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REVIEW

# Animal models of idiopathic membranous nephropathy: Recent advances and future perspectives

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## Abstract

Idiopathic membranous nephropathy (IMN) is one of the main causes of adult nephrotic syndrome. A subset of untreated or inadequately treated patients eventually progress to end-stage renal disease (ESRD), posing a significant clinical challenge. Although the discovery of novel podocyte target antigens has deepened our understanding of IMN pathogenesis, the precise molecular mechanisms remain incompletely elucidated, and effective targeted therapies are still lacking. Animal models play an irreplaceable role in uncovering IMN pathogenesis and developing effective therapies. In recent years, with a deeper understanding of IMN, researchers have successfully established various animal models, including Heymann nephritis (HN), cationic bovine serum albumin (C-BSA), Aminopeptidase A (APA), thrombospondin type 1 domain-containing 7A (THSD7A)-related, and phospholipase A2 receptor (PLA2R)-related IMN models. These models have substantially advanced the simulation of pathological human IMN features. Notably, the development of human PLA2R1-related animal models marks a landmark breakthrough in this field, as these models are the first to recapitulate the immunopathological processes driven by a key human autoantigen in experimental animals. However, current animal models have their own limitations and still cannot fully replicate the complex pathological process of human IMN. This review summarizes recent progress in animal IMN models, analyzes their methods, pathological features, strengths, and limitations, and discusses future directions. Future model development should integrate advanced multi-omics and artificial intelligence (AI) to achieve greater accessibility and precision, enabling the construction of multidimensional models encompassing genetics, environment, and immunity, thereby enabling a leap from "disease simulation" to "personalized treatment".

**KEYWORDS**

animal model, end-stage renal disease, idiopathic membranous nephropathy, phospholipase A2 receptor

Qiuying Liu and Huixian Bian contributed equally to this work.

## 1 | INTRODUCTION

Idiopathic membranous nephropathy (IMN) is a common glomerular disease and is among the main pathological types of adult nephrotic syndrome. Its course follows the "one-third rule"—that is, around one-third of patients spontaneously remit without treatment, one-third present with persistent proteinuria, and one-third develop end-stage renal disease (ESRD) within 5–10 years.[1,2] Despite the increasing diversification of therapeutic strategies for membranous nephropathy (MN) in recent years,[3,4] suboptimal treatment regimens or poor initial responses remain critical hurdles that contribute to disease progression in a subset of patients. Furthermore, MN is characterized by a high risk of recurrence; approximately 30%–40% of patients who achieve clinical remission experience relapse during follow-up,[5] while 30%–50% of kidney transplant recipients face recurrent MN, which severely compromises long-term graft survival.[6,7] To address these clinical challenges, the management of MN has transitioned from conventional protocols toward precision medicine and integrative strategies. The identification of key autoantigens, most prominently PLA2R1, alongside systems biology research incorporating multi-omics and the gut microbiota, has refined our understanding of MN pathogenesis and risk assessment, thereby establishing a robust foundation for individualized therapy.[8,9] Regarding treatment, a risk-stratified approach is now standard, while, simultaneously, biologics and T-cell therapies are expanding the options for refractory disease.[8,10] Moreover, Traditional Chinese Medicine (TCM) contributes to this integrative model through its immunomodulatory and podocyte-protective effects, enhancing remission and reducing toxicity.[8,9,11] An integrated TCM-Western medicine approach represents the future of MN management, requiring further validation through large-scale trials and molecular investigations into therapeutic synergy.[10,11]

The pathological features of MN include glomerular basement membrane (GBM) thickening and subepithelial fuchsinophilic deposits under light microscopy (Figure 1A–C), bright granular staining of immunoglobulin G (IgG) deposition under immunofluorescence microscopy (Figure 1D–F), and electron-dense deposits in the GBM under immunofluorescence microscopy (Figure 1G–I; images are based on pathological data from renal biopsy cases in Shengjing Hospital). The complex pathogenesis of IMN is not yet fully understood. Currently, complement-mediated podocyte damage, caused by antibodies targeting podocyte antigens, is believed to be the main pathogenic mechanism of MN.[12] The core process involves immune complex formation in the GBM (Figure 2). After the target antigen binds to the antibody, immune complexes form and are deposited beneath the glomerular epithelial cells, activating the complement system and causing podocyte damage. In recent years, researchers have identified various podocyte-targeting antigens associated with IMN, including phospholipase A2 receptor (PLA2R),[13] thrombospondin type 1 domain-containing 7A (THSD7A),[14] neural epidermal growth factor-like 1 (NELL-1),[15] semaphorin-3b (Sema3B),[16] high-temperature requirement A serine peptidase 1 (HTRA1),[17] protocadherin 7 (PCDH7)[18] and netrin G1 (NTNG1).[19] Among these, PLA2R and THSD7A are the primary target antigens, accounting for approximately 70%–80% and 1%–5% of the target antigens in IMN, respectively; nonetheless, 10%–20% of IMN-related target antigens have yet to be discovered[20–22] (Figure 3). Although the discovery and clinical application of anti-PLA2R antibodies have brought breakthrough progress in the diagnosis, prognosis and treatment of IMN,[23–28] the specific mechanism of IMN caused by the deposition of immune complexes produced by different target antigen antibodies remains to be further explored.

To further investigate the pathogenesis of IMN and develop effective targeted therapies, various animal models of IMN have been successfully established (Figure 4), including Heymann nephritis (HN), cationic bovine serum albumin (C-BSA), THSD7A- related and PLA2R-related models. Although the latest PLA2R1-related IMN model has made significant progress in simulating the pathogenesis and pathological features of IMN and may represent the most realistic simulation thus far, it cannot fully reproduce the complex pathological processes of human IMN. Furthermore, the technical complexity, high cost, and limited availability of this model restrict its widespread application. Therefore, existing animal IMN models remain unsatisfactory and require further refinement, and new models should be developed to better elucidate the pathogenesis of IMN and establish more effective treatment strategies. This review summarizes the pathological characteristics, advantages, and limitations of various animal IMN models in recent years (Table 1) and discusses the future development directions of animal IMN models.

