Complexities of the glomerular basement membrane

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Abstract | The glomerular basement membrane (GBM) is a key component of the glomerular capillary wall and is essential for kidney filtration. The major components of the GBM include laminins, type IV collagen, nidogens and heparan sulfate proteoglycans. In addition, the GBM harbours a number of other structural and regulatory components and provides a reservoir for growth factors. New technologies have improved our ability to study the composition and assembly of basement membranes. We now know that the GBM is a complex macromolecular structure that undergoes key transitions during glomerular development. Defects in GBM components are associated with a range of hereditary human diseases such as Alport syndrome, which is caused by defects in the genes *COL4A3*, *COL4A4* and *COL4A5*, and Pierson syndrome, which is caused by variants in *LAMB2*. In addition, the GBM is affected by acquired autoimmune disorders and metabolic diseases such as diabetes mellitus. Current treatments for diseases associated with GBM involvement aim to reduce intraglomerular pressure and to treat the underlying cause where possible. As our understanding about the maintenance and turnover of the GBM improves, therapies to replace GBM components or to stimulate GBM repair could translate into new therapies for patients with GBM-associated disease.

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Basement membranes are condensed regions of extracellular matrix (ECM) that underlie continuous layers of cells. They are found in and around most tissues; however, the full scope of their function is unknown. The evolutionary appearance of basement membranes coincided with the emergence of metazoa and, as such, basement membranes are likely to be a fundamental requirement for multi-cellular life[1]. In metazoa, basement membranes are integral regulators of tissue shape, size and health and, when disrupted, they cause a range of diseases. Major and conserved basement membrane components include laminins, type IV collagen (collagen IV), nidogens and the heparan-sulfate proteoglycans (HSPGs) agrin, perlecan and collagen XVIII (FIG. 1). These components establish a cell-adhesive sheet that maintains normal physiology by regulating many facets of cell biology, including cell polarity, proliferation, apoptosis, survival, migration, differentiation and signalling. Thus, basement membranes have a vital role in the general maintenance of tissue homeostasis. In addition, they function as selective barriers between adjacent compartments in various tissues, including in lung alveoli, skin epidermis, gut epithelium, the placenta and the blood–brain barrier. One of the most studied basement membranes is in the kidney glomerulus. The glomerular basement membrane (GBM) separates podocytes from endothelial cells and contributes to the selectivity of the glomerular filtration barrier, which allows water and small molecules to pass into the urinary space but retains macromolecules and cells within the circulation. Indeed, the presence of haematuria or proteinuria is indicative of glomerular barrier dysfunction. Additional matrix compartments coexist within and around the glomerulus, namely the basement membrane of the Bowman capsule, which is produced by the parietal epithelial cells and encapsulates the glomerular tuft, and the mesangial matrix, which is a loose interstitial matrix produced by mesangial cells that connect capillary loops in the glomerulus (FIG. 2). Although the mesangial matrix contains major basement membrane components, it does not exist as a sheet-like structure.

The glomerular filtration barrier and the contribution of the GBM to barrier function was first investigated using ultrastructural and biochemical analyses[2,3]. These studies determined the three-layered structure of the barrier and identified major components but could not provide insights into the global composition of the GBM. More recently, unbiased approaches, such as mass spectrometry-based proteomics, have shown that the composition of basement membranes is tissue specific and revealed that the GBM is distinct from other basement membranes[4]. For example, we now know that specific GBM components, including the α3α4α5 network of collagen IV, which predominates in the mature

Key points

- The glomerular basement membrane (GBM) is composed of many unique components that are likely to be important for appropriate GBM function.
- The application of new imaging and proteomics technologies is enabling greater insight into GBM organization in health and disease.
- The composition of the GBM changes during glomerulogenesis to permit proper development and filtration function.
- The GBM is a major contributor to the size selectivity of the glomerular filter.
- Causes of GBM-associated disease include primary genetic defects in basement membrane components and damage secondary to autoimmune and metabolic diseases, leading to abnormal synthesis and/or turnover of GBM proteins.
- Novel pharmacological and genome-editing approaches might facilitate basement membrane repair and treatment of GBM-associated disease.

GBM and differs from the α1α1α2 network found in most basement membranes, are crucial for glomerular barrier function. Similarly, minor components of the GBM, such as nephronectin and fibulin 1, have restricted tissue localization and likely provide important glomerular-specific functions. Unravelling the underlying reasons for these differences will improve our understanding of the diversity of basement membrane functions and potentially lead to new targeted therapeutic approaches. In this Review, we describe the methods that have enabled the investigation of GBM composition and structure and note how these studies have provided insights into GBM composition and assembly, its developmental transitions and its role in glomerular filtration. We also describe GBM-associated diseases and discuss current and potential future treatments for these disorders.

GBM composition and assembly

Hierarchical assembly

During development, basement membrane assembly proceeds in a hierarchical fashion. In vitro analyses in embryoid bodies demonstrate that basement membrane assembly initiates with the secretion of laminin heterotrimers by endodermal cells into the extracellular space[5]. Polymerization of this laminin generates an immature basement membrane, which consists of a lattice-like sheet that binds to specific receptors on the surface of cells, including integrins, discoid domain receptors (DDRs), α-dystroglycan and syndecans[6]. Maturation of the basement membrane follows with the deposition and polymerization of collagen IV, which establishes a reticular network on the laminin sheet. Formation of the GBM involves the fusion of two basement membranes that are independently generated by endothelial cells and podocytes during glomerulogenesis[7]. Thus, the mature GBM consists of two laminin sheets on either side of a thick, central collagenous network. Most basement membranes are 50–100 nm in width. However, the GBM is much thicker, at 330–460 nm in humans[8] and 50–300 nm in rodents[9,10]. The next stage in basement membrane assembly involves the deposition of the HSPGs agrin and perlecan. In the mature GBM, agrin is the major HSPG[11] expressed by podocytes and tethers laminins to cell surface receptors to augment adhesion. Agrin also connects laminin and collagen IV polymers via nidogen[12]. Glycosaminoglycan (GAG) side chains, which attach to agrin and the other major proteoglycans (perlecan and collagen XVIII), can bind water molecules, which probably contributes to the thickness of basement membranes in living animals[13,14].

Evidence for this hierarchical assembly of basement membranes comes from analysis of protein localization profiles during development in model organisms and the observation of embryonic lethality with the loss of basement membrane components. For example, an elegant genetic study used time-lapse imaging of endogenous GFP-tagged basement membrane proteins in *Drosophila* to demonstrate the temporal hierarchy of basement membrane assembly[15]. This study showed that the laminin-α and laminin-β subunits are expressed in the early *Drosophila* embryo, followed by expression of collagen IV and perlecan. This sequence of events fits the dynamic expression profiles of basement membrane orthologues in embryos of *Caenorhabditis elegans*[16,17] and aligns with our understanding of basement membrane development from knockout studies in vertebrate models. In global laminin-deficient mice, basement membrane assembly is not initiated and embryos do not survive later than embryonic day (E) 5.5 (REF.[18]). By contrast, global depletion of collagen IV in mice causes lethality later, at E10.5–11.5 (REF.[19]). This lethality is not due to a lack of basement membrane assembly but is rather caused by impaired stability as evidenced by the irregular appearance and ruptures of basement membranes throughout the embryo. Taken together, these findings support the hypothesis that the hierarchy of laminin sheet formation followed by the assembly of collagen IV and then HSPGs, as demonstrated in *Drosophila*, may be conserved in vertebrates.

Basement membrane components

Laminins. Laminins are the major non-collagenous (NC) components of basement membranes and exist as heterotrimers composed of α-chains, β-chains and γ-chains. In the mature GBM, the major isoform is laminin α5β2γ1 (also known as laminin 521) (FIG. 1). Globular laminin domains exist at the C terminus of the α-chain of laminin and bind to the α-subunit of integrin receptors on the surface of cells. In podocytes, the major integrin receptor is α3β1, which interacts directly with laminin α5 via laminin G-like (LG) domains. As the laminin sheet assembles, the N-terminal arms of the α-chain, β-chain and γ-chain interact to enable polymerization. The laminin chain arms also contain variable numbers of laminin-type epidermal growth factor-like domains, which are important for the binding of laminin to nidogen[20,21] and may act as a bridge to the collagen IV network, although this link has been disputed[22].

Collagen. Collagen IV is a major component of all basement membranes and is thought to provide tensile and compressive strength. It is encoded by six genes (*COL4A1–6*) in vertebrates, which form three triple helix trimers, α1α1α2, α3α4α5 and α5α5α6. Unlike laminins, the evolutionary appearance of collagen IV is restricted to metazoa[1], suggesting that they were fundamental in the emergence of multicellularity. Collagen IV chains are large proteins that consist of a short, N-terminal

Laminins
A family of glycoproteins that exist as heterotrimers with α, β and γ subunits. Laminin heterotrimers polymerize in the extracellular space to form a sheet that is essential for the formation and function of basement membranes.

