Journal of Translational Autoimmunity 11 (2025) 100307

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.sciencedirect.com/journal/journal-of-translational-autoimmunity

Afucosylated IgG in idiopathic nephrotic syndrome patients with anti-nephrin autoantibodies correlate with disease activity

Sonia Spinelli [a], Andrea Garbarino [a], Francesca Lugani [a], Edoardo La Porta [a,*], Noemi Rumeo [a], Giorgio Piaggio [a], Alberto Magnasco [a], Antonella Trivelli [a], Maria Ludovica Degl'Innocenti [a], Gino Tripodi [b], Simona Granata [c,d], Francesca Leone [e], Elena Zocchi [f], Lorenzo Gallon [g], Gian Marco Ghiggeri [a], Enrico Verrina [a], Gianluigi Zaza [e,h], Giovanni Candiano [a], Maurizio Bruschi [a,f]

[a] Division of Nephrology, Dialysis and Transplantation and Laboratory of Molecular Nephrology, Istituto Giannina Gaslini, IRCCS, Genoa, Italy
[b] Immunohematology and Transfusion Medicine Unit, Istituto Giannina Gaslini, IRCCS, Genoa, Italy
[c] Department of Biology, Ecology and Earth Sciences, University of Calabria, Rende, Italy
[d] Nephrology, Dialysis and Transplantation Unit, Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy
[e] Division of Nephrology, Dialysis and Transplantation, Annunziata Hospital, Cosenza, Italy
[f] Department of Experimental Medicine (DIMES), University of Genoa, Italy
[g] Department of Medicine, University of Illinois Chicago, Chicago, IL, USA
[h] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Italy

A B S T R A C T

Background: Idiopathic nephrotic syndrome (INS) is a glomerular disorder characterized by podocyte injury and proteinuria. Emerging evidence suggests that anti-nephrin autoantibodies (Abs) may contribute to disease pathogenesis in a subset of INS patients. Variation in techniques for detecting anti-nephrin Abs and lack of urinary data contribute to uncertainties of results.

While reduced IgG fucosylation is known to enhance antibody-dependent cellular cytotoxicity in non-INS autoimmune diseases, its role in modulating anti-nephrin autoantibody function and disease severity in INS remains unexplored.

Methods: We studied serum and urine of pediatric and young adult patients with biopsy-proven focal segmental glomerulosclerosis (FSGS) or minimal change disease (MCD) with different disease activity (proteinuria + *vs* proteinuria-). Anti-nephrin autoantibodies were evaluated with either conventional ELISA and immunoprecipitation using recombinant full-length extracellular domain of FLAG tagged human nephrin. Aleuria Aurantia Lectin (AAL) and Ulex Europaeus Agglutinin I (UEA-I) Lectins assessed IgG autoantibody fucosylation.

Results: Anti-nephrin autoantibodies were detected in serum of 11 % of FSGS and 15 % of MCD patients, with a higher prevalence among those with nephrotic-range proteinuria. These autoantibodies were absent in healthy controls as well as in patients with primary membranous nephropathy and class V lupus nephritis. Autoantibody titers correlated with disease activity, decreasing during remission. Immunoprecipitation confirmed results obtained with ELISA. In a subset of anti-nephrin positive patients, the autoantibodies were also detected in urine. Circulating anti-nephrin autoantibodies showed significantly reduced antennary and core fucosylation of IgG.

Conclusions: Our findings confirmed the significance of anti-nephrin autoantibodies as markers of active disease in a small subset of INS patients and showed their presence in urine. ELISA and Immunoprecipitation results correlated. Molecular studies showed that altered IgG fucosylation may contribute to immune-mediated podocyte injury. These insights provide potential biomarkers for disease monitoring and therapeutic targets in INS.

1. Introduction

Idiopathic nephrotic syndrome (INS) is the most common glomerular disease in children and young adults. It is characterized by altered glomerular permeability, leading to proteinuria, hypoalbuminemia, and edema. The pathological spectrum ranges from simple podocyte effacement (which justifies the definition of minimal change disease, or MCD) to focal areas of segmental glomerulosclerosis (FSGS). Corticosteroids induce remission in 70–80 % of cases (steroid-sensitive nephrotic syndrome, SSNS), some of which become steroid-dependent

* Corresponding author. Division of Nephrology, Dialysis and Transplantation and Laboratory of Molecular Nephrology, Istituto Giannina Gaslini, IRCCS, Via Gerolamo Gaslini, 5 16147, Genoa, Italy.
E-mail address: edoardolaporta@gaslini.org (E. La Porta).

https://doi.org/10.1016/j.jtauto.2025.100307
Received 7 May 2025; Received in revised form 29 July 2025; Accepted 8 August 2025
Available online 8 August 2025

(SDNS), while approximately 20 % are steroid-resistant and require further immunosuppression (steroid-resistant nephrotic syndrome, SRNS); a minority of cases remain refractory to any treatment (multidrug resistant nephrotic syndrome, MDRNS).

Although the pathogenesis of INS remains incompletely understood, the efficacy of anti-CD20 antibodies in inducing sustained remission over the past decade supports the hypothesis of an immune-mediated mechanism, possibly involving B cells or their products [1]. Recent studies have identified circulating anti-nephrin autoantibodies as potential pathogenic factors in INS [2–5]. These autoantibodies have been detected during active phases of the disease. They are often associated with punctate IgG deposits on podocytes in renal biopsies from patients with MCD [2,3,5], FSGS [2,5], and recurrent post-transplant FSGS [4].

Nephrin is a key structural component of the slit diaphragm, essential for maintaining selective glomerular permeability, as demonstrated by both in vitro and in vivo studies [6]. It has been hypothesized that the binding of autoantibodies to nephrin disrupts slit diaphragm integrity, thereby contributing to podocyte dysfunction and proteinuria. This pathogenic mechanism supports the idea that, in at least a subset of patients with INS, the disease represents an organ-specific autoimmune condition.

Glycosylation is a critical post-translational modification of immunoglobulins that influences their function [7–10]. IgG molecules undergo N-glycosylation at a conserved site (Asn-297) in the Fc region, essential for modulating interactions with Fc gamma receptors (FcγRs) and the complement system [7,11]. The glycosylation profile of IgG is highly dynamic and influenced by genetic factors, aging, and inflammatory states [12]. Aberrant glycosylation patterns have been associated with autoimmune diseases, alloimmune responses, inflammatory disorders [13], and renal pathologies [7].

Core fucosylation is one of the most critical modifications in IgG Fc N-glycans, significantly impacting antibody-dependent cellular cytotoxicity (ADCC) [14]. Core fucose reduces IgG affinity for FcγRIII, attenuating ADCC, whereas afucosylated IgG demonstrates enhanced binding to FcγRIII, leading to increased immune activation [14–17] and immune-mediated cytotoxicity [18]. Recent studies have highlighted the role of IgG afucosylation in kidney diseases: 1) Hypofucosylation of donor-specific antibodies has been implicated in antibody-mediated kidney graft rejection [7]; 2) In idiopathic membranous nephropathy (IMN), IgG4 exhibits a distinct glycosylation pattern characterized by hypogalactosylation and hypofucosylation, which correlates with disease severity and complement activation [19]; 3) Similar alterations have been observed in other renal diseases, including IgA nephropathy, where aberrant glycosylation contributes to immune complex deposition and glomerular injury [20].

This study focuses on anti-nephrin antibodies in patients with INS and their correlation with disease activity. It also demonstrates their detection in urine for the first time. Finally, we investigated changes in the fucosylation of circulating anti-nephrin IgG and their potential pathogenic association with disease activity.

