Taking the Kidney Personally: The Quest for Novel Antigens of Idiopathic Membranous Nephropathy through Proteomic Approaches – *Per Ardua Ad Astra?*

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1. INTRODUCTION

Membranous nephropathy is one of the most common diseases affecting the glomerulus, an important part of the kidney responsible for filtration of blood. Glomeruli are networks of capillaries that filter blood; normally only small ions and proteins are filtered, while larger proteins like hemoglobin, albumin, and immunoglobulins are retained. The filtration barrier consists of several layers including: capillary endothelial cells, glomerular basement membrane, and specialized cells called podocytes. In patients with membranous nephropathy, this selective barrier is injured, leading to massive loss of proteins in urine (proteinuria). Patients typically present with marked swelling due to protein loss and avid sodium absorption by the kidneys, as well as hypoalbuminemia, hyperlipidemia, lipiduria, and propensity for developing infections and clots (called nephrotic syndrome).

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

Histopathologically, this disorder is characterized by discovery of immune deposits in a specific part of glomerulus between the basement membrane and podocyte (called subepithelium). Membranous nephropathy can be primary (idiopathic) or secondary when caused by malignancy, infections, autoimmune diseases or drugs. Idiopathic membranous nephropathy (IMN) is classically believed to be progressive in one third of cases, while the other two thirds remain stable or improve spontaneously [1].

Tremendous insights into the pathophysiology of IMN have been made recently, with studies that have identified key antigens and cemented our understanding of the mechanisms of this disease. The first insights which paved the way to our current understanding of IMN came from a rat model called Heymann's nephritis [2, 3]. The rats had subepithelial deposits consisting of megalin and megalin-specific circulating antibodies [3].

What puzzled scientists and clinicians was that megalin was not expressed in human podocytes, and could thus not be the target antigen in human IMN [4]. Another rare cause of IMN demonstrated the same principle of injury, and this was the occurrence of IMN in neonates with circulating anti-neutral endopeptidase antibodies [5, 6]. Mothers of these neonates had genetic deficiency in neutral endopeptidase, and through immunization produced antibodies to neutral endopeptidase present in podocytes of the fetus. Disappearance of these antibodies led to cure. The most significant discovery along the same paradigm was that of M-type phospholipase A2 receptor (PLA2R) antibodies as the causative agent in most patients with IMN [7]. PLA2R is expressed in the podocyte and remains the most convincing antigen in IMN. Anti-PLA2R antibody in serum identifies approximately 70 – 80% of cases of IMN in Caucasians [7, 8], African Americans [7] and Chinese patients [9]. Immunostaining of biopsies from patients with IMN detects increased deposition of subepithelial PLA2R with similar sensitivity to serum anti-PLA2R levels [10].

A genome wide association study identified a significant association between PLA2R-related SNPs and IMN [11]. Finally, level of the anti-PLA2R antibody in serum appears to parallel response to Rituximab [12], a chimeric monoclonal antibody against the B cell surface antigen CD20. The level of anti-PLA2R antibody may also correlate with IMN relapse after kidney transplantation [13]. This represents an important pharmacoproteomic finding relating to response to an immunomodulatory agent in IMN. There is also preliminary evidence that this antibody mirrors disease activity [8]. Despite the undeniable potential of anti-PLA2R antibodies as a biomarker of disease in patients with IMN, this antibody does not explain the etiology of the disease in a substantial proportion of cases. It is tempting to speculate that antibodies to distinct podocyte antigens are responsible for these "PLA2R negative" cases.

Indeed, aldose reductase (AR) and superoxide dismutase 2 (SOD2) [14] were discovered as additional podocyte antigens linked with IMN, and are likely responsible for a minority of cases. Interestingly, a recent study demonstrated a connection between antibodies to an exogenous antigen, bovine serum albumin (BSA) and IMN in children with this disease [15]. BSA was found in immune deposits, as well as in the circulation of affected children. Furthermore, anti-BSA circulating antibodies were found in the children. All these examples demonstrate that IMN is a conglomeration of autoimmune diseases in which antibodies are

produced to podocyte specific proteins, or alternatively there is *in situ* formation of immune complexes to antigens deposited at the filtration barrier.