Type IV collagen
Encoded for by six genes in vertebrates (*COL4A1–COL4A6*), type IV collagen provides a scaffold and is present in basement membranes. Type IV collagen proteins form three heterotrimers (α1α1α2, α3α4α5 and α5α5α6) that establish networks in the extracellular space.

Nidogens
Formerly known as entactin, nidogens are dumbbell-shaped proteins found in all basement membranes. Nidogen functions to connect collagens and laminins in the matrix.

Heparan-sulfate proteoglycans
(HSPGs). A large family of extracellular and membrane-attached molecules formed by a major protein that contains one or more covalently attached heparin sulfate glycosaminoglycan chains. Examples of HSPGs in basement membranes include collagen XVIII, agrin and perlecan.

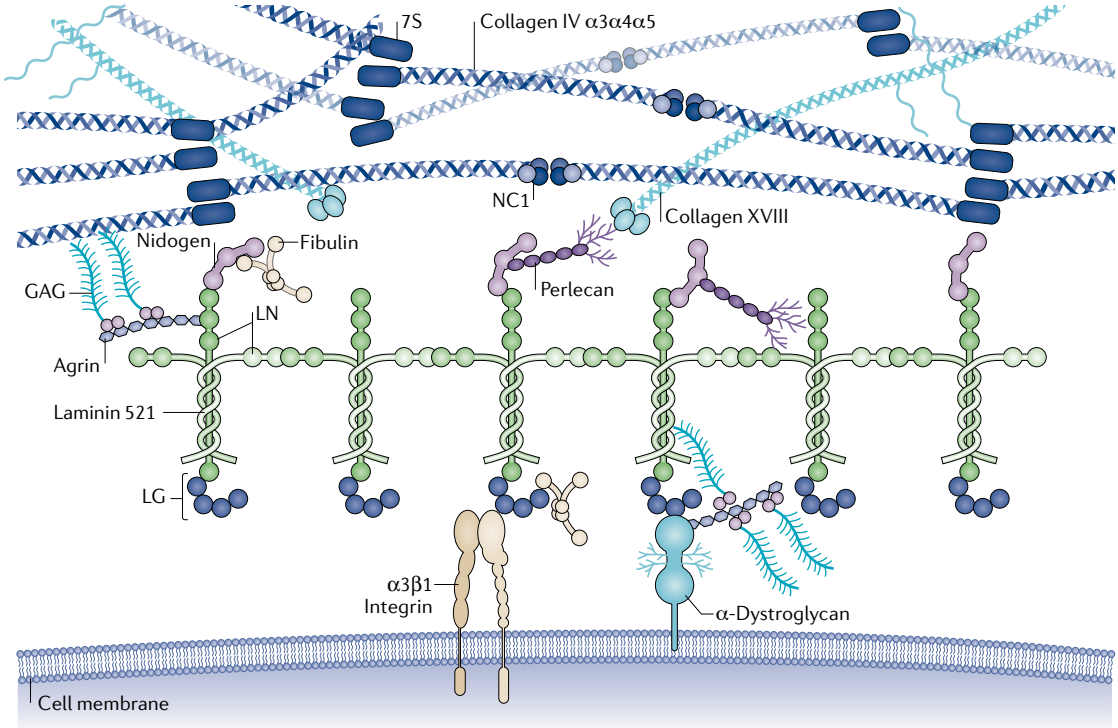

Fig. 1 | **Major components of the GBM.** The major components of all basement membranes include laminins, collagen IV, nidogens and the heparan sulfate proteoglycans agrin, perlecan and collagen XVIII. The glomerular basement membrane (GBM) is enriched in the α3α4α5 isoform of collagen IV as well as in laminin 521, which interacts with cell-surface receptors α3β1 integrin and α-dystroglycan on podocytes and endothelial cells. Minor components such as fibulin 1 are also present in the GBM. 7S, collagen IV 7S domain; GAG, glycosaminoglycan; LG, laminin G-like domain G; LN, laminin N-terminal domain; NC1, non-collagenous domain.

~25 amino acid 7S domain, a long ~1,400 amino acid collagenous domain and a C-terminal ~230 amino acid NC domain. Interestingly, the central collagenous domain (which contains the Gly-X-Y sequences that permit triple helix formation) also contains about 20 short NC (or interruption) domains, which are thought to provide molecular flexibility[23]. Once trafficked out of the cell, the collagen IV chains assemble to form a polymer adjacent to the laminin sheet. The collagen IV network exists as a lattice with interprotomer binding via homophilic covalent crosslinking either between four neighbouring N-terminal 7S domains or between two adjoining NC domains at the C terminus. Of note, the assembly of collagen IV polymers (specifically the oligomerization of NC domains) only occurs outside cells as this process is dependent on chloride ions, which are more abundant in the extracellular space[24]. In the extracellular space, covalent crosslinking at the 7S domains is mediated by lysyl oxidase-like 2 (LOXL2), creating a dodecamer that is a hallmark of collagen IV polymerization[25]. Covalent crosslinking of opposing NC domains occurs via a sulfilimine bond[26]. Interestingly, the formation of this bond is the only known requirement for ionic bromide in animals and involves the haem peroxidase, peroxidasin[27].

Heparan sulfate proteoglycans. HSPGs are thought to provide basement membranes with a negative charge and connect laminin and collagen IV networks, although their roles in GBM function seem to be dispensable as their depletion had no effect on glomerular development or function[28,29]. The three major HSPGs expressed in the GBM during development are perlecan, agrin and collagen XVIII. Agrin is the primary HSPG synthesized by podocytes[29]. The N-terminal domain of agrin binds to laminin-γ1 to stabilize GAG side chains in a regular distribution within the GBM, whereas its C terminus binds to cell-surface receptors such as α-dystroglycan and αvβ1 integrin to mediate cell-matrix adhesion and signalling[30]. Perlecan and collagen XVIII are abundant in most glomerular matrix compartments during glomerulogenesis before becoming restricted to the mesangial matrix and to the Bowman capsule in the mature glomerulus[29,31]. Perlecan is found in the mature GBM but only on the subendothelial side[11] even in agrin-knockout mice[29]. Collagen XVIII is a hybrid collagen–HSPG that is found on both sides of the mature GBM as a polarised molecule but is more abundant in the mesangial matrix and the Bowman capsule basement membrane[32]. Mice deficient in *Col18a1* have impaired renal excretion and show loss of glomerular stiffness, mild mesangial expansion and podocyte foot process effacement but do not show evidence of ultrastructural GBM defects[32,33]. Of note, however, microindentation studies have shown that the GBM in *Col18a1*-deficient mice is 30% softer than that of control mice[30], suggesting a role for collagen XVIII in the mechanical properties of the GBM.

Collagen XVIII
A heparin-sulfate proteoglycan that exists as a homotrimer in basement membranes. It has an important role in maintaining basement membrane integrity by mediating signalling (for example, canonical Wnt signalling) and cell–matrix interactions (such as with integrin and VEGF receptors via a C-terminal endostatin domain).

