Autoantibodies Targeting Nephrin in Podocytopathies: Questions & Answers

[Ask the authors an additional question]

What is nephrin and what role does it play in the kidney?

Nephrin is a key adhesion and signaling protein of the slit diaphragm between podocyte foot processes. Its extracellular part protrudes into the space between podocyte foot processes, where it interacts with other molecules and conveys signals to the podocyte through its transmembrane and intracellular part. Changes in nephrin phosphorylation signal to the podocyte via a multitude of adaptor proteins and downstream signaling pathways and result in substantial cytoskeletal reorganization of the podocyte. Nephrin thereby regulates the form and function of podocytes, making it a key protein of the glomerular filtration process. Cases of nephrin disturbance, for example due to genetic mutations in congenital nephrotic syndrome of the Finnish type or in animal models, clearly illustrate its pivotal role for podocyte function. Nephrin dysfunction leads to the deterioration of glomerular filtration with extensive loss of protein into the urine and massive nephrotic syndrome.

What are anti-nephrin autoantibodies? What effect do they have?

Anti-nephrin autoantibodies are antibodies against the key podocyte slit diaphragm protein nephrin. We detected them in about two-thirds of adult patients with minimal change disease and active nephrotic syndrome before the initiation of immunosuppression, and in 90% of children with idiopathic nephrotic syndrome and active nephrotic syndrome before the initiation of immunosuppression. A smaller portion of patients with primary FSGS also exhibited anti-nephrin autoantibodies. In anti-nephrin positive patients, anti-nephrin autoantibodies correlate with clinical disease activity and disease course during onset, remission and relapse of nephrotic syndrome. In a mouse model of active immunization with murine nephrin, animals developed anti-nephrin autoantibodies, which was followed by changes in nephrin phosphorylation, altered nephrin downstream signaling, and the onset of a rapid nephrotic syndrome with a minimal change disease-like histological appearance. From these findings we concluded that anti-nephrin autoantibodies are one of the permeability factors causing nephrotic syndrome in children and adults with idiopathic nephrotic syndrome, minimal change disease, and primary FSGS.

Why are these results so important?

The discovery of anti-nephrin autoantibodies and demonstration of their role thoroughly changes our understanding of associated types of idiopathic nephrotic syndrome, minimal change disease and primary FSGS, which we classify as anti-nephrin associated podocytopathies. It offers an explanation to affected individuals so they can better understand the nature of their disease, rather than a vague and frustrating "idiopathic" label. In the long run and depending on future studies, anti-nephrin autoantibodies might help to predict patients' risk for different disease courses, guide therapy decisions, and ultimately even set the ground for the development of specific antibody-targeted therapies to avoid the dangerous side effects of the broad immunosuppression that is the current standard of care.

Anti-nephrin antibodies in MCD have been considered controversial. What has changed?

The first signs of a pathological role of antibodies against nephrin in an animal model date back as early as into the late 1980s. Since then, anti-nephrin antibodies have been a matter of debate on different occasions (for example in the context of diabetes and recurrence of nephrotic syndrome after kidney transplantation), reaching a new high with Astrid Weins' discovery of anti-nephrin autoantibodies in patients with minimal change disease in 2022.[1]

The detection of anti-nephrin antibodies, however, has proven challenging, fostering further discussion on the pathological relevance of anti-nephrin antibodies. The establishment of a reliable and sensitive assay for detection and quantification, its application to large cohorts of international origin and different glomerular diseases, the correlation to clinical disease activity and the creation of an animal model of anti-nephrin-mediated podocytopathy were therefore necessary to fully elucidate the role of anti-nephrin autoantibodies in glomerular diseases.

How will this help us find treatments for these conditions?

Of course, the answer to this question is highly speculative. However, the pathobiological understanding of a disease sets the ground for the development of therapeutics which specifically inhibit or counteract underlying pathomechanisms. In the case of anti-nephrin associated podocytopathies, the detection of anti-nephrin antibodies could help us to choose the right patients for antibody-targeting therapies.

