Pax8 are surprising as their expression are considered highly specific to proximal tubule cells. Expression of Grainyhead-like2 (Grhl2) was especially interesting, as it is an important regulator of epithelial-to-mesenchymal transition and apical and basal polarity, including a large number of claudins and cadherins (Figure 1), and therefore it could play an important role in aging podocytes.

The study has several caveats, including the potential activation of stress response genes induced by the harsh digestion needed for podocyte isolation. Decrease in podocyte number can potentially mask the true transcriptional drivers of aging. Such as Fu *et al.*[9] performed bulk RNA-sequencing on isolated glomeruli and sorted podocytes from inducible tagged NPHS2-Cre-GFP mice and showed that podocyte marker gene expression was decreased in disease state. In addition, the fluorescent activated cell sorter and magnetic isolation might have introduced a bias in cellular gene expression, such as cells that did not fit the usual podocyte prototype, for example, hypertrophied or dying cells might not have been used for the analysis. Single-cell analysis could be a powerful tool to resolve such changes. Despite these weaknesses, publicly available datasets from aging kidneys showed good correlation with the results reported by Wang *et al.*[6]

The next steps should include functional validation of the identified pathways, which will necessitate the generation of novel genetically modified animal models and correlate changes in gene expression and phenotype development such as albuminuria and glomerular filtration rate. Correlation between mouse models and human samples will be important to understand the relevance of changes observed in mice for our patient samples.

In summary, this study is a step forward to advance our understanding of podocyte aging. The team has discovered changes in developmental pathways and metabolism and inflammation. Future studies shall analyze podocyte aging in patients, as aging remains the most important risk factor for reduced kidney function and chronic kidney disease.

DISCLOSURE

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Moore's law for membranous nephropathy

Norifumi Hayashi[1,2] and Laurence H. Beck Jr[2,3]

The identification of target antigens in membranous nephropathy has accelerated since the report of M-type phospholipase A2 receptor 1 (PLA2R1). One could say that technological advances have allowed for the demonstration of Moore's law (a doubling every 2 years in the number of transistors that can be fit onto a computer chip) in the field of membranous nephropathy, and that even more antigens can be expected in the near future. In this issue of *Kidney International*, Sethi *et al.* describe semaphorin-3B as a novel target antigen, defining a type of membranous nephropathy with onset in the pediatric population.

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see clinical investigation on page 1253

[1]*Division of Nephrology, Kanazawa Medical University, Uchinada, Ishikawa, Japan;* [2]*Renal Section, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, USA; and* [3]*Department of Medicine, Boston Medical Center, Boston, Massachusetts, USA*

Correspondence: *Laurence H. Beck, Jr., Renal Section, Department of Medicine, Boston University School of Medicine and Boston Medical Center, 650 Albany Street, X-536, Boston, Massachusetts 02118, USA. E-mail: lhbeckjr@bu.edu*

It was not so long ago that there was a disease "entity" known as idiopathic membranous nephropathy (MN) defined pathologically by the membranous lesion in the absence of secondary associations. But just as all histopathologic lesions are now understood to represent a spectrum of underlying etiologies at the molecular level, so too is MN. After a decades-long hunt for

Table 1 | Comparison of target antigens or pathological biomarkers in subtypes of human membranous nephropathy

Feature	PLA2R1	THSD7A	EXT1/EXT2	NELL1	SEMA3B
UniProt ID	Q13018	Q9UPZ6	Q16394, Q93063	Q92832	Q13214
Size (in amino acids)	1463	1657	746/718	810	749
Compartment	Transmembrane glycoprotein	Transmembrane glycoprotein	Glycosyltransferase complex in Golgi	Secreted	Secreted
Evidence for expression by podocyte	Strong	Strong	Moderate (EXT2 > EXT1)	Weak	Strong
Presence in subepithelial deposits	Yes	Yes	Yes	Yes	Yes
Circulating Ab	Yes	Yes	No	Yes	Yes
Predominant subclass	IgG4	IgG4	IgG1 in deposits	IgG1	IgG1, not IgG4
Distinctive associations	Prototype for primary MN	Malignancy in a minority of cases	Lupus or other systemic autoimmune disease	Possible association with malignancy	Pediatric MN; early onset