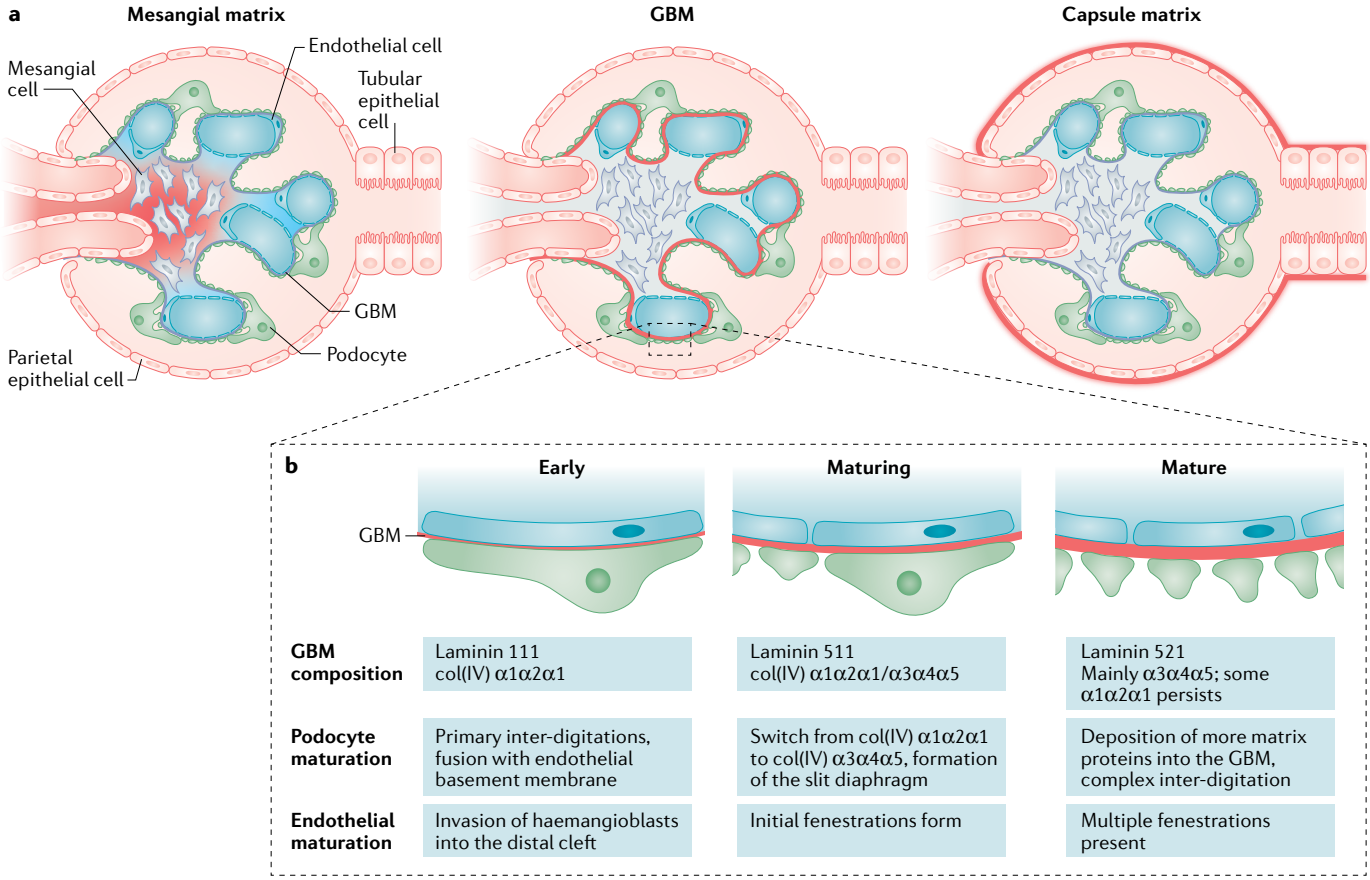

Fig. 2 | **Glomerular matrix compartments and transition of basement membrane components during GBM development. a** | The adult glomerulus contains three major distinct matrix compartments (red): the mesangial matrix, which is synthesized by mesangial cells to support the capillary loops; the glomerular basement membrane (GBM), which is synthesized by podocytes and endothelial cells; and the capsule matrix, which is synthesized by the parietal cells of the Bowman capsule. **b** | The composition of the GBM changes throughout development, with a switch in the composition of early laminin heterotrimers and the collagen IV network, coinciding with maturation of podocytes and the endothelium. col(IV), collagen IV.

Nidogens. Nidogens are dumbbell-shaped sulfated monomeric glycoproteins that bind to collagen IV, HSPGs and laminin-γ1 (REFS[34,35]). Nidogen 1 and nidogen 2 are both found in the GBM[36], with nidogen 1 predominating in the adult GBM[4,37]. Nidogens are localized close to the collagen IV network[38]; they were initially thought to act as crosslinkers for laminins and collagen IV[34,35] and were therefore thought to be essential for basement membrane assembly. Although nidogens are crucial for basement membrane integrity in the lungs and heart, one study found that both nidogens were dispensable for kidney development and GBM assembly in mice, suggesting that nidogens have tissue-specific roles[39]. It is possible that other GBM proteins might compensate for the lack of nidogen in the kidney. Interestingly, in the skin, when collagen IV and laminin supra-structures were isolated from the dermal–epidermal basement membrane by immunomagnetic bead purification[22], the core protein of perlecan was present in the laminin network but the perlecan GAG side chains were present in the collagen IV network. Nidogens were found in both the laminin and collagen IV networks but did not form strong molecular bridges between the two polymers, suggesting that HSPGs, rather than nidogens, might act as the main bridge between laminins and collagen IV. However, as mentioned earlier, the deletion of perlecan or agrin did not affect glomerular architecture or function, suggesting that, like nidogens, HSPGs are dispensable for GBM assembly[28]. However, support for a role of nidogens in GBM assembly and function comes from a study in mice lacking the specific binding site for nidogen 1 within laminin-γ1 (the γ1III4 laminin-type epidermal growth factor-like module). These mice died immediately after birth with renal agenesis or non-functioning kidneys associated with defective glomeruli and focal GBM disruption[40]. Hence, the role of nidogens in GBM assembly is controversial and requires further investigation. To date, no genetic variants in nidogen have been associated with kidney phenotypes in humans.

Studying GBM composition and structure

New insights into GBM composition

A greater understanding of the composition of the GBM is important to elucidate the mechanisms of GBM assembly and function in development, ageing and disease. Over the past 10 years, tissue proteomics and

Plakins
A family of large binding proteins that link cytoskeletal intermediate filaments to other filaments and to junctional complexes such as desmosomes and hemidesmosomes.

genome-based bioinformatics studies have been used to generate species-specific catalogues of extracellular matrix and matrix-associated proteins within various healthy and diseased tissues. Collectively, these studies have helped to define the matrisome — the catalogue of >1,000 mammalian genes that encode matrix and matrix-associated proteins[41,42]. A strategy that involved enrichment of the matrix from isolated glomeruli from human nephrectomy kidneys combined with unbiased mass spectrometry-based proteomics[4,43] identified 144 structural and regulatory human matrix proteins, including 25 proteins known to be associated with the GBM (FIG. 3). As it is not easy to isolate the GBM from other matrix compartments in the glomerulus, these proteins include those within the mesangial matrix and the Bowman capsule basement membrane. Further investigations are therefore required to determine the abundance and role of specific proteins in the GBM.

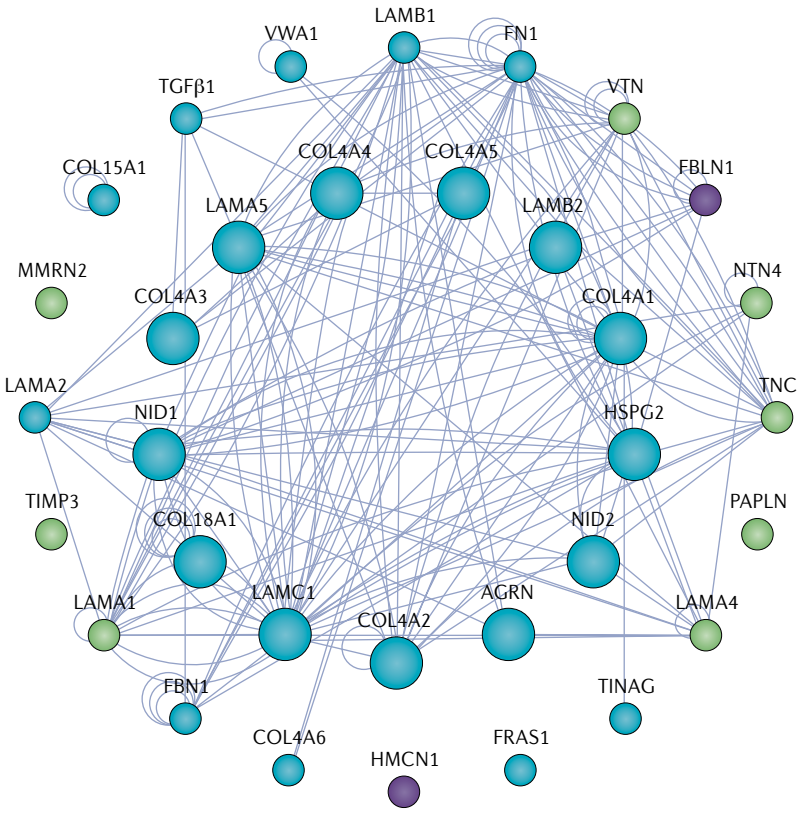

Fig. 3 | **Major and minor components of the GBM.** An interactome — that is, a protein–protein interaction network — of the major and minor components within the glomerular matrix of human and mouse can be generated with the use of mass spectrometry (MS)-based proteomics. Major components include the well-known matrix proteins (for example, collagen IV and laminins), whereas minor components include proteins more recently identified in the glomerular matrix (for example, TINAG) and/or expressed during glomerular development (for example, LAMA1 and LAMB1). The interactome shown here was constructed using STRING v11. Each node represents a protein, and connecting grey lines illustrate an interaction between connected proteins that has been experimentally verified and curated from protein interaction databases. GBM, glomerular basement membrane.