## 2 | TRADITIONAL ANIMAL IMN MODELS

### 2.1 | HN model

The HN model is currently one of the most widely used animal models of IMN. In 1959, Heymann et al.[29] successfully induced proteinuria in rats by injecting homogenate prepared by lavage and established the Heymann nephritis model via homogenization and centrifugation of allogeneic or xenogeneic kidney tissues into experimental rats with complete Freund's adjuvant. At present, the model has been successfully constructed in multiple rat strains, including Sprague–Dawley, Wistar, Munich Wistar, Lewis, and PVG. Heymann nephritis can be divided into two types according to different immunological methods: active Heymann nephritis (AHN) and passive Heymann nephritis (PHN). Specifically, The AHN model is established through the systemic immunization of rats with Fx1A, an antigenic fraction isolated from the rat renal tubular brush border. Conversely, the establishment of PHN is achieved by immunizing xenografts with Fx1A extracted from the brush border of rat proximal renal tubular epithelial cells and then transferring the obtained immune serum antibodies into rats to induce proteinuria.[30,31] In the AHA model, particle deposition of rat IgG was observed in the glomerular capillary wall. After 8–10 weeks, 30%–80% of rats showed significant proteinuria. In the PHN model, at 3–5 days after anti-FX1A antibody injection, IgG, C3, and C5b-9 deposition in the subepithelial tissue was detected and after 7–10 days, the rats showed persistent

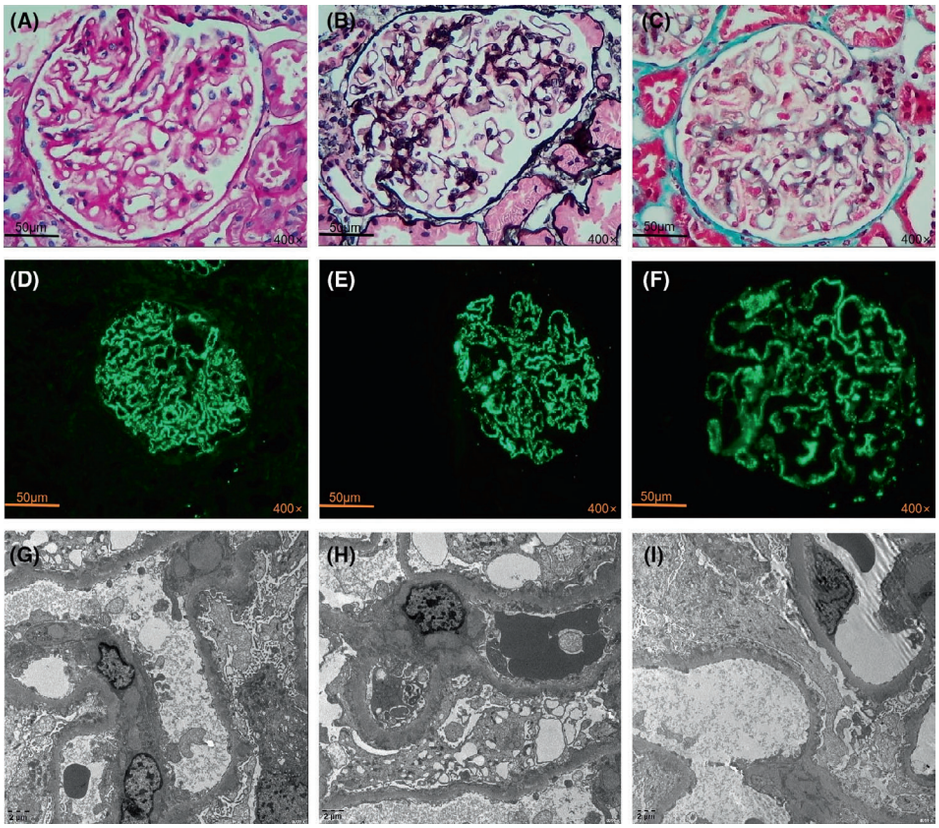


**FIGURE 1** Pathological features of IMN. Light microscopy shows basement membrane thickening and visible spike-like structures (A, B), and subepithelial deposition of fuchsinophilic (C). Immunofluorescence reveals diffuse IgG (+++) staining appearing as fine granular deposits along the capillary loops. IgG+++ (D), IgG4+++ (E), and PLA2R++ (F) are detected. Electron microscopy (G–I) demonstrates segmental homogeneous thickening of the GBM, diffuse fusion of foot processes, and significant deposition of electron-dense material beneath the epithelium and within the basement membrane, along with basement membrane reaction (images are based on pathological data from renal biopsy cases in Shengjing Hospital).

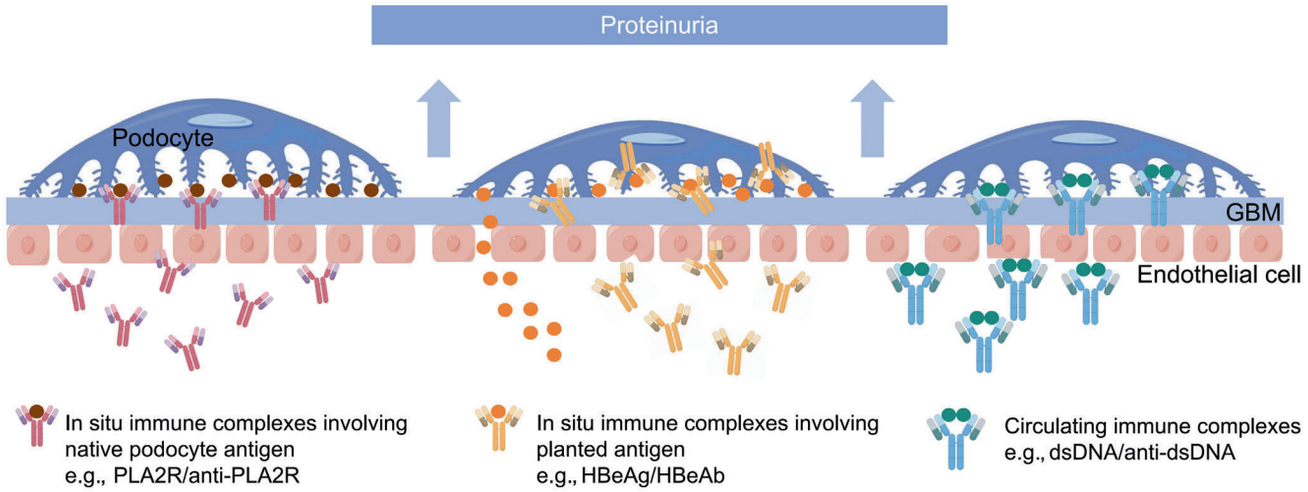


**FIGURE 2** Immune complex formation in the GBM. Schematic of immune complex deposition and complement activation leading to podocyte damage in membranous nephropathy.