EXT1/EXT2, exostosin glycosyltransferases 1 and 2 complex; MN, membranous nephropathy; NELL1, neural epidermal growth factor–like 1; PLA2R1, M-type phospholipase A2 receptor; SEMA3B, semaphorin 3B; THSD7A, thrombospondin type-1 domain containing 7A; UniProt ID, protein identification as per the UniProt database (www.uniprot.org).

target antigens in human MN, M-type phospholipase A2 receptor 1 (PLA2R1) was identified as such in 2009, followed by thrombospondin type-1 domain containing 7A (THSD7A) 5 years later.[1] The past 2 years have provided a flurry of new antigens or biomarkers in MN (Table 1). In fact, it was only earlier this year when another commentary was written in this journal to accompany the latest described autoantigen at that time, neural epidermal growth factor–like 1 (NELL1).[2] And here we are again. It seems that advances in technology have indeed allowed for a Moore's law for MN in that the remaining target antigens are identified at a faster rate and with increasing ease.

In this issue of *Kidney International*, the collaborative team from Mayo Clinic and Paris continue their identification of novel antigens and biomarkers in MN.[3] To their growing list, which includes the exostosin glycosyltransferases 1 and 2 (EXT1/EXT2) complex and NELL1, they now add semaphorin-3B (SEMA3B) as a novel podocyte-expressed antigen in what appears to be a distinct subtype of MN, one with onset in early childhood or adolescence.

A technological shift has allowed for this rapid antigen identification in MN and represents a change in methodology since the identification of PLA2R and THSD7A. In those "early days," patient serum was used for immunoblotting and immunoprecipitation of human glomerular proteins to identify potential bands. Mass spectrometry of trypsinized gel slabs would yield a list of potential antigens that then required sequential screening for serum reactivity until the true target was found.

The current methodology used to identify EXT1/EXT2, NELL1, and now SEMA3B relies on a useful property of the deposits in MN. Antigen-antibody complexes form and accumulate over the course of months, enriching the antigen within the subepithelial deposits. Through the use of laser capture microdissection, numerous glomerular tufts are selectively cut and collected from archival biopsy sections, followed by proteolysis and mass spectrometry. Any deviation from the "normal" repertoire of peptide spectra in MN can be quickly identified as a unique protein. The discovery process is iterative and based on the observation that, for the most part, each type of MN has only a single antigen. Therefore, the pool of biopsies in which there remain unknown antigens to be identified can be narrowed by exclusion of cases with already identified antigens.

What is the importance of identifying another relatively obscure protein? SEMA3B is the likely target antigen in a total of only 11 cases across 3 international cohorts, and 4 of 5 cases tested had demonstrable circulating anti-SEMA3B antibodies. With each new target antigen emerges a slightly novel phenotype of MN. SEMA3B-associated MN has its onset in early childhood or adolescence. Five cases had onset of disease at or before age 2 years; 3 were adolescents; and 3 presented as adults but well before their 50s (the median age of onset for PLA2R-associated MN). Sethi *et al.*[3] report a 10% prevalence of this type of MN in their pediatric MN cohorts, and up to 16% once cases of class V lupus nephritis were removed from the calculation.

The majority of pediatric nephrotic syndrome cases are "classified" by their response to corticosteroids, in that steroid-sensitive nephrotic syndrome most likely reflects minimal change disease and is not biopsied. In contrast, steroid-resistant patients often undergo biopsy and not infrequently exhibit focal segmental glomerulosclerosis, while a small percentage has other lesions such as MN. In this small case series, corticosteroid monotherapy did not seem to be particularly effective for bringing about remission of the MN, which instead required more conventional immunosuppressants for MN such as calcineurin inhibitors and/or rituximab. In this setting, prior serological information pointing to SEMA3B-associated MN might avoid months of ineffective therapy and perhaps obviate the need for kidney biopsy. Other common causes of pediatric MN, such as hepatitis B infection or lupus, already benefit from simple serological screens.

These new findings advance our knowledge about MN in the pediatric population but remind us that there are still many unanswered questions. Why do cases of PLA2R-associated MN peak in middle age yet seem virtually nonexistent before the age of 10 years?