An example of a protein detected in more than one compartment of the glomerular ECM is fibulin 1, which is a secreted matrix glycoprotein found in basement membranes, microfibrils and elastic fibres. Fibulin 1 can bind to other basement membrane proteins such as nidogen 1 and laminin-α and laminin-γ chains; these interactions are important for the structural integrity of these supramolecular matrix scaffolds within tissues[44]. Fibulin 1 is found in both the mesangial matrix and the GBM, and is required for the normal development of glomerular capillaries[44,45]. Mice lacking fibulin 1 develop distended capillary loops in 10–40% of glomeruli and capillary wall malformations but do not show evidence of discrete GBM abnormalities[45]. In *C. elegans*, fibulin assembly is dependent on interaction with hemicentin via epidermal growth factor (EGF)-like domains at specific sites at which two basement membranes attach in the worm body[46,47]. Hemicentin is an evolutionarily conserved matrix glycoprotein and a member of the immunoglobulin superfamily. It is structurally similar to fibulin 1 and has been implicated in cell–cell and cell–matrix adhesion and as a mediator of mitotic cytokinesis[48,49]. In *C. elegans*, hemicentin aggregates with integrins and intracellular plakins to form a specialized linkage system named B-LINK, which mediates the adhesion of adjacent basement membranes at specific uterine attachment sites[50]. A 2020 study investigated whether a similar role in GBM assembly exists for hemicentins in mice; however, hemicentin 1 was not detected by antibody staining in the mature GBM[51]. Furthermore, global deletion of *Hmcn1* or *Hmcn2* had no apparent effect on glomerulogenesis in mice or glomerular function at 10 months of age. Although this study detected hemicentin 1 in the mesangial matrix of mature glomeruli, its role in glomerular function remains unclear.

Global proteomics analyses in healthy mice also demonstrated that glomerular matrix composition differs according to genetic background and sex and correlates with levels of albuminuria[43]. For example, levels of netrin 4, a member of the laminin superfamily, were higher in glomerular matrix extracts from FVB mice than in C57BL/J mice, which are more resistant to kidney disease. A subsequent study[52] demonstrated that full-length netrin 4 forms high-affinity equimolar complexes with laminin-γ1 through laminin N-terminal (LN) domain interactions that block laminin polymerization and basement membrane assembly. That study also found that netrin 4 disrupts preformed laminin 111 ternary node complexes in vitro in a non-enzymatic manner and exerts anti-angiogenic and antitumorigenic roles in vivo through its laminin-binding activity. A specific role for netrin 4 in GBM integrity is yet to be determined, but these studies suggest a possible causal relationship between the higher levels of netrin 4 in FVB mice and higher levels of albuminuria. The global proteomics analysis of healthy mice also showed a 60% overlap between the mouse and human glomerular matrisome as well as the existence of a shared set of known basement membrane components, including collagen IV α3α4α5, laminin 521, nidogens 1 and 2, and the HSPGs agrin, perlecan and collagen XVIII[43], supporting

Serial block-face SEM (SBF-SEM). An imaging approach that uses a scanning electron microscope equipped with an automated ultramicrotome that sequentially cuts resin-embedded samples and scans the block surface in a repetitive manner to yield a stack of aligned images with transmission electron microscopy quality. This approach provides volumetric imaging data of tissue structures in the X, Y and Z axes.

Low-vacuum SEM (LV-SEM). An electron microscopy approach that allows the scanning of non-conductive hydrous samples achieving electron microscopy resolution without prominent charging artefacts.

Helium ion microscopy An electron microscopy approach in which the beam of electrons used for scanning and surface imaging in conventional SEM is replaced by a focused beam of helium ions to minimize sample damage and obtain high magnification images of uncoated soft specimens with improved sub-nanometre resolution and high surface focus and contrast.

Stimulated emission depletion (STED). A super resolution microscopy approach that uses a double laser beam to scan multiple fluorescent markers at the same time. The first beam stimulates fluorescence whilst a second creates a light annulus superimposed on top of the first to deplete fluorescence, creating a small scanning beam below the diffraction limit of resolution.

Stochastic optical reconstruction microscopy (STORM). A super-resolution microscopy approach that uses stochastic photo-switchable fluorescent probes that are activated individually by a weak light source to emit light separately for short time intervals to produce a high-resolution image constructed point-by-point based on the precise location of each individual fluorophore.

the proposal that major GBM components are conserved across species (FIG. 3).

Podocytes and glomerular endothelial cells (GEnCs) are likely to contribute distinct GBM components during kidney development[53–55]. The use of proteomics and transmission electron microscopy to characterize the matrix composition and morphology of matrices derived from human cultured podocytes and/or GEnCs showed that only the co-culture of these two cell types jointly deposited a matrix that is molecularly and structurally similar to the glomerular matrix in vivo[56]. This finding supports the concept that the development and organization of the GBM is facilitated by crosstalk between podocytes and GEnCs.

Methods for visualization

Electron microscopy of the GBM. Advanced electron microscopy techniques have provided detailed insights into the spatial relationship between glomerular cells and the GBM. One form of scanning electron microscopy (SEM), called serial block-face SEM (SBF-SEM), has been used to study the complex ultrastructure of the glomerulus and to analyse the spatial configuration of the GBM in health and across a spectrum of kidney diseases at the nanometre scale[10,57,58]. For instance, use of SBF-SEM demonstrated invasion of podocyte foot processes into damaged areas of the GBM in various mouse models of glomerular disease[10]. Low-vacuum SEM (LV-SEM) has been used to show the presence of spikes on the GBM and subepithelial electron-dense deposits in biopsy samples from patients with membranous nephropathy[59], irregular GBM thickening and a basket-weave appearance in samples from patients with Alport syndrome[60], and GBM thinning and perforation in samples from patients with thin basement membrane nephropathy (TBMN)[60,61]. Another emerging SEM technique, called helium ion microscopy[62], has been used to reveal striking alterations on the surface of glomeruli from *Col4a3*$^{-/-}$ mice, including the deposition of long microfilaments, cytoplasmic bleb-like projections and increased numbers of prominent podocyte bridge-like processes[63]. The use of this technology to investigate basement membranes is an exciting prospect for the future.

Super resolution light microscopy. Super resolution light microscopy enables imaging of tissue structures smaller than the diffraction limit of the illumination source, with electron microscopy-level precision[64]. The best known examples of super resolution light microscopy approaches are stimulated emission depletion (STED) and stochastic optical reconstruction microscopy (STORM)[65]. A landmark study used STORM to map the topographical localization of GBM components at nanoscale resolution in healthy human and mouse. Based on antibody staining, the researchers demonstrated that the GBM has a layered structure in which collagen IV α3α4α5 trimers and nidogens are centrally located, collagen IV α1α2α1 trimers are located close to endothelial cells, and laminin 521 and agrin have their N-terminal domains facing the interior of the GBM and their C-terminal domains oriented towards the surface of endothelial cells and podocytes. They also provided insight into the disruption in GBM organization that occurs in *Col4a3*$^{-/-}$ (Alport) mice in which collagen IV α3α4α5 trimers are no longer detected and, instead, α1α2α1 trimers are spread across the GBM[38]. A subsequent study correlated molecular and structural changes identified by STORM and SEM imaging, respectively, in the podocyte actin cytoskeleton and slit diaphragms in mouse models of glomerular disease. For example, use of this approach identified loss of agrin in the sub-epithelial region of the GBM in *Lamb2*$^{-/-}$ mice that also showed podocyte injury and displacement of the slit diaphragm proteins nephrin and podocin away from the GBM[66]. STORM was also used to study the GBM integration of recombinant human laminin 521 following systemic injection in *Lamb2*$^{-/-}$*Rag1*$^{-/-}$ mice. This study reported accumulation of the recombinant laminin along the endothelial side of the GBM in these mutant mice, demonstrating the utility of super resolution light microscopy for defining molecular localization within the GBM[67].

Tissue expansion. Tissue expansion has also been used to image glomerular structures, including the GBM, slit diaphragms and podocyte foot processes, at nanoscale resolution but using conventional diffraction-limited light microscopy[68]. Several protocols exist for expanding tissue but the basic principles involve the fixation and permeabilization of samples, followed by further embedding of samples within a polymer meshwork and denaturation. The samples are then uniformly expanded up to an average of 4.5 times their original dimension, enabling the subsequent visualization of structures that were previously unresolved at a resolution of 200 nm (REFS[69,70]). Labelling with fluorescent antibodies can be performed before or after the samples are expanded. A 2018 study[71] that performed volumetric analysis and 3D reconstruction of podocyte foot processes imaged using tissue expansion and STED microscopy obtained a resolution of <20 nm within the glomerular filtration barrier and, thus, the GBM.