proteinuria.[32] Analysis of Fx1A components revealed that the pathogenic antigen components were megalin (gp330) and receptor-associated protein (RAP).[33–35] The PHN model closely mimics human IMN, characterized by the subepithelial formation of C5b-9 induced by heterologous anti-Fx1A antibodies and, consistent with human pathophysiology, complement activation in PHN likely proceeds

via both the classical and alternative pathways.[35,36] Consequently, by recapitulating the complement-mediated damage driven by in situ immune complexes, PHN remains a cornerstone animal model for investigating MN pathogenesis and assessing therapeutic strategies.[37–43] For instance, studies employing the PHN model have identified the complement C3a/C3aR axis[37] and pyroptosis[38] as pivotal drivers of podocyte injury. Furthermore, the PHN model provides a reliable experimental platform for evaluating TCM formulas, such as Sanqi Qushi and Shenqi Dihuang decoctions, which have been demonstrated to effectively mitigate renal injury by suppressing the MEK/ERK signaling pathway or enhancing podocyte autophagy, respectively.[42,43]

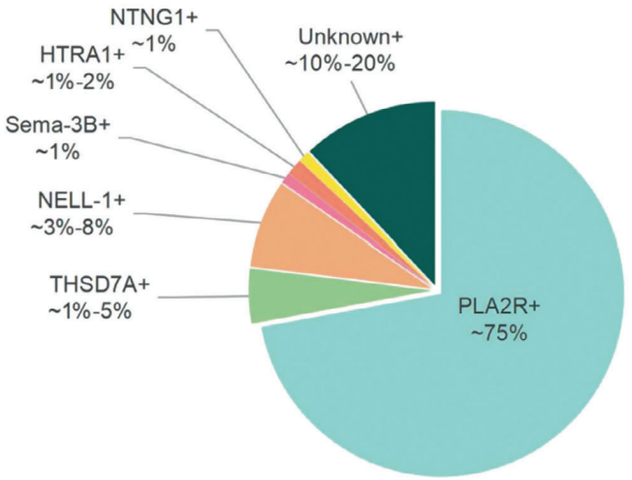


FIGURE 3 Distribution of podocyte antigens in patients with IMN. Pie chart showing the prevalence of identified target antigens in idiopathic membranous nephropathy (IMN). PLA2R and THSD7A remain the primary antigens, while approximately 10%–20% of target antigens are currently unknown.

Although the pathogenesis of Heymann nephritis is similar to that of human IMN, there are some limitations. The primary target antigen of human IMN is PLA2R, whereas the primary pathogenic target antigen of Heymann nephritis is megalin. Furthermore, megalin can act on human podocytes[44] and is expressed on the brush border of proximal tubules,[45] whereas the main pathogenic antigen megalin of Heymann nephritis has not been detected in the subepithelial immune deposits of patients with IMN, suggesting that the main target antigens of human IMN and Heymann nephritis differ. In addition, megalin is highly expressed on the brush border of renal tubules; therefore, immune complex deposition in the tubular area is inevitable after modeling, which differs from human IMN.

## 2.2 | C-BSA model

Batsford and Border successfully induced experimental MN by administering cationic antigens to experimental animals in 1980[46] and 1982,[47] respectively. C-BSA-treated rabbits showed a uniform deposition of particulate IgG, BSA, and C3 on the glomerular capillary wall, accompanied by clinical features similar to those observed in human MN.[47] The principle behind the C-BSA model is that the GBM is negatively charged, allowing positively charged C-BSA to easily