2. MATERIALS and METHODS

2.1. Subject cohort

Serum samples from children and young adults affected by INS with biopsy-proven glomerular disease were obtained from the Division of Nephrology, Dialysis, and Renal Transplantation at the IRCCS Gaslini Children's Hospital and the Division of Nephrology, Dialysis, and Renal Transplantation at Cosenza (UNICAL). The clinical characteristics are reported in Table 1. These cohorts included 34 patients with minimal change disease (MCD) and 46 with focal segmental glomerulosclerosis (FSGS). Additionally, 10 patients with primary membranous nephropathy (MN) and 10 with lupus nephritis (LN) were analyzed for comparison. All LN cases were histologically classified as class V, while all MN cases corresponded to stage II. Among the MN patients, 50 % were positive for circulating antibodies against the phospholipase A2 receptor (PLA2R) antibodies. Samples from 20 healthy donors matched for age and gender were included as additional controls. According to KDIGO guidelines [21], Nephrotic syndrome is defined by the presence of nephrotic-range proteinuria (typically >3.5 g/day in adults or >50 mg/kg/day in children), often accompanied by hypoalbuminemia and edema. For pediatric operational purposes in this study, active disease was defined as a urinary protein-to-creatinine ratio (UPCR) > 2 mg/mg, a threshold commonly used in pediatric nephrology and adopted in previous clinical trials [1]. Complete remission was defined as a negative or trace protein result on the urine dipstick for three consecutive days, confirmed by a urinary protein-to-creatinine ratio (UPCR) of less than 0.2 mg/mg. Relapse was defined by 3+ dipstick for three consecutive days and confirmed by UPCR >2 mg/mg. Of the 80 patients with FSGS or MCD, 12, 54, and 14 had a clinical phenotype of steroid dependence (SDNS), multidrug dependence (MDNS), and multidrug resistance (MRNS), respectively. More in detail, two consecutive relapses defined SDNS within 15 days of steroid discontinuation; MDNS was defined by the need for two oral immunosuppression treatments to maintain clinical remission; MRNS was defined by the absence of complete remission after 12 months of treatment with at least two distinct immunosuppressants. All demographic and clinical characteristics are reported in Table 1. All subjects or their legal guardians provided written informed consent, and the study was conducted following

Table 1
Clinical and demographic characteristics of patients stratified by biopsy findings and treatment response. The clinical and demographic data of the patients studied were grouped according to their biopsy results and response to drug treatment. FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MN, membranous nephropathy; LN, lupus nephritis; SDNS, steroid-dependent NS; MDNS, multidrug-dependent NS; MRNS, multidrug-resistant NS; UPCR, urinary protein-creatinine ratio.

		FSGS				MCD				MN	LN
	Total FSGS MCD	Total	SDNS	MDNS	MRNS	Total	SDNS	MDNS	MRNS	Total	Total
Number (%)	80	34(42.5)	5	22	7	46(57.5)	7	32	7	10	10
Female (%)	35(44)	15(44)	2	10	3	20(43)	3	14	3	5(50)	5(50)
Male (%)	45(56)	19(56)	3	12	4	26(57)	4	18	4	5(50)	5(50)
Age median (range) year	9(2–21)	7(2–21)	10(6–17)	7(2–21)	6(4–21)	8(3–21)	7(4–18)	10(3–21)	10(5–21)	45 (31–51)	39 (28–49)
UPCR>2 mg/mg	40	15	1	7	7	25	3	15	7	10	10
UPCR between 0.2 and 2 mg/mg	40	19	4	15	0	21	4	17	0	0	0
Serum anti-PLA2R positive	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	5	Not tested
Serum anti-Nephrin positive	10	5	1	4	0	5	1	7	0	0	0
Urine anti-Nephrin positive	2	0	0	0	0	2	1	1	0	0	0

institutional guidelines and approved by the local ethics committee of the IRCCS Gaslini Children's Hospital Genoa, Italy (Approval numbers 089REG2015) and of the University/Hospital of Foggia, Italy (Approval numbers 191/CE/2023).

2.2. Recombinant human nephrin

HEK293T cell lysate overexpressing the extracellular domain (aa 23–1056) of FLAG-tagged human nephrin (OriGene Cat# MN004646) was centrifuged at 14,000×*g* for 10 min at 4 °C. Per manufacturer's instructions, the protein was purified using anti-FLAG M2 Affinity Gel (Merck Cat# A2220) and eluted with 3 × FLAG peptide (Merck Cat# F4799). The eluate was concentrated using Amicon Ultra Centrifugal Filters (Merck Cat# UFC9003) to remove excess peptide.

2.3. Quantitation of anti-nephrin autoantibodies in serum and urine

Nunc MaxiSorp™ ELISA plates (ThermoFisher Cat# 442404) were coated with 100 ng/well of recombinant FLAG-tagged nephrin in 50 mM carbonate-bicarbonate buffer (pH 9.6) and incubated overnight (ON) at 4 °C. Uncoated wells were included to control for nonspecific binding. Plates were washed three times with PBST (PBS + 0.05 % Tween-20), blocked ON at 4 °C with SuperBlock (ThermoFisher Cat# 37518), rewashed, and incubated for 1 h at room temperature (RT) with 100 μL of serum diluted 1:100 in SuperT (SuperBlock + 0.05 % Tween) or 90 μL urine plus 10 μL of 10 × PBST. After five PBST washes, 100 μL of HRP-conjugated anti-human IgG Fc antibody (Merck Cat# AP101P, 1:2000 in SuperT) was added for 30 min at RT. Plates were washed again, and 100 μL of TMB substrate (Bio-Rad Cat# 1721066) was added. The reaction was stopped with 100 μL of 100 mM H_2SO_4 upon reaching appropriate signal intensity. Absorbance at 450 nm was measured using a Bio-Rad iMark reader (Cat# 1681130). Nonspecific binding was subtracted using the uncoated well signal. All samples were analyzed in duplicate. Antibody titers were calculated from a standard curve generated with serial dilutions of sheep anti-nephrin IgG (R&D Systems Cat# AF4269); 100 μL of a 1:100 dilution was defined as 2 U/mL. The positivity threshold corresponded to the highest value in healthy controls, as described [2–5]. This conservative approach ensures high specificity and has been commonly adopted in prior studies on anti-nephrin IgG detection. The detection limit was defined as the lowest value distinguishable from blank. Titers were visualized in dot plots comparing positive and negative cases, with each dot representing an individual sample.

2.4. Immunoprecipitation of anti-nephrin autoantibodies

Ten microliters of serum or 900 μL of urine plus 100 μL of 10 × PBST were incubated with 100 ng of recombinant nephrin in 100 μL PBST in low-binding deep-well plates (ThermoFisher Cat# 9504050B) overnight (ON) at 4 °C. After incubation, 5 μL of pre-washed Protein G Mag Sepharose Xtra beads (Merck Cat# GE28978118-AD) were added. Positive and negative controls included 20 ng of sheep anti-human nephrin IgG (R&D Systems Cat# AF4269) and rabbit anti-human albumin IgG (Merck Cat# A3293), respectively. Samples were incubated for 4 h at room temperature (RT) with shaking at 500 rpm to allow formation of IgG–nephrin complexes. Beads were washed five times with PBST using a Magnum FLX magnet plate (Eppendorf Cat# 5075751836). Immunocomplexes were eluted in 30 μL of Laemmli buffer containing 40 mM TCEP (Merck Cat# C4706) and heated at 95 °C for 10 min.

2.5. Western blot

Protein samples were separated on 8–16 % SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad Cat# 1620112) using the Trans-Blot SD semi-dry transfer system (Bio-Rad Cat# 1703940). Membranes were blocked overnight (ON) at 4 °C with 3 % BSA, washed three times with PBST, and incubated ON at 4 °C with sheep anti-nephrin polyclonal antibody (R&D Systems Cat# AF4269, 1:1000 in blocking buffer). After washing, membranes were incubated for 2 h at room temperature with HRP-conjugated donkey anti-sheep IgG (R&D Systems Cat# F0125), washed again, and developed using SuperSignal West Pico Plus substrate (ThermoFisher Cat# 1863096). Images were acquired with the ChemiDoc Imaging System (Bio-Rad Cat# 12003153). Antibody titers were quantified using a standard curve from serial dilutions of sheep anti-nephrin IgG; the signal from 20 ng of antibody incubated with 100 ng of antigen was defined as 1000 U/mL.