Decellularization approaches. Decellularization through chemical fractionation can also be used to analyse matrices by 3D imaging. In 2019, researchers published a detailed protocol for the in situ decellularization of tissues and 3D imaging of the resulting matrix scaffold[72]. With the aid of multi-photon confocal microscopy and labelling with multiple antibodies, the in situ decellularization of tissues enabled the interrogation of matrix proteins within decellularized matrix scaffolds of 33 different murine tissues. The perfusion of mice with decellularization reagents (either 0.5% sodium deoxycholate or 0.1% sodium dodecyl sulfate) enabled the matrix scaffold to be observed in its native 3D context at a submicron level of resolution, with minimal or no structural damage. The application of such innovative techniques to the kidney is expected to provide new insights into the complexities of the GBM.

Protein tagging and live imaging. Developments in technologies that aid our ability to visualize the GBM have improved our understanding of GBM composition, organization and architecture; however, the

Tissue expansion
A sample preparation technique in which a tissue sample embedded within a polymer meshwork (for instance, a hydrogel) is uniformly expanded to enable nanoscale-resolution imaging of preserved tissue structures by immunofluorescence staining and diffraction-limited microscopy.

Haemangioblasts
Multipotent precursor cells that can differentiate into endothelial cells and any cell type within the haematopoietic lineage.

imaging approaches that have been applied so far have used fixed tissue and therefore lack details about the dynamics of the GBM. New approaches that involve the labelling of endogenous basement membrane components and live imaging using model systems have provided interesting insights into basement membrane dynamics, including hierarchical assembly[15] and turnover. For instance, studies with mCherry-tagged collagen IV and live imaging have tracked the spatiotemporal incorporation and turnover of collagen IV in *C. elegans*[73,74]. Indeed, the utility of *C. elegans* has been further demonstrated[75] by a study that used gene editing to tag 29 major basement membrane proteins and receptors with mNeonGreen, providing new insights into the interactions between basement membrane proteins, their dynamics and the changes that occur during tissue maturation. Future approaches that involve the labelling of endogenous GBM components and live imaging in appropriate experimental systems could provide similar insights into GBM dynamics, including turnover and repair in the context of health and disease.

GBM transitions during development

In metanephric kidney development, glomerular precursors are first observed within the distal cleft of the S-shaped body[76,77]. The secretion of VEGF by these primitive glomerular cells stimulates haemangioblasts, which are most likely derived from the neighbouring mesenchyme[78], to form blood vessels that invade the distal cleft. The proximity of the glomerular precursors to the endothelium invokes a panoply of changes that gives rise to podocytes[79]. Mature podocytes are highly arborized, and a key transition in their maturation is the replacement of classical epithelial cell-to-cell tight junctions with podocyte slit diaphragms composed of proteins such as nephrin, NEPH1 and podocin[80–84]. These cell–cell adhesion molecules are first expressed at the S-shaped body stage and function to establish and maintain the slit diaphragm. Glomerular maturation creates a closed renal corpuscle encapsulated by a single layer of parietal epithelial cells (Bowman capsule), which surrounds the capillary tuft and central supportive mesangium.

The GBM is formed from the fusion of two basement membranes that separately underlie the podocyte layer and the endothelium[7]. The fusing of two adjacent basement membranes is not unique to the GBM and occurs in the optic cup[85] and in lung alveoli[86]. The initial fusion of the early endothelial and podocyte basement membranes may require linking molecules. Given its role in basement membrane fusion in the worm, hemicentin was hypothesized to be a possible linking molecule in the GBM, though (as described above) recent evidence suggests hemicentin is not required for GBM formation[49]. In addition, the formation of new GBM also occurs in situ as the glomerulus matures. The GBM assembly that occurs after the invasion of haemangioblasts into the distal cleft is unique in that the composition of the matrix undergoes defined changes that are essential for both filtration and continued development. These compositional transitions have been most intensely studied in terms of the contribution of laminins and collagen IV (FIG. 2b).

Laminin protein nomenclature labels trimers by their subunits, such that laminin-α5β2γ1 is encoded by *LAMA5*, *LAMB2*, and *LAMC1* and written as laminin 521. The major laminin heterotrimers expressed by early glomerular precursors are laminin 111 and laminin 511 (REFS[87,88]), and these are replaced by laminin 521 as capillaries begin to form within the distal cleft of the S-shaped body[89]. The importance of replacing early laminin heterotrimers with laminin 521 is physiologically relevant as humans with pathogenic variants in *LAMB2* develop Pierson syndrome, which is characterized by kidney, neurological and ocular defects[90]. *Lama5*$^{-/-}$ mice exhibit loss of GBM integrity, which results in cessation of glomerulogenesis at the stage at which the laminin-α1 chain is replaced by laminin-α5 heterotrimers[91]. These findings demonstrate that transitions observed in laminin heterotrimer composition in the early glomerulus are required for later glomerular development. However, grafting of *Lama5*$^{-/-}$ metanephroi into wild-type hosts resulted in glomerular vascularization of the transplanted metanephroi[92], although the laminin-α1 chain was still detected in the GBM (predominantly on the podocyte side) and the podocyte slit diaphragm did not form. The observation that the hybrid GBM contained laminin-α5 but predominantly on the wild-type endothelial side[92] suggests that laminin-α5 signalling is necessary for podocyte differentiation and highlights an essential role for the GBM in glomerulogenesis.

Simultaneously to the replacement of laminin 111 and laminin 511 by laminin 521 in the GBM, a switch in the collagen IV network occurs[93] (FIG. 2b). The early endothelial and podocyte basement membranes are composed of a collagen IV α1α1α2 network but, upon fusion of the two basement membranes, an α3α4α5 network is deposited[93,94]. Whether α1α1α2 is still produced by podocytes remains unclear but immunolabelling[53], super resolution microscopy[38] and genetic analyses[95] suggest that the α3α4α5 heterotrimer is exclusively synthesised by podocytes. The reason for this switch in the composition of the collagen IV network produced by podocytes is poorly understood; however, a reduction or absence of the α3α4α5 network causes Alport syndrome, which is characterized by kidney, hearing and ocular phenotypes[96,97]. Although the α1α1α2 collagen IV network persists in human Alport kidneys, the GBM thickness is reduced at early stages of the disease[98], indicating that compensatory α1α1α2 synthesis is insufficient to maintain GBM integrity. In addition, the α3α4α5 heterotrimer is thought to provide additional biomechanical strength relative to that of the α1α1α2 heterotrimer. This increased strength may be conferred by the greater number of cysteine residues available to form intermolecular disulfide bonds between the α3α4α5 chains[99]. The increased number of intermolecular bonds might also confer greater resistance to endopeptidase-mediated proteolysis[98]. Moreover, unique interactions between the α3α4α5 heterotrimer and cell membrane receptors (such as collagen IV binding integrins[100] or discoidin domain receptors[101,102]) or with other matrix proteins (such as other α3α4α5 heterotrimers via disulfide bonding, or with nidogen and HSPGs) might also be important

in strengthening and maintaining GBM integrity in mature glomeruli; however, evidence for this role is currently lacking.

Other GBM components may also undergo developmentally timed changes in their expression and/or composition but their profiles have not been formally investigated for functional significance. For example, immunostaining for perlecan shows that it is highly expressed throughout the immature GBM but is expressed to a lesser extent in the mature GBM, where agrin predominates[27]. Furthermore, a potentially relevant switch in GAG chain composition occurs in the GBM during development[103]. In the early stages of development, chondroitin sulfate is the dominant GAG in the GBM, but the mature GBM is composed mainly of heparan sulfate GAG chains on proteoglycans[103]. This change may be functionally relevant in disease as the thickened GBM that is associated with diabetic kidney disease (DKD) is also abnormally associated with high levels of chondroitin sulfate proteoglycans[104].

The GBM in glomerular filtration

Glomeruli filter approximately 180 litres of blood per day into the tubules, where 99% of the primary filtrate is reabsorbed and the remaining 1–2 litres of waste are excreted. Despite this seemingly simple function, the mechanism of size-selective filtration and the contribution of the GBM to filtration are not fully understood. The complex arrangement of podocyte foot processes prompted early modelling studies to describe the podocyte slit diaphragm as the major determinant of size selectivity. This concept was supported by measurements showing that the average spacing between podocyte foot processes is comparable to the size of albumin[105,106]. However, more recent discoveries have questioned this concept.

The endothelial glycocalyx may also contribute to size-selective filtration in the glomerulus. The glycocalyx is a gel-like layer of proteins and GAG chains (including heparan sulfate, chondroitin sulphate and hyaluronan). It is positioned within the fenestrae of the endothelium and anchors down to the underlying GBM[107]. Studies in rodents show that enzymatic breakdown of GAGs within the endothelial glycocalyx[108] or displacement of the glycocalyx by hypertonic sodium chloride[109] induce an increase in albumin filtration by up to 12-fold. However, the role of the endothelial glycocalyx in glomerular filtration has been questioned by in vivo experiments that implicated the GBM as the major component mediating size-selective filtration.