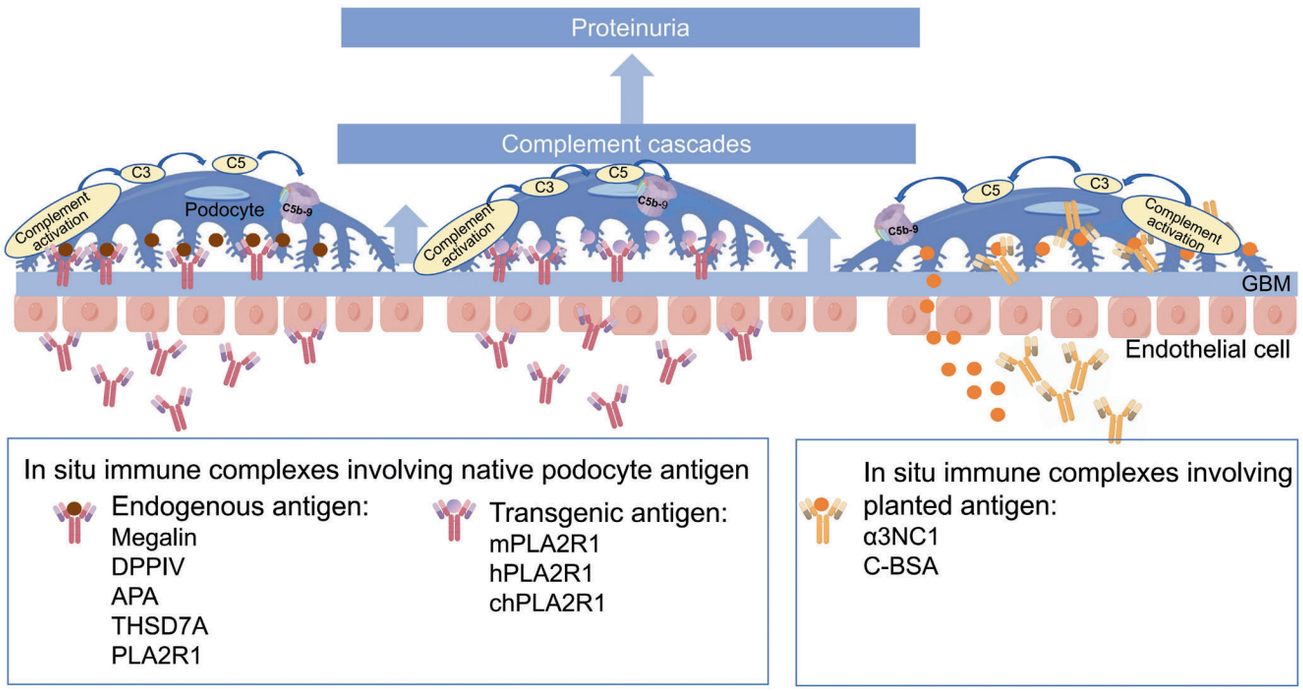


FIGURE 4 Pathogenesis in animal models of IMN. Schematic representation of pathogenic mechanisms in various IMN animal models, including endogenous, transgenic, and planted antigen-related models. These models illustrate the core process of in situ immune complex formation, complement cascade activation, and subsequent podocyte damage leading to proteinuria.

**TABLE 1** Comparison of different animal models of IMN.

| Animal model | Species | Target antigen | Antibody source | Complement activation | Proteinuria | Key limitations | References |
|---|---|---|---|---|---|---|---|
| AHN model | Rat | Megalin, RAP | Endogenous | Classical pathway | Rats had significant proteinuria after 8–10 weeks | Time consuming | [29] |
| PHN model | Rat | Fx1A | Exogenous | Classical pathway and alternative pathway | Rats developed persistent proteinuria after 7–10 days | Differences with the main target antigens of IMN | [30,31] |
| C-BSA model | Rat, mice, rabbit, dog, cat, etc. | C-BSA | Endogenous | Unknown | Dose-dependent severe proteinuria | High mortality after 8-week repeated tail injections | [47–49,53–55] |
| APA model | Mice | APA | Exogenous | Complement-independent | Dose-dependent severe proteinuria lasted at least 16 days | Poor stability, short duration, lacking IMN pathological features | [63] |
| DPPIV model | Rat | DPPIV | Exogenous | Complement-independent | Peaked at 2 days, declined rapidly | Short duration of proteinuria, fast disappearance of IgG | [65] |
| APN model | Mice | Annexin-3 and other antigens | Exogenous | Complement-independent | Severe albuminuria peaked on day 10 | Complement-independent; multiple antigens differ from the main target antigens of IMN | [66,67] |
| α3NC1 model | Mice | α3NC1 | Endogenous | Alternative pathway | Massive proteinuria dependent on alternative complement pathway | Different strains of mice differ in sensitivity | [70] |
| ACE model | Minipig | ACE | Exogenous | Unknown | Unknown | Different from the main target antigen of IMN | [74] |
| THSD7A model | Mice | THSD7A | Exogenous | Lectin pathway | Onset at day 3, peaking at day 14, persisting for 70 days | Applicable to only a few strains of animals | [76] |
| mPLA2R1 model | Mice | mPLA2R1 | Exogenous | Unknown | Nephrotic-range proteinuria | High technical and cost requirements | [81] |
| hPLA2R1 model | Mice | hPLA2R1 | Endogenous | Unknown | Onset at 4 weeks, with deterioration occurring at 12 weeks | High technical and cost requirements | [82] |
| chPLA2R1 model | Mice | chPLA2R1 | Endogenous | Classical pathway, alternative pathway and lectin pathway | Significant proteinuria | High technical and cost requirements | [85] |
| PLA2R1 minipig model | Minipig | hPLA2R1 | Exogenous | Three pathways potentially involved | Mild proteinuria | Low level of proteinuria | [88] |

cross the basement membrane and become the planted antigen; it further induces the corresponding anti-C-BSA antibodies in the circulatory system to accumulate, forming an in situ immune complex. In 1998, Kobayashi et al.[48] successfully induced subepithelial deposition of IgG and C3 characteristic of MN in rats immunized with C-BSA. In 2004, Chen et al.[49] intravenously injected an optimal dose of C-BSA into 8-week-old female ICR, BALB/c, and C57BL/6 mice and found that MN development in mice varied by species over a 4-week period. Among these, ICR and BALB/c mice were relatively more susceptible to human-like models. In ICR mice, pathological examination of kidney tissues showed thickening of the basement membrane, deposition of subepithelial immune complexes, and formation of basement membrane spikes. In addition, the induction of MN exhibited a dose-dependent effect, and excessive doses posed a risk of mortality in the model mice; most ICR mice in the 9 mg/kg group died. The optimal dose for ICR mice is 7 mg/kg, and at doses of C-BSA below 3 mg/kg normal proteinuria is completely restored in mice after discontinuation. The ideal dose for inducing MN in BALB/c mice is 13 mg/kg; however, this dose cannot reliably induce MN in C57BL/6 mice.

The development process of the C-BSA mouse model is similar to that of human IMN, with both being closely related to Th2 cells. In the C-BSA-induced MN mouse model, Th2 cells are significantly associated with hypercholesterolemia and proteinuria.[50] Similarly, human IMN patients exhibit a Th2-type immune response bias, with Th2 cytokines (especially IL-4) promoting B cells to produce IgG4 subclass antibodies, which may be an important component of the pathogenesis of IMN.[51] Moreover, C-BSA not only induces experimental MN in animals but also has a similar immunopathogenic effect in humans. C-BSA, especially that derived from milk, which is not completely digested in food, can enter the human body through the digestive tract to form planted antigens. These antigens form