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[1] Watts AJB, Keller KH, Lerner G, Rosales I, Collins AB, Sekulic M, Waikar SS, Chandraker A, Riella LV, Alexander MP, Troost JP, Chen J, Fermin D, Yee JL, Sampson MG, Beck LH Jr, Henderson JM, Greka A, Rennke HG, Weins A. Discovery of Autoantibodies Targeting Nephrin in Minimal Change Disease Supports a Novel Autoimmune Etiology. J Am Soc Nephrol. 2022 Jan;33(1):238-252. doi: 10.1681/ASN.2021060794. Epub 2021 Nov 3. PMID: 34732507; PMCID: PMC8763186.

The current trend towards treatment with anti-CD20 could enhance and/or develop further towards anti-CD38 antibodies in severe cases. In the future, we envision directly targeting autoantibody-producing cells, which was recently demonstrated as a promising therapeutic approach in PLA2R- and THSD7A-mediated membranous nephropathy. Other approaches such as the specific elimination of pathogenic antibodies, inhibition of their binding to nephrin, or drugs interfering with dysfunctional nephrin signaling are also promising options for future investigation.

This solves a significant mystery about minimal change disease and idiopathic nephrotic syndrome. What questions still remain to be answered?

- First and foremost: How and where do these autoantibodies arise?
- Can we influence or even prevent the onset of anti-nephrin autoimmunity?
- Which factors influence the individual disease course and determine the long-term patient outcome?
- What are the underlying factors in anti-nephrin antibody negative patients?
- What are the molecular consequences of autoantibody binding to nephrin and how can we therapeutically intervene?

How do you envision a disease activity marker impacting patients in the future?

Depending on further (prospective) trials, anti-nephrin antibodies as a disease activity marker could serve several different purposes. First, measuring anti-nephrin antibodies during active nephrotic syndrome could help to diagnose anti-nephrin associated podocytopathy in patients who are ineligible for biopsy, for example due to young age, established anticoagulation medication, or pregnancy. This is particularly important since idiopathic nephrotic syndrome and minimal change disease often affect young patients and many patients with nephrotic syndrome are on anticoagulants.

Second, the assessment of anti-nephrin as a prognostic marker in patients with idiopathic nephrotic syndrome, minimal change disease, and focal segmental glomerulosclerosis will be of high interest. Prospective trials are necessary to determine if anti-nephrin antibodies can be used to predict a patient's response to therapy, disease course, risk of relapse, and so on.

For patients awaiting a kidney transplant, the measurement of anti-nephrin autoantibodies could become an essential part of the pre-transplant workup to reduce the risk of post-transplant recurrence of nephrotic syndrome. During post-transplant follow-up of affected patients, the measurement of anti-nephrin autoantibodies could help to discriminate between recurrence of anti-nephrin associated podocytopathy and other proteinuric disease of the transplanted kidney. A recent study from Japan found a surprisingly high proportion of anti-nephrin positivity in recurrent FSGS patients

(Shirai et al., Kidney Int 2024[2]). However, we believe that the role of anti-nephrin antibodies in recurrent disease after transplantation needs further studies.

Describe the collaboration involved in this international, multi-center project.

Collaboration is key! We are deeply grateful for the trust and support of our collaboration partners across the globe, which were enabled by ISGD! ISGD now represents a hub catalyzing collaborative efforts and joint networks as demonstrated by this work.

Clinical Questions

How do you envision this discovery changing the standard of care for patients with MCD/INS?

Further studies are necessary to understand the characteristics and outcomes of patients with anti-nephrin positive podocytopathies, also in comparison to anti-nephrin negative disease. However, the presence of pathogenic antibodies argues for B cell or plasma cell-targeted treatments, aiming at elimination of the pathogenic factor. We envision that response to treatment may be monitored not only based on disease activity (i.e. proteinuria), but also immunological response (i.e. anti-nephrin levels). It would be very valuable to prospectively evaluate new generation monoclonal antibodies targeting B cells, such as obinituzumab and ofatumumab, for their efficacy in reducing anti-nephrin autoantibodies and proteinuria.

What advice or information would you give to your patients today?