The gel-like resemblance of the GBM[110] led to the proposal that the GBM follows the gel permeation principle formalized by Ogston in 1958, which describes how permeation into gels is related to molecular size[111]. This principle suggests that the GBM determines the permeation of macromolecules through the filter in a size-dependent manner through diffusion, whereas water and ions would pass through the filter by flow generated from hydraulic pressure[110]. In this model, the fenestrated endothelium and the podocyte slit diaphragms do not act by size selectivity but rather provide resistance to the capillary fluid flow. Support for this gel permeation–diffusion model came from a 2017 study that used transmission electron microscopy to follow the fate of variably sized gold nanoparticles following their injection into the superior mesenteric artery of mice[112]. Large nanoparticles (equivalent in size to IgG dimers) permeated into the subendothelial GBM but did not enter its central region, whereas smaller nanoparticles (equivalent in size to IgG monomers) could partially permeate into the central region. Nanoparticles equivalent in size to albumin, which is much smaller than IgG monomers, could permeate across the GBM. The researchers also showed that proximal tubules could completely absorb albumin-sized nanoparticles following tail vein injection at low concentrations. However, the injection of high concentrations of albumin nanoparticles saturated the reabsorption capacity of the proximal tubules, resulting in albuminuria. Interestingly, the gold nanoparticles used in these experiments frequently aggregated upstream of the slit diaphragms at the approximate position of the podocyte glycocalyx. They did not accumulate at the endothelial glycocalyx or at the protein bridges that form the slit diaphragm, leading to the conclusion that the GBM (acting as a relatively dense gel) and the podocyte glycocalyx are the two major sites that determine size selectivity in the glomerulus[112]. However, the reason why the podocyte glycocalyx acts as a barrier but the endothelial glycocalyx does not is unclear and requires further experimental insight.

The gel permeation–diffusion model of glomerular filtration is therefore supported by experimental evidence; however, it is interesting to note that the absence of podocyte nephrin precludes the formation of the slit diaphragm and causes massive proteinuria[113,114] but has no known effect on GBM composition. Rather than providing evidence for an essential role of the slit diaphragm in size selectivity, this finding may imply a requirement for the slit diaphragm in the compression and tension of the GBM to maintain its physiological thickness. Indeed, a gel compression model[115] proposes that podocyte foot process effacement limits GBM compression, leading to a subtle but physiologically relevant shift from radial compression to circumferential tension. This shift reduces compression of the GBM, opening its meshwork and increasing the average pore size, enabling passage of larger macromolecules. Genetic models of kidney disease, such as *Lamb2*$^{-/-}$ or *Col4a3*$^{-/-}$ mice, show podocyte foot process effacement and GBM thickening, which is consistent with a requirement for interdigitating podocyte foot processes in the maintenance of GBM compression. In support of this model, a new study combined morphometric analyses of glomerular filters prior to and after the onset of albuminuria in a mouse model induced to develop glomerular barrier dysfunction[116]. Together with mathematical modelling, the researchers demonstrated that compression of the GBM by the 'buttress force' of podocyte foot processes facilitates permselectivity and that, when this function is perturbed, capillary dilatation results, the GBM becomes more porous and albuminuria ensues.

The GBM and cellular glycocalyx maintain a net negative charge across the filtration barrier owing to the negative charge of HSPGs and their GAG side chains.

This net anionic charge led to the suggestion that negatively charged molecules (such as albumin) would be repelled by the GBM and glycocalyx and selectively retained within the capillaries. However, this theory has been contested by several studies showing that depletion of HSPGs in the glomerulus does not affect glomerular function. For instance, one study showed that the deletion of both agrin and perlecan reduced the net negative charge of the GFB in mice but did not induce proteinuria[28]. Similarly, the removal of heparan sulphate side chains by glomerular overexpression of heparanase reduced GAG-associated anionic sites fivefold but did not cause changes in the glomerular ultrastructure or function[117]. In zebrafish, mutation of the *ext2* gene, encoding exostosin glycosyltransferase 2, which polymerizes GAG side chains attached to HSPGs, led to fewer anionic sites in the GBM but also did not induce proteinuria[118]. Together, these findings suggest that the role of charge selectivity in the glomerular filter is likely to be minor. However, despite the evidence in support of this conclusion, anionic molecule tracers have more difficulty in passing the glomerular filter than do neutral or cationic tracers[119], suggesting that we do not fully understand the role of charge selectivity in the glomerulus.

Human disease with GBM involvement

Overt morphological changes in the GBM are seen in many glomerular diseases (FIG. 4) and are caused by a multitude of molecular and biophysical mechanisms. Notable changes in GBM composition, arrangement and function occur in genetic diseases as well as in acquired diseases, including autoimmune diseases such as anti-GBM disease and membranous nephropathy, and metabolic diseases such as DKD.

The spectrum of Alport syndrome

Alport syndrome represents a spectrum of phenotypes caused by variants in the *COL4A3*, *COL4A4* and *COL4A5* genes. Patients typically present with early persistent haematuria followed by progressive proteinuria and declining kidney function, often accompanied by hearing loss and ocular complications. These hallmarks are caused by progressive deterioration in the function of specific basement membranes and the progression of tissue fibrosis[120]. Early Alport syndrome is characterized by a thin GBM. However, as the disease progresses, the GBM becomes segmentally thickened, split and lamellated, giving rise to the characteristic basket-weave appearance[10,120,121]. Podocyte foot process effacement and invasion into the GBM have also been observed[10,121,122]. The *COL4A3*, *COL4A4* and *COL4A5* variants lead to inadequate incorporation of the collagen IV α3α4α5 network into the GBM during glomerulogenesis, which leads to retention of the collagen IV α1α1α2 network. As the α1α1α2 trimers have about half the number of cysteine residues (and hence fewer disulfide bonds) than the α3α4α5 trimers[123], the abnormal GBM might have reduced mechanical resistance to the hydrostatic pressures that drive glomerular filtration. It has been proposed that the mechanical load imposed by filtration and the reduced compression imposed by effaced podocyte foot processes might therefore contribute to a progressive thickening of the GBM and thereby induce the onset of haematuria and proteinuria[115]. The persistent α1α1α2 network in Alport syndrome is also thought to be more susceptible to proteolysis by matrix-degrading enzymes[97,124,125] and can induce abnormal outside-in signalling via collagen IV binding receptors, such as α1 and α2 integrins, and DDR1. Deletion or inhibition of these receptors in *Col4a3*$^{-/-}$ mice reduces podocyte injury and GBM damage and therefore delays progression in this model of Alport syndrome[126–128]. An accumulation of ectopic laminin-α1 in thickened segments of the GBM has also been described in *Col4a3*$^{-/-}$ mice, associated with progressive loss of permeability to ferritin[129], and might contribute to reduced podocyte adhesion[130].

COL4A5 is located on the X chromosome, and therefore males with X-linked Alport syndrome (XLAS) are more severely affected than females. Females with XLAS have a single abnormal *COL4A5* gene and an attenuated kidney phenotype with thin GBMs and some deposition of α5 subunits in the GBM[131]. Homozygous or compound heterozygous variants in *COL4A3* or *COL4A4* cause autosomal recessive Alport syndrome in both males and females. However, similar to XLAS in women, heterozygous variants in *COL4A3* or *COL4A4* cause TBMN. GBM thickness in these individuals uniformly increases from a maximum of 180 nm in children (2–11 years old) up to 200–264 nm in adults in at least 50% of the GBM in most glomeruli, which is still significantly thinner than that of normal adults (about 320 nm in women and 370 nm in men)[132,133]. Typically, TBMN manifests as persistent microscopic haematuria in childhood; however, it is no longer considered benign as it is associated with progression to proteinuria and impaired kidney function in adults, with up to a 30% lifetime risk of kidney failure. Unlike XLAS in men and autosomal recessive Alport syndrome, the α3α4α5 network is present in the mature GBM in TBMN but the GBM is diffusely thinned and use of LV-SEM has demonstrated the existence of irregular holes along the GBM surface[61]. The GBM thinning is likely explained by a gene dosage effect, whereby podocytes that carry one abnormal copy of *COL4A3*, *COL4A4* or *COL4A5* produce fewer α3α4α5 trimers than podocytes that carry normal copies of the three genes[132]. Individuals with heterozygous variants in any of these genes should receive lifelong renal surveillance to screen for modifiable risk factors such as hypertension and proteinuria[120].

Pierson syndrome

Pierson syndrome is a rare genetic disorder caused by variants in *LAMB2*, which result in a complete or partial lack of laminin 521 heterotrimers in the GBM, eye and muscle tissue. The clinical features of Pierson syndrome include nephrotic syndrome with progression to kidney failure within the first year of life, neurological defects and microcoria[134]. Studies of *Lamb2*$^{-/-}$ mice have contributed enormously to the pathophysiological underpinnings of GBM dysfunction in Pierson syndrome. These mice develop normally in utero and assemble a structurally intact GBM in which the lack of laminin 521 is compensated for by ectopic laminins

Microcoria
A congenital disorder of the eye characterized by small pupils with a diameter of less than 2 mm.