2.6. Confocal microscopy

Five-micrometer cryosections were prepared from kidney biopsies of selected INS patients positive or negative for circulating anti-nephrin autoantibodies. Sections were fixed in 95 % ethanol for 10 min, washed with PBS, and blocked for 60 min at room temperature (RT) with PBS containing 2 % BSA, 2 % FBS, and 0.2 % fish gelatin. After PBS rinses, sections were incubated for 1 h at RT with sheep anti-human nephrin antibody (R&D Systems Cat# AF4269, 1 μg/mL), followed by donkey anti-sheep IgG–Alexa Fluor™ 568 (Invitrogen Cat# A-11015) or mouse anti-human IgG (Abcam Cat# ab200699, 1:750), then goat anti-mouse IgG–Alexa Fluor™ 488 (Invitrogen Cat# A-11001). All Alexa Fluor™ conjugates were used at 1:500. Slides were washed with PBS and mounted using ProLong Gold Antifade (ThermoFisher Cat# P36930) with #1.5 coverslips. Imaging was performed using a Leica TCS SP5 confocal microscope with 458–633 nm lasers and a 40 × oil immersion objective. Background-subtracted images were analyzed with Fiji ImageJ v2.9.0 (NIH, Bethesda, MD, USA).

2.7. Enzyme-linked immunosorbent assay (ELISA) for fucosylated IgG

Nunc MaxiSorp™ ELISA plates (ThermoFisher Cat# 442404) were coated with 100 ng/well of goat anti-human IgG (Merck Cat# I2136) in 50 mM carbonate-bicarbonate buffer (pH 9.6) and incubated overnight (ON) at 4 °C. Uncoated wells served as background controls. Plates were washed three times with PBST, blocked ON at 4 °C with SuperBlock (ThermoFisher Cat# 37518), rewashed, and incubated ON at 4 °C with 100 μL of 1:100 diluted serum or 90 μL urine plus 10 μL of 10 × PBST, standard, or positive/negative controls in SuperT. After five washes, 100 μL of Aleuria Aurantia Lectin (AAL; Vector Laboratories Cat# B1395-1) or biotinylated Ulex Europaeus Agglutinin I (UEA-I; Vector Laboratories Cat# B1065-2), both diluted 1:1000 in SuperT, were added and incubated ON at 4 °C. Plates were washed, incubated with 100 μL of streptavidin-HRP (ThermoFisher Cat# 434301, 1:10,000 in SuperT) for 30 min at room temperature (RT), washed again, and developed with 100 μL of TMB substrate (Bio-Rad Cat# 1721066). The reaction was stopped with 100 μL of 100 mM H_2SO_4. OD450 was measured using a Bio-Rad iMark reader (Cat# 1681130). Fucose levels were determined using a standard curve from serial dilutions of the highest-titer healthy donor serum, arbitrarily defined as 100 U/mL. The detection limit corresponded to the lowest fucose value distinguishable from blank.

Lectin specificity was verified in two control experiments. In the first, lectins were preincubated for 30 min at RT with 100 mM L-fucose (Merck Cat# F2252) or D-lactose (Merck Cat# 61345) before addition to ELISA plates with healthy donor serum; L-fucose, but not lactose, blocked lectin binding. In the second, 100 μg of serum from the highest-titer donor was diluted to 100 μL in PBS and treated with PNGase F (New England Biolabs Cat# P0704, 1 U/μg protein) ON at 37 °C to remove N-glycans [22]. Samples were then concentrated using Amicon Ultra filters (Merck Cat# UFC5100) to remove the enzyme. Treated samples were reanalyzed with lectin-based ELISA under the same conditions. Fucose levels were visualized as dot plots, with each dot representing one sample.

2.8. Statistical analysis

Statistical analyses were performed using OriginPro (version 2024b). Depending on distribution, continuous variables are reported as median with interquartile range (IQR) or mean ± standard deviation (SD). Categorical variables are expressed as counts and percentages. Incidence rates described the frequency of outcome events. The Mann-Whitney U or Kruskal-Wallis test was used to compare continuous variables; Fisher's exact test was applied for categorical data. Spearman's rank correlation assessed associations between proteinuria and either anti-nephrin autoantibody titers or fucose levels. Receiver operating characteristic (ROC) curves evaluated assay performance, with area under the curve (AUC) and 95 % confidence intervals (CI) classified as: 0.5 (no discrimination), 0.5–0.6 (fail), 0.6–0.7 (poor), 0.7–0.8 (fair), 0.8–0.9 (good), and 0.9–1 (excellent). Survival analysis was conducted using Kaplan-Meier curves with group comparisons by log-rank test. A two-tailed p-value ≤ 0.05 was considered statistically significant.

3. Results

Fig. 1 shows the study workflow. Serum and urine anti-nephrin autoantibodies were measured by ELISA in 100 NS patients (80 INS, 10 MN, 10 LN) and 20 healthy controls (panel A). Immunoprecipitation confirmed antibody binding to recombinant nephrin (panel B). IgG fucosylation was then assessed in all sera using lectin-based ELISA to explore its correlation with INS severity (panel C).

3.1. Circulating anti-nephrin autoantibodies and clinical correlation

ELISA and immunoprecipitation assessed anti-nephrin IgG.

ELISA: ELISA was performed in 46 FSGS (57.5 %) and 34 MCD (42.5 %) patients, along with 10 MN, 10 LN, and 20 age- and sex-matched healthy controls (Fig. 1A). The positivity threshold (0.78 U/mL), based on the highest value in controls, yielded 98.97 % specificity (CI 94.4–99.9 %) and 100 % sensitivity (CI 72.2–100 %) in the ROC analysis. None of the MN or LN patients were positive (Fig. 2A). Among INS patients, 10/80 (12.5 %) had detectable autoantibodies, 5/46 (11 %) FSGS, and 5/34 (15 %) MCD (Fig. 2A–C). No statistically significant difference was observed in antibody titers between the MCD and FSGS groups.

By treatment response, 2/12 (17 %) SDNS and 8/54 (15 %) MDNS were positive, while none of the 14 MRNS patients were (Fig. 2A–D). Furthermore, antibody titers did not differ significantly between SDNS and MDNS subgroups. When stratified by proteinuria, 10/40 (25 %) patients with UPCR >2 mg/mg were positive; none were positive with UPCR between 0.2 and 2 mg/mg (Table S1). Among the 40 patients with UPCR >2 mg/mg, 31 met the criteria for nephrotic-range proteinuria, and of these, 6 (19 %) were anti-nephrin positive. No demographic differences were observed between antibody-positive and -negative patients. Antibody-positive individuals showed a trend toward shorter relapse-free intervals (9 vs. 11 months; Fig. S1); however, this difference did not reach statistical significance (log-rank test, P = 0.5; Fig. S1). All positive cases became negative during complete remission (UPCR <0.2