The immediate effect of the identification of anti-nephrin antibodies as specific disease markers that strongly correlate with disease activity is that we for the first time have a blood biomarker at hand, allowing to make a specific and pathobiology-based diagnosis. However, the assays to detect this antibody are time-consuming and expensive and it will take time until anti-nephrin antibody measurement becomes routinely available. In the future, we believe that anti-nephrin antibody measurement will allow us to make a noninvasive diagnosis, to guide the treatment of affected patients, and to help to reduce disease recurrence in patients after kidney transplantation.

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[2] Shirai Y, Miura K, Ishizuka K, Ando T, Kanda S, Hashimoto J, Hamasaki Y, Hotta K, Ito N, Honda K, Tanabe K, Takano T, Hattori M. A multi-institutional study found a possible role of anti-nephrin antibodies in post-transplant focal segmental glomerulosclerosis recurrence. Kidney Int. 2024 Mar;105(3):608-617. doi: 10.1016/j.kint.2023.11.022. Epub 2023 Dec 16. PMID: 38110152.

How does a discovery like this progress from academic research to a lab test that any doctor can order?

Currently, this is a purely academic test, developed in the lab at the University of Hamburg. We expect diagnostic companies to develop assays that may be more broadly available. This process takes time, on average a few years. Bringing an assay from the research setting to commercial availability is a complex process requiring:

- Technology transfer and scientific / engineering development
- Clinical trials to assess safety and efficacy
- Regulatory approval process with FDA, EMA and other regulators
- Insurance / health system coverage determinations

How would the detection of anti-nephrin antibodies guide your decisions about which therapies to use with your patients?

Until follow-up prospective studies have been conducted, we won't have a definitive answer to this question. However, there are B cell-targeted therapies available which may have an impact on anti-nephrin antibodies. In our study, we present the history of three patients with high anti-nephrin antibody levels that were treated with rituximab. This led to a drop in anti-nephrin antibodies and proteinuria in all three cases. One of the three cases had a two-year follow-up after rituximab treatment and their disease did not relapse during this time (in contrast to several relapses before receiving rituximab). We currently use rituximab for relapsing or frequently relapsing disease, and when patients do not respond to glucocorticoids at initial diagnosis.

If patients are refractory to rituximab, this could mean that CD20-negative immune cells are the main disease drivers. These could, for example, be plasma cells which escape the action of rituximab. In this direction, a recent study described considerable success of combined B cell and plasma cell depletion using both rituximab and daratumumab in multidrug-dependent and multidrug-resistant nephrotic syndrome (Angeletti A et al., Am J Transplant 2024[3]).

Do you think this is a target that new drugs could address? Will we see new therapeutics developed to counteract ANAAs specifically?

We are convinced that the knowledge on ANAAs and their pathogenic role in podocytopathies will catalyze the development of compounds specifically targeting the antigen, the antibodies, and the antibody-producing cells.

[3] Angeletti A, Bin S, Magnasco A, Bruschi M, Cravedi P, Ghiggeri GM. Efficacy of combined rituximab and daratumumab treatment in posttransplant recurrent focal segmental glomerulosclerosis. Am J Transplant. 2024 Apr;24(4):688-692. doi: 10.1016/j.ajt.2023.12.010. Epub 2023 Dec 14. PMID: 38101474.

Do you expect the ability to detect anti-nephrin autoantibodies to change biopsy practices in nephrotic syndrome?

It is currently too early to say that the ability to detect anti-nephrin antibodies should change biopsy practices in patients with nephrotic syndrome. However, we think that the detection of anti-PLA2R antibodies in membranous nephropathy is a good example of how autoantibody detection can guide further diagnostic steps. The current KDIGO guidelines recommend to *not* perform a kidney biopsy if anti-PLA2R is present, kidney function is normal and stable, and no other findings such as positive ANA are present. We envision that the ability to detect anti-nephrin autoantibodies might have similar value in the future.

Scientific Questions

What were the diagnostic criteria for MCD/INS in the study?

Adult patients were included in our minimal change disease cohort if they had histologically proven minimal change disease lesions as determined by the local nephropathologist. In the presence of any histological signs of beginning capsule synechia or sclerosis patients were classified as Focal Segmental Glomerulosclerosis, which was further subclassified into primary and non-primary depending on diffuse foot process effacement and/or nephrotic-range proteinuria (urinary albumin-to-creatinine ratio (UACR) or urinary protein-to-creatinine ratio (UPCR) > 3.5 g/g) with hypalbuminemia (serum albumin < 3 g/dl). Children were diagnosed with idiopathic nephrotic syndrome by clinical phenotype and laboratory findings, independent of potentially undertaken kidney biopsy.