THSD7A-associated MN has been described in a 4-year-old but is more often found in adults. Rare entities such as antenatal alloimmune MN caused by the transfer of maternal antibodies to neutral endopeptidase to the fetus, or infantile MN caused by antibodies to cationic bovine serum albumin have been described, but a major antigen in pediatric MN has yet to be identified. This study by Sethi *et al.*[3] points us in the right direction and suggests that comprehensive analysis of other pediatric MN repositories using similar techniques could help identify further antigens in this demographic group.

The characteristics of SEMA3B-associated MN have some interesting features such as the presence of IgG-containing immune deposits within the tubular basement membrane that do not stain for SEMA3B. This phenotype may be evanescent, as the feature disappeared from subsequent biopsies in 1 subject, despite the persistence of the SEMA3B-containing glomerular deposits. An older report describes an 11-month-old with apparent idiopathic MN and tubular basement membrane deposits in whom autoantibodies against a 58 kDa antigen was present;[4] it is possible that these 2 disease phenotypes could be part of the same spectrum.

In general, the absence of IgG4 and the existence of other immunoglobulin deposition such as IgM and IgA within the subepithelial deposits on renal biopsy suggest a secondary cause of MN. In SEMA3B-associated MN, only a single case (a 28-year-old woman) exhibited IgG4-predominant disease, whereas the remainder showed an absence of IgG4 or an IgG1 predominance. Several of the other cases also showed positivity for C1q, IgM, and IgA, and 3 cases exhibited tubuloreticular inclusions. Such features in pediatric disease do not necessarily rule out primary disease, as atypical features such as IgA, IgM, and mesangial deposits have been reported in cases of pediatric PLA2R-associated MN.[5] However, the unusual biopsy findings and the young age of the patients with SEMA3B-associated MN suggests an underlying immune pathogenesis distinct from that of PLA2R-associated MN. There were occasionally additional autoimmune phenomena such as type 1 diabetes or idiopathic thrombocytopenic purpura, but none of these patients had outright lupus.

Circulating antibodies from patient sera reacted with recombinant SEMA3B by immunoblotting and were correlated with disease activity. An unusual finding was that the human autoantibodies detected recombinant SEMA3B only in its reduced state. This is a finding that will need to be confirmed with native SEMA3B from human glomeruli. However, if verified, the result is quite intriguing, suggesting that the autoantibodies fail to recognize the native protein and may require another event to present a novel or cryptic epitope. In this small cohort of SEMA3B-associated MN, at least 3 participants may have had a genetic component (2 sibling brothers and another whose father had MN of unspecified etiology). A heritable polymorphism could potentially yield a neoepitope in these selected cases that might only be reproduced by experimental reduction of the wild-type protein. Alternatively, as suggested by Sethi *et al.*,[3] the exposure of a cryptic epitope by environmental or pathophysiological factors might be the cause. All class-3 semaphorins contain at least 2 conserved cleavage sites,[6] and it is possible that a regulated cleavage event could occur *in vivo* to expose the epitope.

What does it take to confirm a novel candidate as a true target antigen in MN? Antibodies to alpha-enolase, aldose reductase, and superoxide dismutase can be found in a significant proportion of MN cases, but these intracellular antigens are generally not considered the primary target antigens for disease[7] but could instead represent neoantigens induced by podocyte injury. Sethi *et al.*,[3] make a good case for SEMA3B as a target antigen due to its detection in only 3 biopsies out of thousands analyzed by mass spectrometry; the specific presence of SEMA3B that colocalizes with IgG within immune deposits by confocal microscopy; and the presence of circulating anti-SEMA3B during active disease but not during remission. In addition, SEMA3B is normally expressed by the human podocyte as a secreted protein that should be available to circulating autoantibodies. For most of the known MN autoantibody/autoantigen systems, however, Koch's postulate regarding reproduction of disease (in this case, with human autoantibodies) in a healthy host has not been fulfilled. Human anti-THSD7A antibodies can cause mild disease in mice on passive transfer,[8] and introduction of a naïve donor kidney into a recipient with circulating anti-PLA2R can transfer disease to the new kidney.[9] We await further studies assessing the pathophysiology of these newer antigens.