2. PHARMACOPROTEOMICS IN PRACTICE?

In this issue of CPPM, original research by Ligabue *et al.* takes advantage of proteomics-based technology to define novel markers of IMN. Proteome is dynamic as it undergoes changes in response to the individual cell's metabolic state and reception of intracellular and extracellular signal molecules. This dynamic nature of the proteome may also be due to the post-translational modifications and proteolytic processing. Proteomics research has quickly evolved to become crucial for medical sciences and along with the information contained in the human genome sequences, it has the potential to lead to discovery of biomarkers for diseases and novel targets for drug development. However, it has been recognized that unbiased proteome mining of complex biofluids such as blood and urine is difficult and has not yet led to major breakthroughs in discovery of disease biomarkers [16, 17].

Ligabue *et al.* employed a novel approach consisting of centrifugation of renal biopsy samples to extract fluid they termed “interstitial fluid” (IF). IF is fluid that bathes and surrounds cells. It consists of sugars, salts, fatty acids, amino acids, enzymes, hormones and cellular waste products in water. Ligabue *et al.* analyzed IF samples extracted from renal biopsies of patients with IMN, one deceased kidney donor, and a number of other renal diseases including tubular, glomerular, and urologic pathologies. The proteome of IF samples was analyzed by electrospray ionization – Quadrupole –Time of flight (ESI-Q-TOF) mass spectrometer, and label free quantification was performed by extracted ion current derived peak areas of individual peptides. Proteins differentially expressed in IMN compared to other renal pathologies were identified. Protein characterization using tandem mass spectrometry and label free quantification have greatly improved the accuracy and throughput of analysis of thousands of proteins. By employing these methodologies the authors focused on three significantly over-expressed proteins amongst biopsies with IMN, because these three were functionally related and had not been linked to IMN before. These three proteins included: four and a half LIM domain protein 1 (FHL1), LIM domain-binding protein 3 (LDB3), and PDZ and LIM domain 5 protein (PDLI5). The authors then went on to show that these proteins were expressed in the glomerulus, although FHL1 expression appeared to be no different between cases and controls by immunofluorescence. LDB3 and PDLI5 had increased expression in glomeruli of patients with IgA nephropathy and IMN compared to controls, and they were further demonstrated to be expressed in podocytes by dual staining immunofluorescence. The Human Protein Atlas (<http://www.proteinatlas.org/>) reports no LDB3 expression in normal human glomeruli, which would support the findings of this study. However, contrary to observations regarding PDLI5, there is strong expression of PDLI5 in some normal glomeruli, suggesting that this protein may not be specific to glomerular diseases such as IgA nephropathy and IMN, and that inter-individual variation in its expression may be significant. Nonetheless, it is informative that the IF proteome deciphered by the authors contained podocyte-derived proteins. It is also interesting that the list of 83 differentially expressed proteins contained many proteins involved in oxidative stress, and that at least one protein previously linked to IMN was found amongst the candidates (AR).

3. LOOKING FORWARD

Despite the novelty and potential strength of the approach, there are many lingering unanswered questions. One of them relates to how we should interpret the Western blots of sera and urines of patients with IMN, IgA nephropathy and healthy controls.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an intracellular housekeeping protein that should be present in low amounts in serum and urine. It will thus not reflect the massive increases in urine protein excretion rate that are typical of IMN. This then leaves us to ponder whether densitometry differences between LDB3 and PDLI5 and albumin (as the most highly abundant urine protein) would have been different between patients with IgA, IMN and normal controls. While we commend the authors for searching for their top candidates in biofluids in order to make them clinically relevant, it remains unclear whether comparison between IMN and the two other groups, namely normal controls and IgA nephropathy has clinical merit. Patients with IMN will typically present with nephrotic syndrome, and glomerular diseases other than IgA nephropathy such as focal and segmental glomerulosclerosis, minimal change disease, membranoproliferative glomerulonephritis, and secondary membranous nephropathy are on the differential diagnosis, thus begging their use as a comparator group. Finally, if we are to believe the well documented paradigm of immune response responsible for development of IMN, the real step forward in demonstrating pathogenicity of these proteins would be to show circulating antibodies specific to the proteins, and only present in patients with IMN and not those with secondary membranous or other causes of glomerular disease, as well as to demonstrate these protein-specific antibodies in subepithelial immune deposits in the glomeruli of patients with IMN.