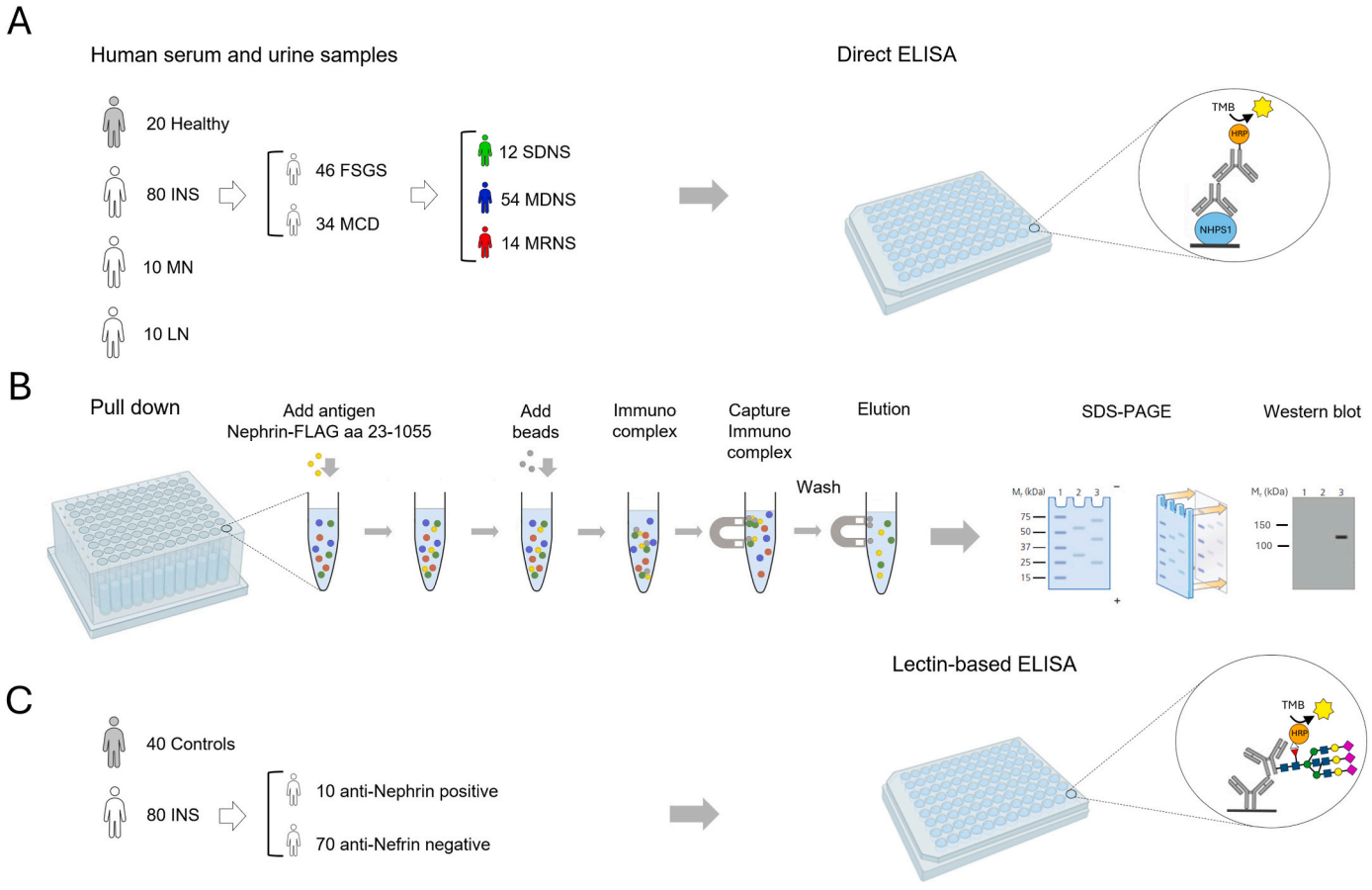

Fig. 1. Schematic overview of the workflow for the characterization of anti-nephrin autoantibodies. Panel A. Paired serum and urine samples from 20 healthy donors, 100 NS patients were analyzed using an ELISA assay to detect anti-nephrin autoantibodies. Panel B. The same paired samples were analyzed using a pull-down assay to verify whether the identified anti-nephrin positive samples could also immunoprecipitate the extracellular domain of human nephrin. Panel C. The fucose content of IgG from the same serum samples was measured using an ELISA assay using the fucose-binding lectins. INS, Idiopathic nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MN, membranous nephropathy; LN, lupus nephritis; SDNS, steroid-dependent NS; MDNS, multidrug-dependent NS; MRNS, multidrug-resistant NS.

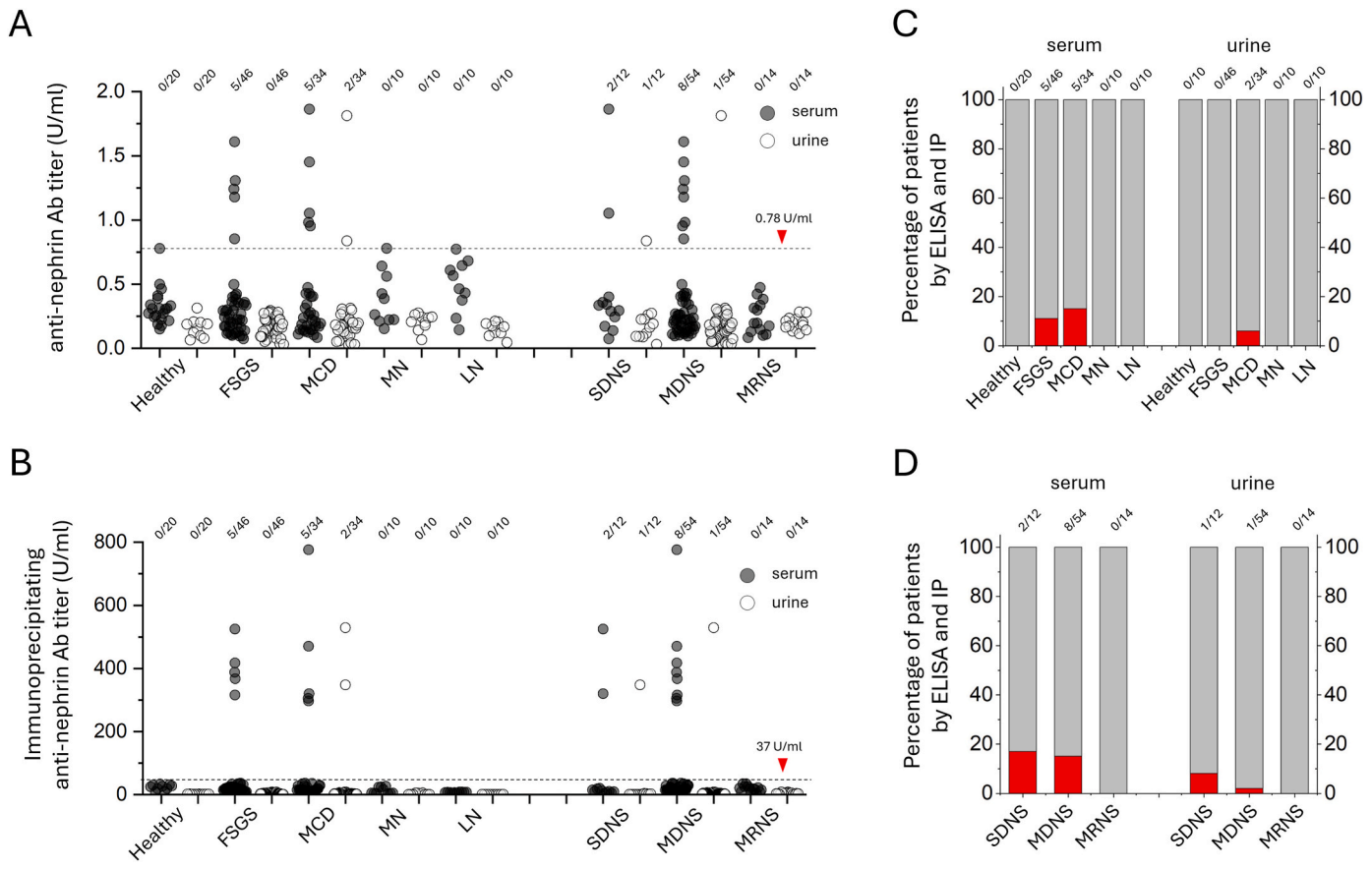

Fig. 2. Circulating antibodies against the extracellular domain of nephrin in idiopathic nephrotic syndrome (INS) patients. Panel A shows the levels of circulating anti-nephrin antibodies measured by ELISA in 10 healthy donors and 100 INS patients stratified based on biopsy. FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MN, membranous nephropathy; LN, lupus nephritis. Additionally, in patients with FSGS and MCD, anti-nephrin levels were further stratified based on the type of INS. SDNS, steroid-dependent NS; MDNS, multidrug-dependent NS; MRNS, multidrug-resistant NS. Panel B shows the titer of immunoprecipitating anti-nephrin antibodies in the serum and urine of the same subjects as in panel A. Panel C shows the prevalence of circulating anti-nephrin autoantibodies in the investigated cohorts of children and young adults with INS, with red and light gray colors representing positive and negative percentages, respectively. Panel D shows the prevalence of circulating anti-nephrin autoantibodies in FSGS and MCD samples stratified based on their pharmacological response. The dotted line indicates the statistically significant threshold.