In summary, the detection of additional target antigens allows both clinicians and scientists to better classify MN at a molecular level. It is likely that many of the lessons and conceptual models learned from PLA2R-associated MN will carry over to these newly described forms. The work by Sethi *et al.*[3] continues to move the field forward step by step. This ever-increasing number of antigen-antibody systems will require us to reexamine the terms "primary" and "secondary" as we all struggle with the next challenge of how to optimally put these newfound molecular phenotypes of MN into clinical use.

DISCLOSURE

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Atypical hemolytic uremic syndrome associated with a factor B genetic variant and fluid-phase complement activation: an exception to the rule?

Marina Noris[1] and Giuseppe Remuzzi[1]

Gain-of-function variants in *CFB* encoding factor B (FB), a component of the alternative pathway C3 convertase, have been reported in a minority of patients with aHUS and result in massive C3 activation. Zhang *et al.* describe the functional characterization of a novel FB variant (p.Ser367Arg) that they identified in 2 unrelated aHUS pedigrees who had undetectable C3 levels. The mutant FB caused strong C3 cleavage in fluid-phase but also C3 deposition on cell surface. This commentary addresses the implications of these findings for understanding the complexity of complement-related genetic renal diseases.

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see clinical investigation on page 1265

Atypical hemolytic uremic syndrome (aHUS) is a rare form of thrombotic microangiopathy (TMA) that manifests with nonimmune anemia, low platelet count, and acute renal failure.[1] Several studies have clarified that aHUS is caused by dysregulation of the alternative pathway of complement, resulting in excessive deposition of C3b and of the terminal membrane attack complex C5b-9 on cell surfaces.[2] In about 50% of patients, the disease is associated with loss-of-function genetic abnormalities that result in impaired function of the complement regulatory proteins factor H (FH), membrane cofactor protein (MCP), factor I (FI), and thrombomodulin or anti-FH autoantibodies.[1]

Gain-of-function variants in the genes (*C3* and *CFB*) encoding the 2 components of the alternative pathway C3 convertase, C3 and factor B (FB) have also been reported in patients with aHUS.[1] C3 genetic abnormalities have been found in about 5% to 10% of patients, and in functional studies around 75% of mutant proteins demonstrated defective binding to complement regulators, which translates to a decreased proteolytic inactivation of C3b by FI in the presence of FH or MCP (cofactor activity).[3] Gain-of-function variants in *CFB* are rarer and have been identified in <2% of patients with aHUS.[4]

Factor B interacts with C3(H_2O), formed by spontaneous hydrolysis of C3 in the alternative pathway (tick-over), or with C3b that is generated by C3 cleavage through any of the 3 complement pathways, to form the alternative pathway proconvertase complexes (C3(H_2O)B and C3bB). The latter are converted into the active convertase enzymes C3(H_2O)Bb and C3bBb through cleavage of FB to Bb and Ba by factor D (Figure 1a).

Most aHUS-associated FB variants cluster in the von Willebrand type A (vWA) domain of Bb, often close to the Mg^{2+} adhesion site (MIDAS) (Figure 1b).[4] Both the vWA and the MIDAS domains are involved in the interaction with C3b and in the stability of the C3 convertase complex. The pathogenetic variants in the MIDAS could affect the binding of the Mg^{2+} ion, which is critical for C3/FB interactions. Functional studies have revealed that aHUS-associated FB variants affecting vWA and MIDAS domains result in faster association and stronger binding to C3b, and/or to a more stable C3 convertase that is resistant to accelerated decay by the complement regulators, in particular by FH.[5,6] The result is increased enzyme activity, with the formation of massive amounts of C3 activation products.

Of relevance, published studies have documented that the C3 convertases formed by aHUS-associated FB variants are also more active on the cell surface. Indeed, mutant C3 convertases were more efficient in causing sheep erythrocyte cell lysis in a complement-dependent hemolytic assay.[6] In addition, serum from patients with *CFB* abnormalities or

[1]*Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Bergamo, Italy*

Correspondence: *Marina Noris, Istituto di Ricerche Farmacologiche Mario Negri, IRCCS, Via Camozzi 3, 24020 Ranica Bergamo, Italy. E-mail: marina.noris@marionegri.it*