In the patients who had active MCD or INS but did *not* have anti-nephrin antibodies, do you think there is a separate etiology?

Apart from potentially a few false-negative patients in our measurements, we expect a separate etiology in anti-nephrin negative patients. Most obviously, one could think of antibodies targeting distinct podocyte proteins, but other as yet unknown permeability factors could also play a role. It is our scientific duty to dig deeper and identify the underlying cause in anti-nephrin negative patients, too.

Minimal change vs. FSGS vs. nephrotic syndrome — are these all really just anti-nephrin autoantibody-mediated podocytopathies? Should we be reclassifying these diseases?

In our opinion, diseases should be classified based on their endotype, e.g., their pathogenic cause. Therefore, anti-nephrin positive MCD, FSGS or INS should be classified as anti-nephrin associated podocytopathies with (or, in case of INS, without the knowledge of) a MCD or FSGS histotype. However, there may be additional disease

factors playing a role, in anti-nephrin positive as well as negative patients. It will be essential to better understand which factors are responsible for the histological as well as clinical phenotype, particularly in terms of the clinical disease course. We need to elucidate which patients with anti-nephrin associated podocytopathy are likely to respond to which therapy and which are at risk for acute or chronic kidney failure.

What was the geographic background / genetic diversity of the individuals analyzed in the study? Do you think the prevalence of anti-nephrin autoantibodies as a cause of MCD/INS would differ across populations?

In our adult cohorts, ethnicity was unfortunately not reported by supplying biobanks. However, people of color were most likely underrepresented in this study due to limited sample availability in supplying (European) biobanks. In our pediatric patients, we had ethnic information for 142 individuals. They comprised 23 Asian, 26 sub-Saharan African, 24 Middle Eastern/North African, 60 Caucasian, and 9 mixed ethnicity pediatric patients, selected due to sample availability in supplying (European) biobanks. Due to the limited data, we are not able to comment on differences across populations. This could be an interesting area for follow-up studies.

Do you have any hypotheses about what causes certain individuals to develop anti-nephrin antibodies?

Any comments are purely speculative in nature, since we did not assess the origin of measured anti-nephrin autoantibodies in our study. However, the occurrence of idiopathic nephrotic syndrome in children has been linked to upper respiratory tract infections, which could potentially trigger anti-nephrin autoimmunity through unknown mechanisms. Interestingly, we found one child in our control cohort positive for anti-nephrin autoantibodies – and this child had almost 4 times as many infections during the year before sampling compared to the average of all other pediatric controls.

It appears that anti-nephrin autoantibodies cause disease symptoms at relatively low antibody concentrations and may not trigger traditional immune responses. Could you discuss the significance of this finding?

While the pathogenic effect of autoantibodies in podocytopathies known so far mostly depends on traditional immune responses, the detrimental effect of anti-nephrin autoantibodies is most likely due to nephrin dysfunction upon antibody binding. Although the observed change in phosphorylation offers interesting pathomechanistic explanations, the exact mechanism causing nephrin dysfunction upon antibody binding remains elusive so far. The absence of any signs of traditional pathological antibody effects mediated by immune cells or the complement system argues for anti-nephrin associated podocytopathies as a new classification of antibody-mediated podocytopathies.

Outside of podocytopathies, there are other examples of antibody-induced damage due to the interference with the antigen's physiological functions instead of traditional immune response. For example, autoantibodies in myasthenia gravis impact neuromuscular junction function via different mechanisms including cross-linking of acetylcholine receptors and triggering their endocytosis, interfering with receptor activation by acetylcholine (the classical effect of a receptor antagonist) or by inhibiting acetylcholine receptor clustering through interaction with muscle-specific kinase.

What comes next? What other interesting questions does this research suggest for follow-up studies?

All of the above! We welcome collaboration with additional centers for future studies, and we especially emphasize the need for prospective studies so these findings can be translated into clinical practice to improve patients' kidney health and quality of life.