in situ immune complexes with IgG1 and IgG4, leading to the occurrence of membranous nephropathy.[52] The C-BSA animal model offers advantages, such as low cost and ease of operation. Moreover, the C-BSA model exhibits broad species applicability, extending beyond mice and rats to include rabbits, cats, dogs, and other species.[47–49,53–55] Thus, it has become one of the most widely utilized approaches for elucidating the pathogenesis and evaluating novel treatments for MN in recent years.[56–62] Based on the C-BSA-induced rat models and zymosan-activation serum (ZAS)-treated podocytes, researchers have demonstrated that aryl hydrocarbon receptor (AhR) signaling drives oxidative stress and inflammation in MN by functioning as a key upstream regulator that activates NF-κB while suppressing Nrf2-mediated antioxidant responses.[59] Furthermore, Sirtuin 6 (Sirt6) has been shown to protect podocytes from injury by inhibiting the Wnt1/β-catenin pathway, thereby antagonizing the renin-angiotensin system (RAS).[60] Recent investigations utilizing this model have further elucidated the role of the "gut-kidney axis" in MN, revealing that the depletion of intestinal *Lactobacillus* leads to an imbalance in tryptophan metabolites, which serves as a primary trigger for intrarenal AhR activation.[61,62] These insights, elucidated through the C-BSA model, not only uncover novel molecular mechanisms but also provide strong preclinical evidence supporting AhR antagonists, Sirt6 activators, and gut microbiota modulation as promising therapeutic targets for MN.

While the C-BSA model has gained widespread adoption due to its cost-effectiveness and operational simplicity, it is nonetheless constrained by certain practical limitations. Specifically, the requirement for repeated tail vein injections imposes significant physiological stress on experimental animals, and the notably high mortality rate observed after 8 weeks remains a critical challenge for long-term studies.

## 2.3 | Aminopeptidase A model

Aminopeptidase A (APA) is a hydrolase present in mouse kidneys, notably on the membrane of the glomerular visceral epithelium and the brush border of the proximal tubules. It participates in the metabolic regulation of angiotensin II. Researchers utilized the fusion of Lou rat spleen cells from the brush border membrane of the kidneys of immunized mice with mouse myeloma cells to screen and obtain the monoclonal antibody ASD-4 targeting APA, belonging to the IgG1 subclass. The study found that after a single intravenous injection of purified ASD-4 into normal BALB/c mice, its binding distribution and pathophysiological effects were observed. The results showed that ASD-4 rapidly bound to the glomerular capillary wall, followed by a membrane-like granular distribution, accompanied by binding to the brush border and basolateral membrane of the proximal tubules, leading to severe albuminuria that was dose-dependent and lasted for at least 16 days. Microscopically, podocyte foot processes were significantly fused, but there was no obvious inflammatory cell infiltration or complement deposition, suggesting that this monoclonal antibody can induce severe proteinuria without significant immune inflammatory reactions. This antibody also significantly inhibited APA enzyme activity, indicating that its mechanism of action may be related to interfering with the local RAS in the kidneys.[63]

Another study has shown that injection of anti-APA monoclonal antibody can cause changes in podocyte cytoskeleton proteins CD2AP and Podocin.[64] Therefore, the mechanism of ASD-4-mediated proteinuria may involve two aspects: First, antibody binding inhibits APA activity, leading to ineffective degradation of angiotensin II, enhancing its impact on glomerular hemodynamics and causing increased permeability of the filtration barrier[63]; second, the antibody directly interferes with the structure and function of podocyte foot processes, disrupting the integrity of the filtration barrier and leading to protein leakage.[64]

The advantage of this model is that a single intravenous injection can rapidly induce proteinuria; however, its shortcomings include short duration, poor stability, and lack of other characteristic pathological features of IMN beyond immune complex deposition.

## 2.4 | Anti-dipeptidyl peptidase IV model

Dipeptidyl peptidase IV (DPPIV), also known as gp108, is another major antigen of Fx1A localized at the tubular brush borders. DPPIV is expressed by podocytes and can be detected in brush border extracts of proximal renal tubular epithelial cells and circulating serum. Following intraperitoneal injection of DPPIV-immunized rabbit serum into 6-week-old Lewis rats, rabbit IgG deposits in the glomerular capillaries for 4–8 h, and proteinuria occurs within 8 h. Two days later, proteinuria peaks and then rapidly declines.[65] The study indicates that the mechanism by which anti-DPPIV antibodies directly bind to glomerular membrane antigens to induce proteinuria may be related to antibody interference with the interaction between DPPIV and extracellular matrix (such as collagen), rather than through complement activation or inflammatory cell infiltration. Unlike human IMN and megalin-induced Heymann nephritis models, proteinuria and IgG deposition occur in the absence of glomerular C3 deposition, and this method induces a shorter appearance of urinary protein and faster disappearance of IgG. Therefore, this is not an ideal animal model for human IMN.

## 2.5 | Anti-podocyte nephritis (APN) model

In 2007, Meyer et al.[66] successfully induced severe proteinuria in mice by intravenously injecting rabbit polyclonal anti-mouse podocyte antibodies following pre-immunization with Freund's adjuvant. Their results showed that antibody-treated mice had the highest proteinuria level on day 10, accompanied by the deposition of IgG and complement in the glomeruli. The number of podocytes significantly decreased, whereas the number of T cells increased in the glomeruli. IgG and complement C3 were linearly deposited in the glomeruli, indicating that immune complex formation was associated

with podocyte injury. Nevertheless, despite complement depletion, proteinuria still occurred, suggesting that complement activation is not necessary for proteinuria formation in this model. In 2011, their team studied immune-mediated podocyte nephropathy and nephrotic syndrome caused by subepithelial immune complexes. After injection of polyclonal sheep anti-mouse podocyte antibodies, the mice exhibited typical symptoms of nephrotic syndrome, including severe edema, proteinuria, hypoalbuminemia, hypercholesterolemia, and hypertriglyceridemia.[67] Proteinuria showed a biphasic pattern, with a significant initial increase in urinary protein followed by a second significant increase on day 10. Histological examination showed that the podocytes were swollen, and electron microscopy revealed that podocyte foot processes disappeared in 60%–80% of podocytes; however, no damage to the GBM occurred. In addition, the study identified 20 potential podocyte antigens including membrane-bound proteins and cytoskeleton-related proteins such as Annexin-3 through affinity chromatography combined with mass spectrometry technology. Using these data, they successfully constructed a reproducible model of immune-mediated podocyte injury and observed the formation of subepithelial immune complex. Nevertheless, they also found that the mechanism of podocyte damage appeared to be independent of the complement system, as mice lacking complement C3 also exhibited podocyte damage and proteinuria.