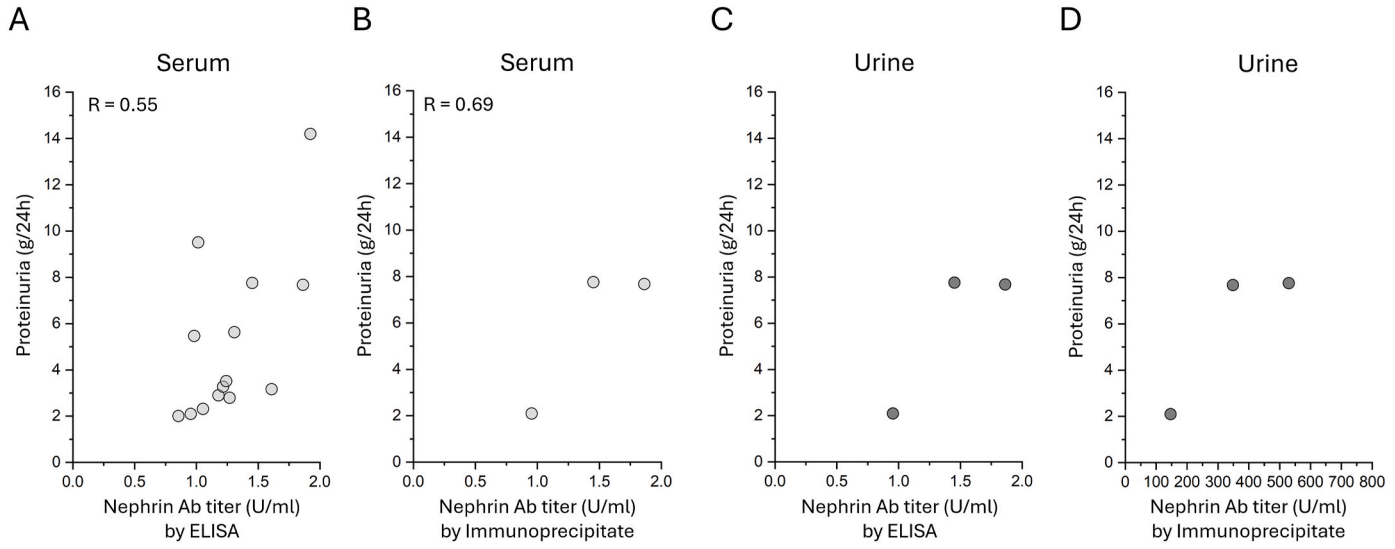

Fig. 3. Correlation between anti-nephrin antibody titers and urinary proteinuria in patients with anti-nephrin-associated MCD or FSGS. Panels A and B show the correlation between anti-nephrin antibody titers in serum, measured by ELISA (A) or immunoprecipitation (B), and 24-h proteinuria. Panels C and D show the corresponding correlation between urinary anti-nephrin antibody titers, measured by ELISA (C) or immunoprecipitation (D), and proteinuria. R = Spearman's correlation coefficient.

mg/mg), and in some, longitudinal analysis confirmed fluctuations with disease activity (Fig. S2).

Furthermore, a separate ROC analysis using histological diagnosis (MCD/FSGS vs. controls) as the classification variable yielded an AUC of 0.6 ($P = 0.16$), indicating that anti-nephrin antibody levels do not effectively distinguish between these histological subtypes or from control groups. These findings reinforce the notion that anti-nephrin autoantibodies are not broadly associated with a specific histological diagnosis but rather occur in a limited subset of patients with MCD or FSGS, typically during phases of active disease.

Immunoprecipitation: Immunoprecipitation confirmed ELISA results, detecting nephrin-binding IgG only in ELISA-positive samples. Western blot revealed a single band matching recombinant nephrin's molecular weight, confirming specificity (Fig. S2B and C). No bands were seen in antibody-negative samples. Signal intensity was markedly higher in sera with antibody titers ≥37 U/mL (Fig. 2B). Anti-nephrin titers correlated with 24h proteinuria, with Spearman's correlation coefficients of 0.55 (ELISA, $P < 0.05$; Fig. 3A) and 0.69 (immunoprecipitation, $P < 0.05$; Fig. 3B). In a representative MCD case, longitudinal immunoprecipitation data over two years showed a strong correlation between antibody levels and disease activity (Fig. S3).

3.2. Anti-nephrin antibodies in urine

ELISA and immunoprecipitation were performed on samples from the entire cohort to assess urinary anti-nephrin IgG.

ELISA: Of the 10 serum-positive patients, only 2 showed detectable urinary antibodies by ELISA. Both had MCD, one SDNS, one MDNS, and were sampled during active disease (Fig. 2A). All other serum-positive patients and all controls were negative in urine.

Immunoprecipitation: The results mirrored the ELISA. Only the two ELISA-positive urine samples immunoprecipitated recombinant nephrin (Fig. 2B). No signal was detected in other cases, supporting that urinary anti-nephrin IgG is rare and limited to active MCD with high proteinuria. Due to the small number of cases with detectable urinary anti-nephrin antibodies, no statistically significant correlation could be established between their presence in urine and clinical or biochemical parameters (Fig. 3C and D).

3.3. Colocalization of anti-nephrin autoantibodies with nephrin in renal biopsy

Kidney biopsy analysis was performed in a subset of INS patients for whom renal tissue was available at the same time point as serum and urine sampling during the active disease. Tissue was available for 3 out of the 10 patients positive for circulating anti-nephrin IgG (2 with MCD and 1 with FSGS). In all 3 cases, confocal microscopy revealed punctate IgG deposits that colocalized with nephrin in every evaluable glomerulus. Colocalization was defined as the consistent overlap of IgG and nephrin signals in all glomeruli available from a given biopsy section (minimum of two glomeruli per patient).

Among the anti-nephrin-negative patients, biopsy tissue was available for 7 individuals (4 with MCD and 3 with FSGS). None of these patients showed colocalization between IgG and nephrin in any of the examined glomeruli. Fisher's exact test confirmed a statistically significant association between the presence of circulating anti-nephrin autoantibodies and glomerular IgG/nephrin colocalization ($P < 0.001$). This colocalization provides further evidence for a potential pathogenic role of anti-nephrin autoantibodies in mediating podocyte injury. Representative images are shown in Fig. S4.

3.3.1. IgG fucosylation

Serum: To validate lectin specificity, we first tested IgG from serum of healthy donors. AAL and UEA-I recognize fucose residues on N-glycans: AAL preferentially binds core α1,6-linked fucose, while UEA-I targets α1,2-linked fucose on galactose (Fig. 4A). Lectin specificity was confirmed by preincubation with L-fucose, which inhibited binding, whereas lactose had no effect (Fig. 4B and C). PNGase F treatment of IgG