In conclusion, while PLA2R is the most convincing antigen in most patients with IMN, there are without doubt other podocyte antigens that are targets of the immune response in the minority of patients with IMN. Moreover, the antigens responsible for secondary membranous nephropathy are unknown. Renal proteomic approaches such as the novel one utilized in the study by Ligabue *et al.* will open the door to better understanding of podocyte and other glomerular basement membrane proteins (or to proteins deposited at the filtration barrier) which may allow us to identify the cryptic antigens involved in both primary and secondary membranous nephropathy. Renal proteomics may be similarly utilized to better understand proteins involved in other glomerular or tubulointerstitial diseases and this may one day help achieve the ultimate goal of the development of personalized biomarkers that can be used to more accurately diagnose renal diseases and better understand heterogeneity in underlying disease mechanisms. Ultimately, these biomarkers may be used to guide treatment decisions and to monitor response to currently available therapies and novel ones in the renal drug development pipeline. Attention will need to be paid to understanding intra- and inter-individual differences between and within different ethnic groups, determining sensitivity, specificity as well as positive and negative predictive values of novel renal biomarker tests, and to using appropriate comparison groups that reflect real-life clinical questions.

The road ahead is arduous, but as the Latin proverb *Per Ardua Ad Astra* in the title suggests, only great efforts could result in great achievements, such as the ultimate goal of bringing personalized medicine into the clinic.

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ABBREVIATION

IF Interstitial fluid

References

1. Cattran DC. Idiopathic membranous glomerulonephritis. *Kidney Int.* 2001; 59:1983–94. [PubMed: 11318974]
2. Heymann W, Hackel DB, Harwood S, et al. Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. *Proc Soc Exp Biol Med.* 1959; 100:660–4. [PubMed: 13645677]
3. Kerjaschki D, Farquhar MG. Immunocytochemical localization of the Heymann nephritis antigen (GP330) in glomerular epithelial cells of normal Lewis rats. *J Exp Med.* 1983; 157:667–86. [PubMed: 6337231]
4. Whitworth JA, Leibowitz S, Kennedy MC, et al. Absence of glomerular renal tubular epithelial antigen in membranous glomerulonephritis. *Clin Nephrol.* 1976; 5:159–62. [PubMed: 1277597]
5. Debiec H, Guignon V, Mougenot B, et al. Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med.* 2002; 346:2053–60. [PubMed: 12087141]
6. Debiec H, Nauta J, Coulet F, et al. Role of truncating mutations in MME gene in fetomaternal alloimmunisation and antenatal glomerulopathies. *Lancet.* 2004; 364:1252–59. [PubMed: 15464186]
7. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med.* 2009; 361:11–21. [PubMed: 19571279]
8. Hofstra JM, Beck LH Jr, Beck DM, et al. Anti-phospholipase A(2) receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol.* 2011; 6:1286–91. [PubMed: 21474589]
9. Qin W, Beck LH Jr, Zeng C, et al. Anti-phospholipase A2 receptor antibody in membranous nephropathy. *J Am Soc Nephrol.* 2011; 22:1137–43. [PubMed: 21566055]
10. Hoxha E, Kneissler U, Stege G, et al. Enhanced expression of the M-type phospholipase A2 receptor in glomeruli correlates with serum receptor antibodies in primary membranous nephropathy. *Kidney Int.* 2012; 82:797–804. [PubMed: 22673885]
11. Stanescu HC, Arcos-Burgos M, Medlar A. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. *N Engl J Med.* 2011; 364:616–26. [PubMed: 21323541]
12. Beck LH Jr, Fervenza FC, Beck DM, et al. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol.* 2011; 22:1543–50. [PubMed: 21784898]
13. Stahl R, Hoxha E, Fechner K. PLA2R autoantibodies and recurrent membranous nephropathy after transplantation. *N Engl J Med.* 2010; 363:496–8. [PubMed: 20818871]
14. Prunotto M, Carnevali ML, Candiano G, et al. Autoimmunity in membranous nephropathy targets aldose reductase and SOD2. *J Am Soc Nephrol.* 2010; 21:507–19. [PubMed: 20150532]
15. Debiec H, Lefeu F, Kemper MJ, et al. Early-childhood membranous nephropathy due to cationic bovine serum albumin. *N Engl J Med.* 2011; 364:2101–10. [PubMed: 21631322]
16. Konvalinka A, Scholey JW, Diamandis EP. Searching for new biomarkers of renal diseases through proteomics. *Clin Chem.* 2012; 58:353–65. [PubMed: 21980170]
17. Ray S, Reddy PJ, Jain R, et al. Proteomic technologies for the identification of disease biomarkers in serum: advances and challenges ahead. *Proteomics.* 2011; 11:2139–61. [PubMed: 21548090]