, which defines these conditions as antinephrin-associated podocytopathies. Among the patients with minimal change disease or idiopathic nephrotic syndrome who had active disease before immunosuppressive treatment, the prevalence of these antibodies was 69% and 90%, respectively. Antinephrin autoantibodies were not detected in serum or plasma samples from patients with IgA nephropathy without minimal changes, patients with ANCA-associated glomerulonephritis, patients with lupus nephritis, or healthy controls. However, we detected antinephrin autoantibodies in one patient with nonprimary FSGS and one patient with PLA2R-associated membranous nephropathy. We speculate that some cases that had been classified as nonprimary FSGS actually were primary FSGS in patients who were in partial remission at the time of sampling, thereby escaping our classification criteria for primary FSGS. We also speculate that the case of antinephrin-positive membranous nephropathy might represent a rare case of concomitant membranous nephropathy and minimal change disease (as described anecdotally)[22] or a yet-undescribed case of intermolecular epitope spreading at the damaged podocyte cell membrane. In our study, one child in the control group — the child in that group who had the most upper respiratory infections — showed unexpected reactivity to nephrin, which suggests that positive results can occur in frequently infected children.

Analyses of follow-up samples identified circulating antinephrin autoantibodies as a new and reliable biomarker for monitoring disease in patients with antinephrin-associated podocytopathies, since their presence was correlated with disease activity at initial study enrollment and during follow-up in both adults and children. Antinephrin autoantibodies occasionally disappeared before the resolution of proteinuria, and in some patients proteinuria lessened even though antinephrin autoantibodies remained. This phenomenon may indicate the sensitivity limits of the assay, delayed healing of the glomeruli, or the activation of intrinsic repair mechanisms that counter the effects of autoantibodies.

In three patients with antinephrin-associated podocytopathy, B cell–targeted therapy with rituximab was shown to deplete antinephrin autoantibodies and induce clinical remission. This finding suggests that rituximab-induced remission in patients with nephrotic syndrome may be a consequence of autoantibody depletion, potentially in addition to off-target T cell–mediated effects, as discussed previously.[23-25]

The detection of antinephrin autoantibodies has proved challenging. Traditional methods such as direct Western blotting, which is effective in identifying autoantibodies in patients with membranous nephropathy,[5,26] have not been effective in identifying antinephrin antibodies, possibly because of the low abundance or affinity of these autoantibodies; a highly sensitive assay is therefore needed. Our immunoprecipitation assay, which enriches IgG, facilitated the specific and reliable qualitative identification of antinephrin autoantibodies. Our newly developed hybrid technique, which combines immunoprecipitation with a nephrin ELISA, also allowed for quantitative measurement of antinephrin autoantibody levels. Such antibody quantification can potentially transform the management of nephrotic syndromes by providing noninvasive alternatives for

diagnosis, enhancing treatment evaluation, and improving kidney-transplantation strategies in patients with recurrent primary FSGS.[10]

The strong correlation between disease activity and the detection of antinephrin autoantibodies, along with the specificity of these autoantibodies among glomerular diseases, suggests that these antibodies are not merely a by-product of podocyte damage but play a causal role in the pathobiologic characteristics of the disease. The pathogenicity of antinephrin autoantibodies was also illustrated by the development of antinephrin autoantibodies and nephrotic syndrome in mice that were immunized with nephrin. In contrast to immune models of membranous nephropathy,[27] disease in our model was triggered by a single immunization and manifested rapidly at low antibody concentrations. Furthermore, we discovered that antinephrin autoantibodies induced phosphorylation of nephrin at tyrosine Y1191, a process critical for mediating nephrin endocytosis and maintaining podocyte cytoskeletal integrity,[18,20,21] which resulted in profound actin and cytoskeletal alterations. Our findings suggest a new type of antibody-mediated kidney disease, in which low-level autoantibodies, like those targeting nephrin, disrupt kidney function without triggering traditional immune responses.

Our study revealed the widespread presence of antinephrin autoantibodies in patients with nephrotic syndrome across age groups, established these autoantibodies as indicators of disease activity, and suggested the direct pathogenicity of these autoantibodies. Together, these discoveries open new avenues for further prospective studies and pathophysiologically tailored medical interventions for glomerular diseases.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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