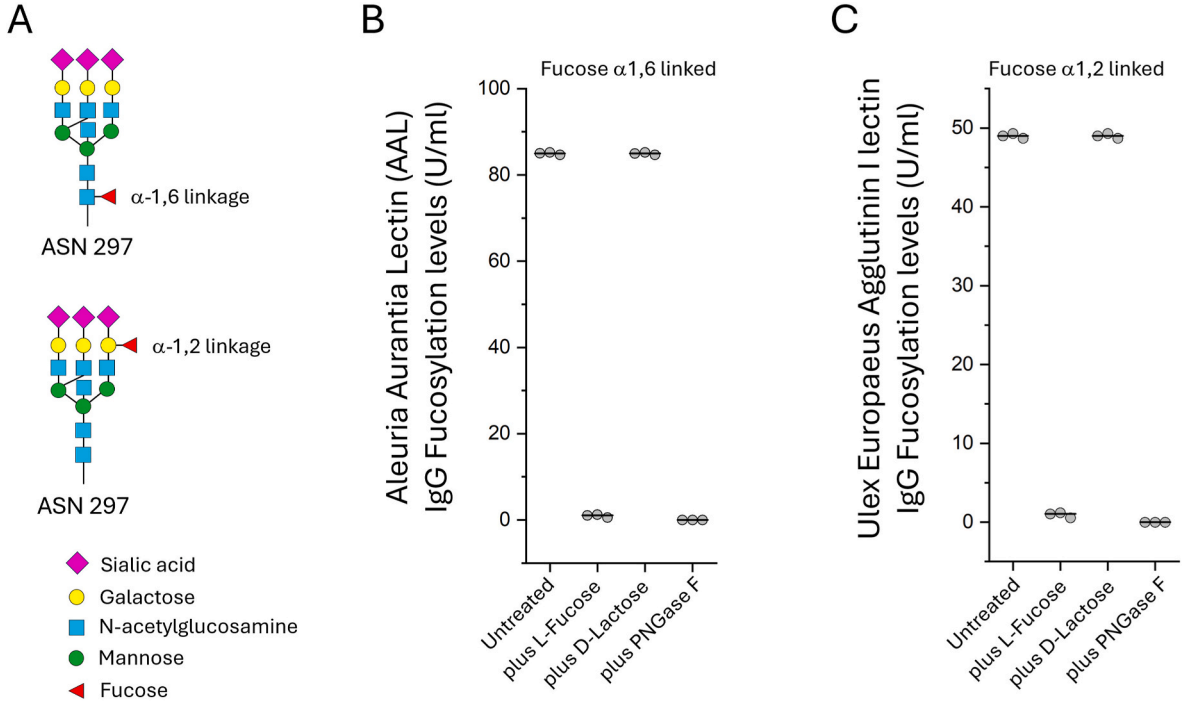

Fig. 4. Evaluation of IgG fucosylation in healthy donor samples using biotinylated lectins under competitive and enzymatic treatments. Panel A shows a schematic representation of N-glycans linked to Asn297 of IgG, depicting core (α1,6-linked) and antennary (α1,2-linked) fucosylation. Panels B and C show the levels of fucosylated IgG from serum of healthy donors, either untreated or preincubated with L-fucose or D-lactose to compete for binding to AAL (Panel B) or UEA-I (Panel C) or pretreated with PNGase F to enzymatically remove N-glycans (Panels B and C). These experiments were performed to confirm lectin specificity in the detection of IgG fucosylation under defined conditions.

further reduced the signal, confirming binding to N-glycan structures.

Sera from anti-nephrin-positive patients showed significantly reduced binding to both lectins compared to negative and healthy controls, indicating lower levels of both core (α1,6-linked) and antennary (α1,2-linked) IgG fucosylation (Fig. 5A and B). We defined operational thresholds for hypofucosylation as ≤53.4 U/mL for core fucosylation (AAL) and ≤35.3 U/mL for antennary fucosylation (UEA-I), based to the highest values observed among anti-nephrin antibody–-positive patients. Notably, UEA-I values showed a wider range, approximately 35 U/mL compared to 10 U/mL for AAL, indicating broader alteration in antennary fucosylation. When applied to the entire patient cohort, only one anti-nephrin-negative patient, with biopsy-proven FSGS and nephrotic-range proteinuria, met both criteria. Although patients with FSGS showed a trend toward lower fucosylation levels compared to those with MCD, these differences did not reach statistical significance for either AAL or UEA-I binding. This may be due to the limited number of anti-nephrin-positive individuals within each histological subgroup, as well as heterogeneity in treatment status and disease stage.

Fucosylation levels measured by the two assays were strongly correlated (Spearman's correlation coefficient = 0.85, P = 0.002), indicating that core and antennary afucosylation frequently co-occur in the same individuals. Moreover, IgG fucosylation levels showed a strong inverse correlation with 24-h proteinuria (Spearman's correlation coefficient = −0.97, P < 0.001; Fig. 5C and D).

Using the hypofucosylation thresholds to discriminate anti-nephrin-positive patients, ROC analysis yielded an AUC of 0.996, with 100 % specificity and 97.2 % sensitivity. These findings identify IgG hypofucosylation as a distinct and potentially clinically relevant feature in anti-nephrin-positive patients, despite the limitations posed by the small number of positive cases.

Urine: To extend our findings to the urinary compartment, we performed lectin-based ELISA assays for core (AAL) and antennary (UEA-I) fucosylation on IgG from urine samples. Compared to healthy controls, urinary IgG from serum anti-nephrin-positive patients showed significantly reduced binding to both lectins (Fig. 5E and F). AAL binding was significantly decreased in anti-nephrin-positive patients compared to healthy controls (P = 0.001), while UEA-I binding was lower in all patients with NS. No statistically significant correlation was observed between AAL and UEA-I titers in urine. Notably, the two individuals with detectable anti-nephrin antibodies in urine exhibited the lowest fucosylation values for both AAL and UEA-I, supporting the notion that hypofucosylated IgG may be selectively enriched in this compartment.

4. Discussion

This study examined serum and urinary anti-nephrin autoantibodies and their IgG fucosylation in children and young adults with biopsy-proven FSGS or MCD. Although the presence of anti-nephrin antibodies in MCD is increasingly recognized, reported prevalence varies due to differences in detection methods. Hengel et al. [2] showed that conventional direct ELISA often fails to distinguish between negative and positive cases reliably. Liu et al. [23] further showed that polyHis-tagged antigens may bind nonspecifically to IgG, leading to false positives. These methodological pitfalls, highlighted in multiple studies, must be carefully considered when assessing the presence of

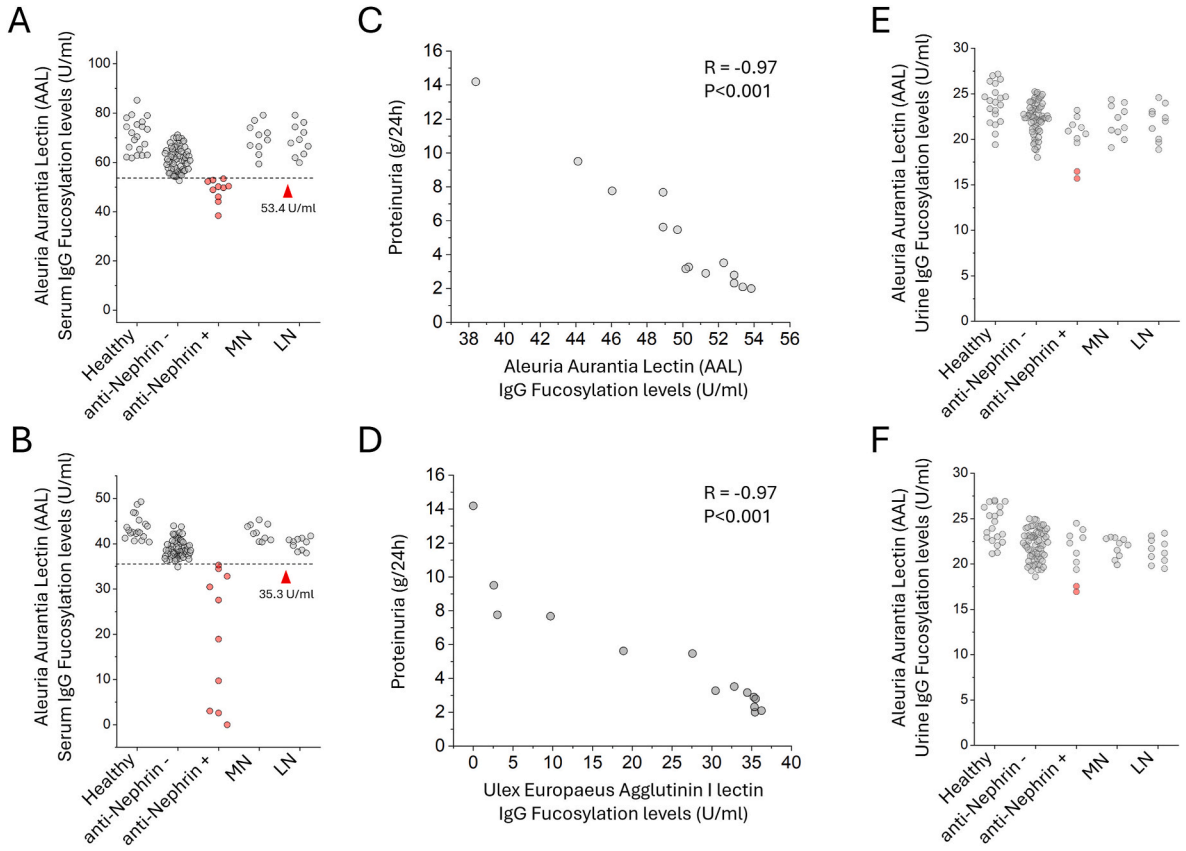

Fig. 5. Evaluation of fucose in IgG by enzyme-linked immunosorbent assay (ELISA) with biotinylated lectins. Panels A and B show the levels of core-fucosylated IgG (α1,6-linked fucose) and antennary-fucosylated IgG (α1,2-linked fucose), respectively, measured using AAL and UEA-I lectins in the serum of healthy donors and NS patients, stratified by circulating anti-nephrin autoantibodies. Panels C and D display the inverse correlation between 24-h proteinuria and serum IgG fucosylation levels, as measured by AAL (Panel C) and UEA-I (Panel D), in patients with anti-nephrin–associated MCD or FSGS. Panels E and F show the corresponding levels of core and antennary fucosylation in IgG from urine samples, measured by AAL and UEA-I, and stratified by anti-nephrin antibody status. Red dots indicate patients positive for anti-nephrin autoantibodies, as confirmed by both ELISA and immunoprecipitation.