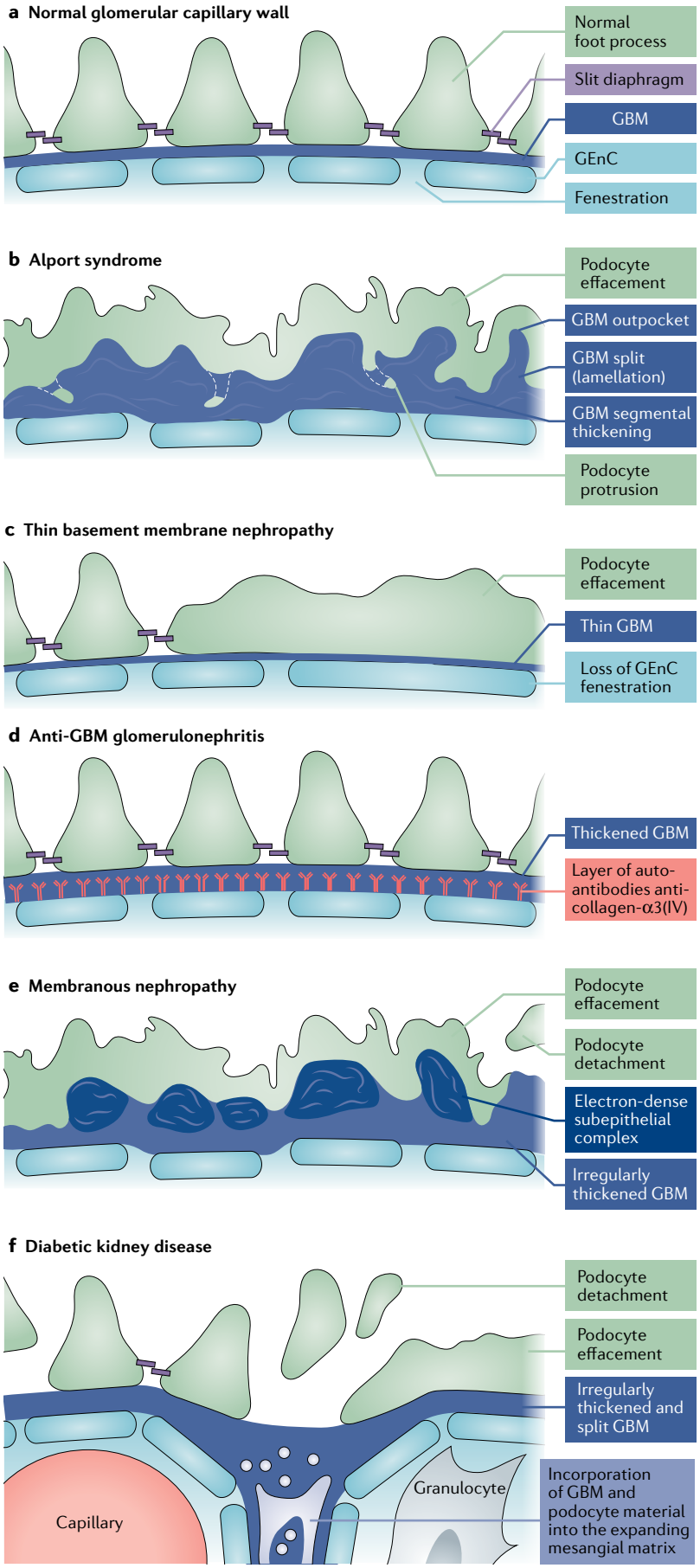

Fig. 4 | **Morphological defects of the glomerular basement membrane associated with disease. a** | The glomerular filtration barrier at the glomerular capillary wall is composed of interdigitating podocyte foot processes, the protein bridges that form the slit diaphragm, and a fenestrated glomerular endothelial cell (GEnC) layer, separated by a thick central glomerular basement membrane (GBM). The composition and arrangement of these components are affected in numerous glomerular diseases. **b** | In Alport syndrome, the GBM is irregularly thickened and split or lamellated. Extensive podocyte foot process effacement is observed along with frequent podocyte protrusions invading the GBM. **c** | Thin basement membrane nephropathy is characterized by an abnormally thin GBM, with some degree of podocyte foot process effacement. **d** | In anti-GBM disease, anti-collagen IV-α3 autoantibodies are typically found deposited along the thickened GBM. **e** | The dominant features of membranous nephropathy include the deposition of large electron-dense immune complexes on the subepithelial surface of the GBM, which is extensively damaged and thickened, and effacement or detachment of podocytes from the capillary wall. **f** | Advanced diabetic kidney disease is associated with extensive GBM thickening, podocyte foot process effacement, retraction and detachment from the GBM. In addition, GBM and podocyte-derived material is often incorporated into the expanding mesangial matrix.

111, 211, 332 and, more commonly, 511 but not at sufficient levels to secure the long-term maintenance of glomerular permselectivity. Newborn *Lamb2*$^{-/-}$ mice develop massive proteinuria shortly after birth that progresses to nephrotic syndrome 3 weeks after birth[135–137]. Severe podocyte foot process effacement is detected at 2 weeks after birth and, although the GBM is ultrastructurally intact, it displays disorganization of anionic sites and an increased permeability to ferritin[137]. Notably, since proteinuria appears before signs of morphological change in the GBM or podocytes, it has been suggested that proteinuria in *LAMB2* deficiency is primarily caused by a permselectivity defect in the GBM[137]. This concept is supported by the observation that the degree of ferritin permeability in the GBM in *Lamb2*$^{-/-}$ mice is not dependent on normal podocyte morphology. Interestingly, an ultrastructural analysis of kidney biopsy samples from patients with a spectrum of glomerular diseases that cause nephrotic syndrome revealed the presence of tunnels and cavities throughout the nephrotic GBM apparently large enough to allow unrestricted protein leakage even in the absence of GBM thickening[138]. Since laminins are essential for basement membrane assembly, it has been proposed that the lack of laminin 521 could permit a similar fluid flow through the GBM as a result of altered structural organization due to the defective laminin network, thereby causing massive early proteinuria[136,137,139]. An alternative explanation is that the signalling axis from laminin 521 in the GBM via α3β1 integrins to the podocyte cytoskeleton is critical for the maintenance of podocyte function and glomerular permselectivity. Indeed distinct basement membrane ligands have been shown to influence both cell shape and the assembly of adhesion complexes in podocytes[128], indicating that the matrix ligand matters. Furthermore, forced podocyte-specific overexpression

Advanced glycation end products
(AGEs). Proteins or lipids that are modified by the non-enzymatic binding of a reactive sugar to basic amino acids such as lysine or arginine.

of *Lamb1* in *Lamb2*$^{-/-}$ mice restored glomerular function and attenuated GBM damage and podocyte injury in a dose-dependent manner[139]. This finding suggests that outside-in signalling is also key for intact glomerular function and that the density of laminin polymers within the GBM may be an important factor.

Autoimmune disease

Anti-GBM disease. In addition to genetic disorders, autoimmune disease can also cause abnormalities in basement membranes. Anti-GBM disease, which is a feature of Goodpasture syndrome, is a rare, immune-mediated condition caused by autoantibodies against conformational and cryptic epitopes in collagen IV. The dominant target antigen is in the NC1 domain of the α3 chain[140], and the disease is characterized by the linear deposition of anti-α3(IV)NC1 IgG (and often complement component C3) along the full length of the GBM with the absence of electron-dense deposits[141–143]. Patients with anti-GBM disease (mostly adults) develop glomerular injury characterized by a crescentic or focal necrotizing glomerulonephritis and a rapid decline in kidney function.

Membranous nephropathy. GBM defects also occur in autoimmune diseases that target podocyte antigens. Membranous nephropathy is the most common cause of immune-mediated nephrotic syndrome in adults and its idiopathic form is associated with autoantibodies against surface antigens expressed by podocytes, predominantly the phospholipase A2 receptor (70% of cases) and thrombospondin type 1 domain containing 7A (2–5% of cases)[144]. The GBM of patients with membranous nephropathy is profoundly thickened, with subepithelial electron-dense deposits and characteristic matrix spikes between the deposits. The dense deposits contain IgG (mostly IgG4) and complement components (C3, C4 and C5b–9), which are trapped within the subepithelial surface of the GBM, although the mechanism for this trapping remains unclear. Spontaneous remission occurs in 30–40% of cases but 40% of patients with persistent proteinuria progress to kidney failure within 10 years[144–146]. Of note, membranous nephropathy is associated with loss of GBM heparan sulfate GAGs in patients[147] and in a rat model of membranous nephropathy[148]. Given that heparan sulfate GAGs regulate complement activation via interactions with complement regulatory proteins, a decreased heparan sulfate content in the GBM might be critical for glomerular damage induced by in situ activation of the complement system[149].