In summary, this mouse model is a complement-independent, anti-podocyte antibody-mediated subepithelial immune complex formation mouse model, revealing the involvement of multiple podocyte antigens and new insights into the immunopathological process. It provides a powerful tool for studying the mechanism of podocyte injury and screening potential therapeutic targets.

## 2.6 | Collagen fragment (α3NC1) model

The non-collagen domain 1 of the α3 chain of type IV collagen (α3NC1) is a normal component of GBM and has been shown to be expressed in mouse podocytes.[68,69] Zhang et al.[70] established a mouse model of membranous nephropathy based on the non-collagen domain 1 fragment of immune α3(IV) collagen. This model induces typical nephrotic syndrome manifestations in DBA/1 mice and their isogenic strains lacking activating Fcγ receptors.[70] Proteinuria begins to appear 6–7 weeks after α3NC1 immunization, and after 9–13 weeks, the disease enters the clinical stage, with severe glomerular crescent formation, extensive renal tubular interstitial damage, and extensive macrophage infiltration, accompanied by massive proteinuria, GBM thickening, and podocyte damage.[70,71] The mechanism of this model may involve the formation of plant antigens through a mechanism similar to that of C-BSA. Its renal pathological changes are basically similar to those of human IMN: under electron microscopy, subepithelial immune complex deposition, GBM thickening, and podocyte foot process disappearance are observed. Immunofluorescence reveals the deposition of mouse IgG, C3, and C5b-9. Studies have found that the pathological damage is mainly mediated by subglomerular subepithelial immune complex deposition and complement activation, rather than Fcγ receptor-mediated inflammatory cell activation.

Another study found that although both wild-type mice and mice deficient in factor B were capable of forming subepithelial immune complexes in the glomeruli and exhibited similar IgG deposition, mice deficient in factor B did not develop proteinuria. In mice deficient in C5, proteinuria was reduced but not completely eliminated, suggesting that the alternative pathway may be necessary for pathogenic complement activation in this model. Inhibiting the alternative pathway, especially factor B, may effectively block complement-mediated kidney damage, which is superior to simply inhibiting C5.[72]

The main advantage of this model is that it can better simulate the pathological changes in human MN-like disease. In addition, because of easy access to mouse genome data combined with genetic engineering technology, specific transgenic mouse strains can be constructed, which can be used to explore the mechanism and treatment of MN-like disease at the molecular biology and targeted drug levels. However, this model has limitations in the selection of animal strains. In previous studies, DBA/1 mice immunized with α3(IV) NC1 protein for 11 weeks developed pathological changes with crescentic glomerulonephritis. C57BL/6 and AKR mice developed a chronic disease course, resulting in kidney damage comparable to that in DBA/1 mice within 6 months. The NOD mice showed almost no significant pathological changes.[73] Therefore, mice with a DBA/1 background alone have a high success rate, and other mouse strains are not recommended for establishing such models.

## 2.7 | Anti-angiotensin-converting enzyme (ACE) model

In normal pig kidneys, angiotensin-converting enzyme (ACE) is strongly expressed in multiple structures, including the brush border of the proximal renal tubules and the epithelium of the glomerular capillaries. This is in contrast to the situation in which ACE is not detected in other animal models, such as rabbits, rats, and mice. By injecting heterologous antibodies targeting angiotensin-converting enzyme (ACE) into miniature pigs, a diffuse subepithelial immune complex deposition resembling human IMN was successfully induced in the glomeruli, revealing the role of ACE as an antigen on the surface of podocyte in the glomeruli.[74] The study found that after injection of anti-ACE antibodies, immune complexes rapidly formed on the glomerular capillary wall, and these immune complexes co-localized with ACE, indicating that the immune complexes were locally formed on the surface of podocytes rather than deposited in circulation. The formation of these immune complexes was accompanied by damage to the brush border of the proximal convoluted tubule and morphological changes in cells, suggesting that the anti-ACE antibody-mediated immune response may be involved in the mechanism of kidney damage. However, given the lack of anti-brush border antibodies and primary tubular damage in human IMN,

this model may only partially reflect the pathogenesis of human diseases. This study revealed the potential role of anti-ACE antibodies in the pathological formation of glomerular immune complexes. The deposition of immune complexes and complement activation may promote damage to the glomerular filtration barrier, thereby affecting renal function.

This model has the advantages of a short modeling period and a long subepithelial immune complex deposition time; however, it is not ideal because it differs from the primary pathogenesis of IMN.

## 3 | HUMAN PODOCYTE TARGET ANTIGEN-RELATED ANIMAL MODELS

### 3.1 | THSD7A-related model

THSD7A is the second most important target antigen of podocytes and is involved in IMN. It is highly expressed not only in human podocytes as PLA2R1, but also in rodent podocytes.[75] In 2016, Tomas et al.[76] injected mice with serum containing anti-human THSD7A (hTHSD7A) antibodies and demonstrated that anti-hTHSD7A autoantibodies could specifically bind to mouse THSD7A (mTHSD7A). Mice injected with serum containing anti-THSD7A antibodies developed significant proteinuria on day 3. Proteinuria increased further after 14 days and remained elevated for 70 days. Granular colocalization of human IgG-mTHSD7A and immune complex deposition were observed in the subepithelial portion of the glomerular filtration barrier. In addition, immunofluorescence staining analysis showed granular accumulation of complement C3 under the epithelium. Thus, this was the first described animal model involving podocyte antigen associated with human IMN. Another study found that patients with membranous nephropathy exhibit significantly elevated expression of anti-THSD7A antibodies and related lectin pathway components such as mannose-binding lectin (MBL), and MBL-associated serine protease 1/2. When serum containing anti-THSD7A antibodies was injected into mice, it activated the lectin complement pathway, leading to increased proteinuria, impaired renal function, and typical IMN pathological changes.[77]

In 2017, Tomas et al.[78] successfully induced specific antibodies against THSD7A human and murine homologues by immunizing experimental rabbits with mTHSD7A and hTHSD7A cDNA. After intravenously injecting 1.4 mg of anti-THSD7A IgG antibody into BALB/c mice, they observed typical clinical manifestations of nephrotic syndrome, including significant proteinuria, edema, and hyperlipidemia. At 14 days after injection, immunohistochemical and ultrastructural analysis of IgG and THSD7A revealed the typical characteristics of IMN. However, when researchers immunized C57BL/6, DBA/J1 mice, and SD rats with the same dose, no similar clinical manifestations were observed, and there was no significant proteinuria. Although only a few animal strains can be used, this model has clear advantages over previous models. Specifically it overcomes the deficiency of previous models lacking common target antigens in human IMN and avoids the deposition of tubular immune complexes, as observed in models such as the HN model.