anti-nephrin autoantibodies. To overcome these limitations, we employed two complementary strategies to quantify anti-nephrin autoantibodies: a direct ELISA and immunoprecipitation followed by Western blotting, utilizing the recombinant extracellular domain of human nephrin as the capture antigen. To avoid artifacts related to polyHis tag interactions [23], we used an affinity-purified full-length extracellular domain of human nephrin tagged with a FLAG peptide and expressed in human cells. Non-specific binding was minimized using a protein-free chemical blocker and short incubation times. We selected Protein G-functionalized magnetic beads for immunoprecipitation for their high binding capacity, reproducibility, and ease of use.

To ensure high specificity, we adopted a conservative positivity threshold based on the highest value observed among healthy controls, consistent with previous studies [2,3,23]. This value also coincided with the highest signals recorded in disease control patients with LN and MN, conditions not associated with anti-nephrin antibodies. While alternative statistical methods might improve sensitivity, they would compromise specificity and potentially misclassify control samples. Importantly, all ELISA-positive samples were confirmed by immunoprecipitation and, in selected cases, by IgG/nephrin colocalization in kidney tissue, supporting the biological relevance of the chosen threshold.

Our results confirmed the presence of circulating anti-nephrin autoantibodies in approximately 10 % of patients with INS overall, rising to about 20 % among those with nephrotic-range proteinuria. This relatively low frequency, compared to some previous studies, may reflect both clinical heterogeneity and methodological differences. In our cohort, most patients had already initiated treatment before the first available serum sample was collected, which may have been sufficient to lower antibody titers just below the positivity threshold in some cases. Nevertheless, our positivity rate is consistent with that reported in studies using similar antigen constructs [23] or nephrin/IgG colocalization techniques [5].

Positivity was observed exclusively in patients with a UPCR >2 mg/mg, with no antibodies detected in those with lower proteinuria levels, suggesting an association with disease activity. While ELISA and immunoprecipitation yielded concordant results, the latter demonstrated greater sensitivity. However, despite its analytical advantages, the technical complexity of immunoprecipitation limits its utility in routine diagnostic settings.

Furthermore, for the first time, we also detected anti-nephrin antibodies in the urine of two MCD patients with nephrotic-range proteinuria (>5 g/24 h). While urinary loss of antibodies is expected in NS due to increased glomerular permeability, the detection of antigen-specific antibodies such as anti-nephrin IgG appears uncommon. We propose that urinary anti-nephrin antibodies may reflect immune-mediated podocyte injury and contribute to the understanding of disease activity. However, larger studies are needed to validate their diagnostic value.

A third novel finding of our study is that IgG N-glycans in anti-nephrin-positive patients show a significant reduction in fucosylation, particularly of both antennary and core fucose residues. These two forms of afucosylation were strongly correlated with each other, suggesting that they frequently co-occur in the same individuals. Furthermore, these glycan modifications appear to be functional in disease severity, as indicated by their strong inverse correlation with 24h proteinuria levels in serum samples. In line with these findings, urinary IgG from anti-nephrin–positive patients also showed markedly reduced binding to both AAL and UEA-I lectins. Notably, the two individuals with detectable anti-nephrin antibodies in urine exhibited the lowest levels of IgG fucosylation in this compartment. These results suggest that the absence of fucose may contribute to altered glomerular handling of these molecules, leading to their selective enrichment in the urinary compartment.

This pattern was not seen in patients with MN or LN, despite prior reports of subclass-specific afucosylation in these diseases [7,19]. Importantly, our assay measures global IgG glycosylation and is not subclass-specific. As such, afucosylation of minor IgG subclasses (e.g., IgG4 in MN) is likely masked by the more abundant IgG1 and IgG2 fractions. In LN, glycosylation changes are characterized by decreased galactosylation and sialylation, along with an increase in bisecting N-acetylglucosamine [7].

Prior studies have linked antennary fucosylation defects to immune dysregulation [11] and pro-fibrotic signaling [24]. Given that core afucosylated IgG enhances antibody-dependent cellular cytotoxicity (ADCC) by increasing affinity for FcγRIIIa on effector cells such as NK cells [17], our findings suggest a direct pathogenic role of afucosylation in nephrin autoimmunity. This mechanism, well-established in autoimmune and inflammatory diseases, leads to tissue damage via heightened ADCC [12,25,26]. Notably, afucosylated IgG can show up to a 50-fold increase in FcγRIIIa binding [14], a feature already exploited in therapeutic monoclonal antibodies to enhance cytotoxic efficacy [18]. However, while afucosylated IgG is known to enhance FcγRIIIa--mediated effector functions, particularly ADCC, our findings do not imply irreversible injury to the podocytes. Most children and young adults with NS, especially those with MCD, experience reversible podocyte dysfunction. Nevertheless, our data suggests that in a subset of patients with anti-nephrin antibodies, afucosylated IgG may promote transient podocyte dysfunction via immune signaling or sublethal stress, rather than full-blown cytotoxicity. This concept aligns with emerging views that antibody effector functions exist on a spectrum and that ADCC-like signaling may occur below the threshold of cell death. In this context, afucosylation may contribute to reversible disruption of the glomerular filtration barrier and proteinuria, particularly in children and young adults. From a diagnostic standpoint, IgG fucosylation distinguished anti-nephrin-positive from -negative patients with high accuracy. Monitoring glycosylation patterns could complement antibody titers in assessing disease activity.

Our findings raise important mechanistic questions. Fucosylation is a modifiable trait, influenced by glycosyl transferases, glycosyl hydrolases, and cellular activation states. Its functional consequences have been studied in autoimmunity and cancer, with the aim of reducing ADCC [18]. In contrast, the effects on tissue damage in NS remain poorly characterized, but may involve immune amplification via FcγRIIIa, particularly in anti-nephrin-positive cases. Therapies targeting this axis, such as FcγR blockers or glycoengineering approaches targeting enzymes to restore proper fucosylation in B cells, may offer new strategies to modulate disease activity and preserve podocyte integrity.

In conclusion, this study identifies a distinct glycosylation profile in anti-nephrin-positive INS patients, marked by reduced fucosylation and associated with disease activity. These findings provide novel mechanistic and translational insights into podocyte-specific autoimmunity, paving the way for the development of biomarkers and targeted therapeutic strategies in glomerular disease.

CRediT authorship contribution statement

Sonia Spinelli: Investigation, Data curation. **Andrea Garbarino:** Investigation, Data curation. **Francesca Lugani:** Writing – original draft, Funding acquisition. **Edoardo La Porta:** Writing – original draft, Funding acquisition. **Noemi Rumeo:** Writing – review & editing, Data curation. **Giorgio Piaggio:** Writing – review & editing. **Alberto Magnasco:** Writing – review & editing, Resources. **Antonella Trivelli:** Writing – review & editing, Investigation. **Maria Ludovica Degl'Innocenti:** Writing – review & editing. **Gino Tripodi:** Writing – review & editing, Formal analysis. **Simona Granata:** Writing – review & editing. **Francesca Leone:** Writing – review & editing. **Elena Zocchi:** Writing – review & editing, Investigation. **Lorenzo Gallon:** Writing – review & editing, Data curation. **Gian Marco Ghiggeri:** Writing – review & editing, Investigation. **Enrico Verrina:** Writing – original draft, Funding acquisition. **Gianluigi Zaza:** Writing – original draft, Funding acquisition. **Giovanni Candiano:** Writing – original draft, Conceptualization.