### 3.2 | PLA2R-related model

#### 3.2.1 | Mouse PLA2R1 (mPLA2R1)-related mouse model

PLA2R is a glycoprotein located on podocytes in healthy humans and is a type I transmembrane receptor belonging to the mannose receptor family.[79,80] A previous study detected PLA2R in the immunoprecipitates of 70%–80% of patients with IMN and identified it as the primary target antigen of IMN.[20–22] Therefore, the establishment of animal models of PLA2R-related IMN is essential to better understand the pathophysiological mechanisms of human PLA2R-related IMN. However, PLA2R has not been detected in the glomeruli of healthy rodents or rabbits, and the lack of PLA2R expression in mouse podocytes has hindered the establishment of PLA2R-related animal IMN models.

In 2020, Meyer-Schwesinger et al.[81] constructed a transgenic mouse strain capable of expressing full-length mPLA2R1 on podocytes. They transferred rabbit anti-mPLA2R1 antibody into mice and found that only those receiving anti-mPLA2R1 IgG showed significant antibody binding and complement C3 aggregation on the GBM, leading to complement activation, proteinuria, hypercholesterolemia, and histopathological signs of MN. This model, which represents the first mPLA2R1 transgenic mouse model, provides a valuable system for further in-depth research on the pathogenic mechanisms of IMN and lays the foundation for the successful establishment of human PLA2R1 (hPLA2R1) transgenic animal models.

#### 3.2.2 | Human PLA2R1 (hPLA2R1)-related model

Tomas et al.[82] addressed the issue of the lack of hPLA2R1 expression in commonly used experimental animal models by generating a knock-in transgenic mouse model that specifically expressed full-length hPLA2R1 on podocytes and successfully reproduced the human IMN phenotype. This represents an important step toward the establishment of a PLA2R-related human IMN mouse model. The results showed that within 3 days of birth, hPLA2R1 was strongly expressed in the podocyte membrane and cytoplasm of mature glomeruli in hPLA2R1-positive mice. Although the integrity of the glomerular filtration barrier was confirmed by electron microscopy 7 days after birth, circulating anti-hPLA2R1 antibodies were detected in some mice at 3 weeks of age. These antibodies specifically recognize cysteine-rich domains and the C-type lectin-like domain, which are epitopes recognized by human anti-PLA2R1 antibodies.[83,84] Notably, the antibody levels increased over time, with antibodies beginning to appear at 3 weeks of age and detected in all hPLA2R1-positive mice at 6 weeks of age, accompanied by a decrease in serum albumin, an

increase in serum lipids, and an increase in urea. At 5–6 weeks of age, hPLA2R1-positive mice began to develop ascites and their health deteriorated. At 12 weeks of age, all animals exhibited severe glomerular and tubulointerstitial damage, including typical granular IgG deposition in the glomeruli under immunofluorescence microscopy and subepithelial electron-dense deposition under electron microscopy. These results indicated that the mouse model could effectively simulate the pathological characteristics and immune mechanisms of human membranous nephropathy. In addition, hPLA2R1 transgenic mice developed MN only on a background of mature B and T lymphocytes. If mated with $Rag2^{-/-}$ mice, which lack mature B and T cells, hPLA2R1-positive mice deficient in mature lymphocytes failed to develop anti-hPLA2R1 antibodies and developed no symptoms such as proteinuria, which emphasizes the crucial role of the antibody-mediated autoimmune response in the development of MN. Therefore, this model provides an important basis for exploring antigen-specific therapeutic strategies and helps to deepen understanding of the autoimmune pathogenesis of membranous nephropathy.

In the mPLA2R1 transgenic mouse model constructed by Meyer-Schwesinger et al.[81] mPLA2R1 overexpression in podocytes in the hPLA2R1 transgenic mouse model constructed by Tomas et al.[82] did not lead to the formation of an autoantibody against mPLA2R1 owing to the endogenous expression of mPLA2R1 in the thymus, which maintains tolerance. In contrast, the hPLA2R1 transgenic mouse model might have produced a large number of anti-hPLA2R1 antibodies within a short period because the model was intentionally designed to express hPLA2R1, which is highly specific to podocytes and not expressed in other kidney cells or the thymus. Ectopic expression of hPLA2R1 on the surface of podocytes is continuously recognized by the immune system as a new antigen. Due to a lack of central tolerance to hPLA2R1 in the thymus, this recognition triggers the production of circulating anti-hPLA2R1 antibodies. These antibodies rapidly bind to the hPLA2R1 antigen on the podocyte surface, leading to the formation of immune deposits and the appearance of large amounts of proteinuria. This hPLA2R1 transgenic model may be the fastest IMN animal model reported to date. However, rapid disease progression limits its use in long-term studies and detailed analyses of its underlying pathophysiological mechanisms.

### 3.2.3 | Chimeric PLA2R1 (chPLA2R1)-related model

To address the loss of immune tolerance and rapid disease progression observed in hPLA2R1 transgenic mice, Tomas et al.[85] generated transgenic mice expressing chPLA2R1. This chimeric protein consists of three hPLA2R1 domains (cysteine-rich, fibronectin type II, and C-type lectin-like domain1) and seven mPLA2R1 domains (C-type lectin-like domain2-8), which are specifically expressed in podocytes.[86,87] Compared with wild-type mice, these mice were born healthy and showed no significant lesions in the renal tubules or glomeruli during the first 6 months. Subsequently, researchers have successfully induced the production of anti-hPLA2R1 antibodies and typical renal manifestations by actively immunizing mice with hPLA2R1. The mice immunized with hPLA2R1 exhibited significant proteinuria, decreased serum albumin, and increased serum cholesterol and triglyceride levels, which are typical characteristics of nephrotic syndrome. Histological analysis showed GBM thickening, loss of foot process, and subepithelial electron density deposits under the electron microscope in immunized mice. These results indicate that chPLA2R1-positive mice can develop typical pathological features of MN associated with hPLA2R1 after immunization with hPLA2R1. Further research revealed that the complement system was activated in the kidneys of immunized mice, particularly with significant deposition of components C1q and C4d from the classical and lectin pathways. To investigate the role of complement in the disease, researchers crossed chPLA2R1-positive mice with knockout mice lacking the key complement component C3. The results showed that mice deficient in C3 exhibited significantly reduced proteinuria and nephrotic syndrome after immunization, suggesting that the complement system plays a crucial pathogenic role in the pathogenesis of PLA2R1-related IMN.

In summary, this model not only exhibits typical histological characteristics of nephrotic syndrome and membranous nephropathy, but also reveals the crucial pathogenic role of the complement system, especially the C3 component, in disease pathogenesis. This innovative model provides an important platform for deepening our understanding of the pathogenesis of PLA2R1-related membranous nephropathy and developing targeted treatments.

### 3.2.4 | PLA2R1-related minipig models

Recently, Reinhard et al.[88] found that human anti-PLA2R1 antibodies can bind to PLA2R1 in miniature pigs. When antibody-positive plasma from PLA2R1-associated MN patients was transferred to minipigs, the pigs developed histological features similar to early human MN, including activation of the complement cascade and low levels of proteinuria. Colocalization of human IgG and porcine C3 was observed by immunofluorescence staining, and electron microscopic observations revealed subepithelial electron-dense immunodeposition in the glomeruli of miniature pigs. Proteomic analysis showed that the levels of complement system proteins in the glomeruli of the experimental miniature pigs increased significantly and that proteinuria occurred during the experiment, which was not observed in the control group, indicating that human anti-PLA2R1 antibodies were pathogenic in miniature pigs, although only mild proteinuria was observed. These findings not only reveal the ability of human anti-PLA2R1 antibodies to induce MN in miniature pig models but also validate their feasibility as a model for studying autoimmune diseases and provide new insights into the pathogenesis of IMN.

These findings lay a solid foundation for the further exploration of the pathogenesis and potential treatment strategies for PLA2R1-related IMN and demonstrate significant clinical value.

## 4 | CONCLUSIONS AND PROSPECTS

The etiology and pathogenesis of IMN have not yet been fully elucidated. Its complex pathological process involves the dynamic interaction of multiple factors, including genetic susceptibility (e.g., single-nucleotide polymorphisms (SNPs) in target antigen genes),[89–91] environmental exposure (e.g., PM2.5),[92–94] and immune regulation imbalance (including complement activation and immune cell interactions).[95,96] In recent years, animal models have made progress in mimicking the pathogenesis of IMN (Figure 4). In particular, the establishment of target antigen-related animal models, such as THSD7A and PLA2R models, represents a major breakthrough in the study of IMN.[97,98] Nevertheless, these models still face challenges in fully replicating the pathogenesis of IMN. In addition, due to the complex construction technology and high cost of PLA2R-related transgenic animal models, only a small number of researchers currently use them. Classical models such as PHN and C-BSA remain the preferred choice for most researchers.

To further develop animal IMN models, researchers should strive for breakthroughs in both "accessibility" and "precision." On the one hand, technological improvements, such as mass production of genetically engineered mice, are required to facilitate an easier construction of models, making them more accessible to a broad range of researchers and promoting their application to in-depth studies focusing on specific etiologies or mechanisms of IMN. On the other hand, the models should be constructed to more closely resemble the characteristics of patients with IMN (e.g., high prevalence of middle-aged and elderly patients or higher proportion of males than females) while considering genetic factors, environmental exposures, and molecular mechanisms (e.g., epitope spreading,[99–101] complement activation pathway[36,95,102] and immune cell infiltration[103–105]). Moreover, artificial intelligence (AI) and machine learning (ML) are profoundly transforming the research paradigm of experimental animal models. By integrating multi-omics data, AI can optimize experimental design, significantly enhancing the predictive power of preclinical models and clinical translation efficiency.[106,107] For example, researchers have developed a machine learning platform that successfully predicted the transporter function of the SLC25A45 gene and verified its regulatory effect on carnitine synthesis and fatty acid oxidation in knockout mice[108]; in addition, ML has been utilized to optimize drug administration regimens in IMN, such as tailoring rituximab therapy, significantly improving therapeutic efficacy.[109] These advances are accelerating the development of new therapies in precision medicine, driving preclinical research in a more efficient, scalable, and personalized direction.

In conclusion, an effective strategy for future IMN animal model research lies in a multidimensional framework that integrates "genetic-environmental-immune" information with advanced omics and artificial intelligence technologies. This approach allows for deeper insights into the cascade mechanisms of molecular networks under specific etiologies, such as the pathogenesis of target antigen-associated IMN driven by the synergy of SNPs mutations in the context of PM2.5 exposure. The combination of these technologies not only provides a theoretical basis for precise diagnosis and treatment but also promotes medical research from "disease simulation" to "individualized treatment".

### AUTHOR CONTRIBUTIONS

**Qiuying Liu**: conceptualization; project administration; writing – original draft. **Huixian Bian**: writing – original draft. **Jianhua Liu**: resources. **Lina Wu**: resources. **Zhijie Zhang**: resources. **Hongli Zhao**: writing – review and editing. **Xiaosong Qin**: conceptualization; resources; writing – review and editing.

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### CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### ETHICS STATEMENT

Ethical approval for the clinical samples was obtained from the Ethics Committee of Shengjing Hospital, China Medical University (No. 2021PS885K). The animal study was approved by the Ethics Committee of Shengjing Hospital, China Medical University (IACUC No. 2023PS678K(T1)).

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