Maurizio Bruschi: Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization.

Key points

- Anti-nephrin IgG correlates with disease activity and is detectable in serum and, rarely, in urine.
- Afucosylated IgG identifies anti-nephrin-positive patients and inversely correlates with proteinuria.
- IgG afucosylation may enhance pathogenicity in INS, offering new biomarker and therapeutic perspectives.

Funding

This work was supported by the Ministero della Salute, Ricerca Corrente, 5xmille (5M-2019–23680420), European Union – Next Generation EU (Missione 4, Componente 2, Investimento 1.1 "Fondo per il Programma Nazionale della Ricerca (PNR) e Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN)". Bando 2022 (2022FH7889), and Fondazione Malattie Renali del Bambino ETS.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maurizio Bruschi reports financial support was provided by Giannina Gaslini Institute. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtauto.2025.100307.

Data availability

Data will be made available on request.

References

[1] P. Ravani, M. Colucci, M. Bruschi, M. Vivarelli, M. Cioni, A. DiDonato, et al., Human or chimeric monoclonal Anti-CD20 antibodies for children with nephrotic syndrome: a superiority randomized trial, J. Am. Soc. Nephrol. 32 (2021) 2652–2663.
[2] F.E. Hengel, S. Dehde, M. Lasse, G. Zahner, L. Seifert, A. Schnarre, et al., Autoantibodies targeting Nephrin in podocytopathies, N. Engl. J. Med. 391 (2024) 422–433.
[3] A.J.B. Watts, K.H. Keller, G. Lerner, I. Rosales, A.B. Collins, M. Sekulic, et al., Discovery of autoantibodies targeting Nephrin in minimal change disease supports a novel autoimmune etiology, J. Am. Soc. Nephrol. 33 (2022) 238–252.
[4] Y. Shirai, K. Miura, K. Ishizuka, T. Ando, S. Kanda, J. Hashimoto, et al., A multi-institutional study found a possible role of Anti-nephrin antibodies in post-transplant focal segmental glomerulosclerosis recurrence, Kidney Int. 105 (2024) 608–617.
[5] V. Raglianti, M.L. Angelotti, L. Cirillo, F. Ravaglia, S. Landini, V. Palazzo, et al., Anti-slit diaphragm antibodies on kidney biopsy identify pediatric patients with steroid-resistant nephrotic syndrome responsive to second-line immunosuppressants, Kidney Int. 106 (2024) 1124–1134.
[6] L.A. New, C.E. Martin, R.P. Scott, M.J. Platt, A. Keyvani Chahi, C.D. Stringer, et al., Nephrin tyrosine phosphorylation is required to stabilize and restore podocyte foot process architecture, J. Am. Soc. Nephrol. 27 (2016) 2422–2435.
[7] A. Beyze, C. Larroque, M. Le Quintrec, The role of antibody glycosylation in autoimmune and alloimmune kidney diseases, Nat. Rev. Nephrol. 20 (2024) 672–689.
[8] C. Blochl, E.M. Stork, H.U. Scherer, R.E.M. Toes, M. Wuhrer, E. Dominguez-Vega, Fc proteoforms of ACPA IgG discriminate autoimmune responses in plasma and synovial fluid of rheumatoid arthritis patients and associate with disease activity, Adv. Sci. (Weinh.) (2025) e2408769.
[9] L.B. Giron, Q. Liu, O.S. Adeniji, X. Yin, T. Kannan, J. Ding, et al., Immunoglobulin G N-glycan markers of accelerated biological aging during chronic HIV infection, Nat. Commun. 15 (2024) 3035.
[10] C. Reily, T.J. Stewart, M.B. Renfrow, J. Novak, Glycosylation in health and disease, Nat. Rev. Nephrol. 15 (2019) 346–366.
[11] K. Mao, J. Luo, J. Ye, L. Li, F. Lin, M. Zhou, et al., 2-D-gal targets terminal fucosylation to inhibit T-cell response in a mouse skin transplant model, Transplantation 107 (2023) 1291–1301.
[12] S. Trzos, P. Link-Lenczowski, E. Pochec, The role of N-glycosylation in B-cell biology and IgG activity. The aspects of autoimmunity and anti-inflammatory therapy, Front. Immunol. 14 (2023) 1188838.
[13] I. Endreffy, G. Bjorklund, L. Szerafin, S. Chirumbolo, M.A. Urbina, E. Endreffy, Plasma alpha-L-fucosidase activity in chronic inflammation and autoimmune disorders in a pediatric cohort of hospitalized patients, Immunol. Res. 65 (2017) 1025–1030.
[14] I. Quast, B. Peschke, J.D. Lunemann, Regulation of antibody effector functions through IgG Fc N-glycosylation, Cell. Mol. Life Sci. 74 (2017) 837–847.
[15] J. Golay, A.E. Andrea, I. Cattaneo, Role of Fc core fucosylation in the effector function of IgG1 antibodies, Front. Immunol. 13 (2022) 929895.
[16] R.L. Shields, J. Lai, R. Keck, L.Y. O'Connell, K. Hong, Y.G. Meng, et al., Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human fcgamma RIII and antibody-dependent cellular toxicity, J. Biol. Chem. 277 (2002) 26733–26740.
[17] J.J. Oosterhoff, M.D. Larsen, C.E. van der Schoot, G. Vidarsson, Afucosylated IgG responses in humans - structural clues to the regulation of humoral immunity, Trends Immunol. 43 (2022) 800–814.
[18] N.A. Pereira, K.F. Chan, P.C. Lin, Z. Song, The "less-is-more" in therapeutic antibodies: afucosylated anti-cancer antibodies with enhanced antibody-dependent cellular cytotoxicity, mAbs 10 (2018) 693–711.
[19] C. Chinello, N. de Haan, G. Capitoli, B. Trezzi, A. Radice, L. Pagani, et al., Definition of IgG subclass-specific glycopatterns in idiopathic membranous nephropathy: aberrant IgG glycoforms in blood, Int. J. Mol. Sci. 23 (2022).
[20] V. Dotz, A. Visconti, H.J. Lomax-Browne, F. Clerc, A.L. Hipgrave Ederveen, N. R. Medjeral-Thomas, et al., O- and N-Glycosylation of serum immunoglobulin A is associated with IgA nephropathy and glomerular function, J. Am. Soc. Nephrol. 32 (2021) 2455–2465.
[21] C. K. D. W. G. Kidney Disease, Improving global outcomes. KDIGO 2024 clinical practice guideline for the evaluation and management of chronic kidney disease, Kidney Int. 105 (2024) S117–S314.
[22] G. Navarro, M. Gomez-Autet, P. Morales, J.B. Rebassa, C. Llinas Del Torrent, N. Jagerovic, et al., Homodimerization of CB(2) cannabinoid receptor triggered by a bivalent ligand enhances cellular signaling, Pharmacol. Res. 208 (2024) 107363.
[23] P. Liu, S. Liu, V. Dalal, J. Lane, E. Gessaroli, E. Forte, et al., Evaluation of methodologies in Anti-nephrin autoantibody detection, Kidney Int. (2025). S0085-2538.
[24] G. Deng, L. Chen, Y. Zhang, S. Fan, W. Li, J. Lu, et al., Fucosyltransferase 2 induced epithelial-mesenchymal transition via TGF-beta/Smad signaling pathway in lung adenocarcinaoma, Exp. Cell Res. 370 (2018) 613–622.
[25] M.D. Larsen, E.L. de Graaf, M.E. Sonneveld, H.R. Plomp, J. Nouta, W. Hoepel, et al., Afucosylated IgG characterizes enveloped viral responses and correlates with COVID-19 severity, Science 371 (2021).
[26] S.L. Sneed, B.B. Reese, A.F. Laureano, S. Ratnapriya, I. Fraschilla, K.L. Jeffrey, et al., An engineered immunomodulatory IgG1 Fc suppresses autoimmune inflammation through pathways shared with i, v. immunoglobulin. J Clin Invest 134 (2024).