Diabetic kidney disease

GBM thickening is an early morphological feature of DKD followed by mesangial matrix expansion and glomerulosclerosis, accompanied by the development of proteinuria and a progressive decline in kidney function[150–153]. A 2019 study identified GBM thickness as a predictor of renal survival in patients with DKD[154]. Ultrastructural studies of the GBM in patients with advanced DKD have revealed dramatic changes, including denudation and abnormal folding of the GBM and the presence of shallow crater-like cavities and tunnels in fragmented segments of the GBM[151,155]. GBM thickening is generally thought to result from matrix dysregulation, involving the overproduction of matrix components and low rates of turnover in response to the activation of inflammatory and profibrotic pathways linked to high glucose levels. The diminished expression and activity of matrix-degrading enzymes and their natural inhibitors might also contribute to the accumulation of matrix in DKD[153,156–158]. The depletion of intracellular ATP in response to high glucose levels also decreases the de novo synthesis and post-translational processing of HSPGs and heparan sulfate GAGs, which become scarce in the thickened GBM[152,153,159]. The severity of GBM damage in DKD is also associated with the formation of advanced glycation end products (AGEs). For example, collagen IV is highly susceptible to damage by AGEs at multiple functional sites[160,161], and AGEs can disrupt cell–matrix interactions and enhance the number of crosslinking bonds within basement membrane proteins in a way that disturbs their assembly and turnover[160–162].

DKD is known to have an underlying genetic component[163]. In the past few years, two variants in *COL4A3* have been associated with disease progression in DKD. The first, a low-frequency single nucleotide polymorphism (rs34505188 causing an Arg408His substitution), was detected in African Americans with DKD and was predicted to be pathogenic owing to its localization within a highly conserved region of the gene[164]. The second, a common single nucleotide polymorphism (rs55703767 causing an Asp326Tyr substitution), was detected in Europeans with type 1 diabetes mellitus. This variant might provide protection against albuminuria and GBM thickening as the amino acid substitution could destabilize the α3α4α5 trimer, resulting in a more flexible network that resists the forces induced by hyperfiltration and is more accessible to proteolytic degradation, enabling faster turnover[165,166]. Further functional studies of these *COL4A3* variants, in addition to variants in other basement membrane genes, could improve the understanding of basement membrane biology and the roles of GBM components in glomerular disease.

Therapy for GBM-associated disease

No curative therapies currently exist for GBM-associated disease; however, a number of treatments can prolong kidney survival (TABLE 1). Renin–angiotensin–aldosterone system inhibitors are the current mainstay of therapy for Alport syndrome and aim to reduce intraglomerular pressure and thereby mechanical load on glomerular capillaries; this therapy reduces proteinuria and delays kidney failure by a decade or more[120]. New therapy prospects that aim to delay disease progression have also emerged from preclinical studies (TABLE 1).

One line of investigation is the assessment of approaches that target fibrosis. Some of these agents, such as TGFβ inhibitors and collagen IV receptor blockers, are still in the preclinical phase of investigation, whereas others, including a microRNA-21 inhibitor[167], are currently in clinical trials. Future strategies to correct basement membrane defects could also address protein trafficking (with the aid of chemical chaperone)

Exon skipping therapy
A technology that can be used to correct the coding reading frame of a mRNA transcript from a mutated gene in order to produce a functional protein.

Joubert syndrome
An inherited ciliopathy caused by mutations in more than 30 genes required for normal ciliary function.

in Alport syndrome[168] or deleterious post-translational modifications such the formation of AGEs in diabetes[153]. In support of this, studies have shown that attenuation of AGE signalling through the receptor (RAGE) ameliorated oxidative stress, GBM thickening, glomerulosclerosis and albuminuria in rodent models of DKD[153,169].

Approaches to correct the defects in GBM composition to re-establish function and prevent disease progression are also an avenue of investigation. For example, transgenic expression of the human *COL4A3–COL4A4* locus rescued the Alport syndrome phenotype of *Col4a3*$^{-/-}$ mice[170]. Similarly, transgenic expression of podocyte-specific *Col4a3* after the onset of proteinuria in *Col4a3*$^{-/-}$ mice partially rescued the collagen IV network and the ultrastructure of the GBM[171]. These findings suggest that gene replacement therapies might be effective in Alport syndrome. Indeed, the therapeutic potential of gene-targeting strategies is also currently being investigated for genetic kidney disease (FIG. 5). A 2019 study reported the successful use of CRISPR–Cas9 gene editing in cultured urine-derived podocytes from patients with Alport syndrome[172]. This approach achieved homologous correction in 59% of variants in *COL4A5* (Gly624Asp) and 44% of variants in *COL4A3* (Gly856Glu)[172]. Exon skipping therapy can also be induced by CRISPR–Cas9 or with splice-blocking antisense oligonucleotides. A study in a mouse model of Joubert syndrome showed that systemic treatment with a splice site masking-antisense oligonucleotide partially rescued full-length transcript and protein levels of CEP290 and ameliorated cystic kidney disease[173]. Exon skipping has also been used to target truncating variants in exon 21 of *COL4A5* in a mouse model of Alport syndrome[174]. The intervention resulted in successful collagen IV α3α4α5 trimer assembly as well as in GBM localization and extended kidney survival in mice. These findings provide optimism for human translation and for the treatment of severe truncating variants in *COL4A5*.

Recombinant proteins can also be efficiently delivered to the GBM, and this approach represents another potential therapeutic option for GBM-associated disease (FIG. 5). Daily injection of pre-albuminuric *Lamb2*$^{-/-}$ mice with recombinant human laminin 521 restored GBM permselectivity and delayed proteinuria[67]. The human laminin 521 stably accumulated on the endothelial side of the GBM but did not accumulate in the GBM on the podocyte side. Despite this encouraging observation, these mice still developed nephrotic syndrome, perhaps as a consequence of absent laminin 521 engagement with podocyte adhesion receptors. Vascular delivery of smaller GBM-repair proteins is another potential strategy. Linker proteins, which are small versions of full-length matrix proteins (for example, αLNNd), or chimeric-engineered peptide sequences (for example, mini-agrin) are also being used to rescue function and to identify mechanisms by which amino acid substitutions in laminin LN domains affect basement assembly (FIG. 5). In vitro experiments demonstrated that the linker protein βLNNd can re-establish the polymerization of a non-polymerizing laminin 111 that bears a mutation in its β1 LN domain (LMβ1Ser68Arg, which is homologous to LMβ2-Ser80Arg that leads to Pierson syndrome) and can assemble with collagen IV and nidogen[175]. These findings suggest that engineered linker proteins could also be an effective approach to repair GBM defects in conditions such as Pierson syndrome. The challenges for this approach include mapping the precise interactions of GBM components, identifying the location of key binding domains for targeting defective proteins, and

Table 1 | **Current and investigative therapies for diseases with GBM involvement**

Target	Type	Mechanism of action	Development stage	Ref.
Available therapies				
RAAS	RAAS inhibitors (ARBs, aldosterone blockers)	Reduction in intraglomerular pressure	Current standard of care	Gross et al.[178]
Therapies under development				
Fibrosis	Anti-miR-21 oligonucleotide	Reduces fibrosis in Alport syndrome by silencing microRNA-21	Preclinical study (*Col4a3*$^{-/-}$ mouse model of Alport syndrome); clinical trial ongoing	Gomez et al.[179]; NCT02855268
	Collagen IV receptor inhibitors	DDR1 selective inhibitor; reduces DDR1 phosphorylation	Preclinical animal study (*Col4a3*$^{-/-}$ mouse model of Alport syndrome)	Richter et al.[180]
	RAGE blocker	Single-stranded DNA aptamer against RAGE; blocks AGE–RAGE signalling, oxidative stress, inflammation and ECM accumulation	Rat model of diabetic kidney disease (induced with streptozotocin)	Matsui et al.[169]
ECM biology	Gene replacement therapy	Inducible expression of GBM genes	Preclinical animal study (*Col4a3*$^{-/-}$ mouse model of Alport syndrome)	Lin et al.[171]
	Chemical chaperone-based therapy	Sodium 4-phenylbutyrate; increases *COL4A5* mRNA levels and enhances the deposition of COL4A5	Preclinical in vitro study (fibroblasts from XLAS male patient)	Wang et al.[168]

AGE, advanced glycation end product; ARBs, angiotensin-receptor blockers; DDR1, discoidin domain receptor type 1; ECM, extracellular matrix; GBM, glomerular basement membrane; RAAS, renin–angiotensin–aldosterone system; RAGE, receptor for advanced glycation end products; XLAS, X-linked Alport syndrome.

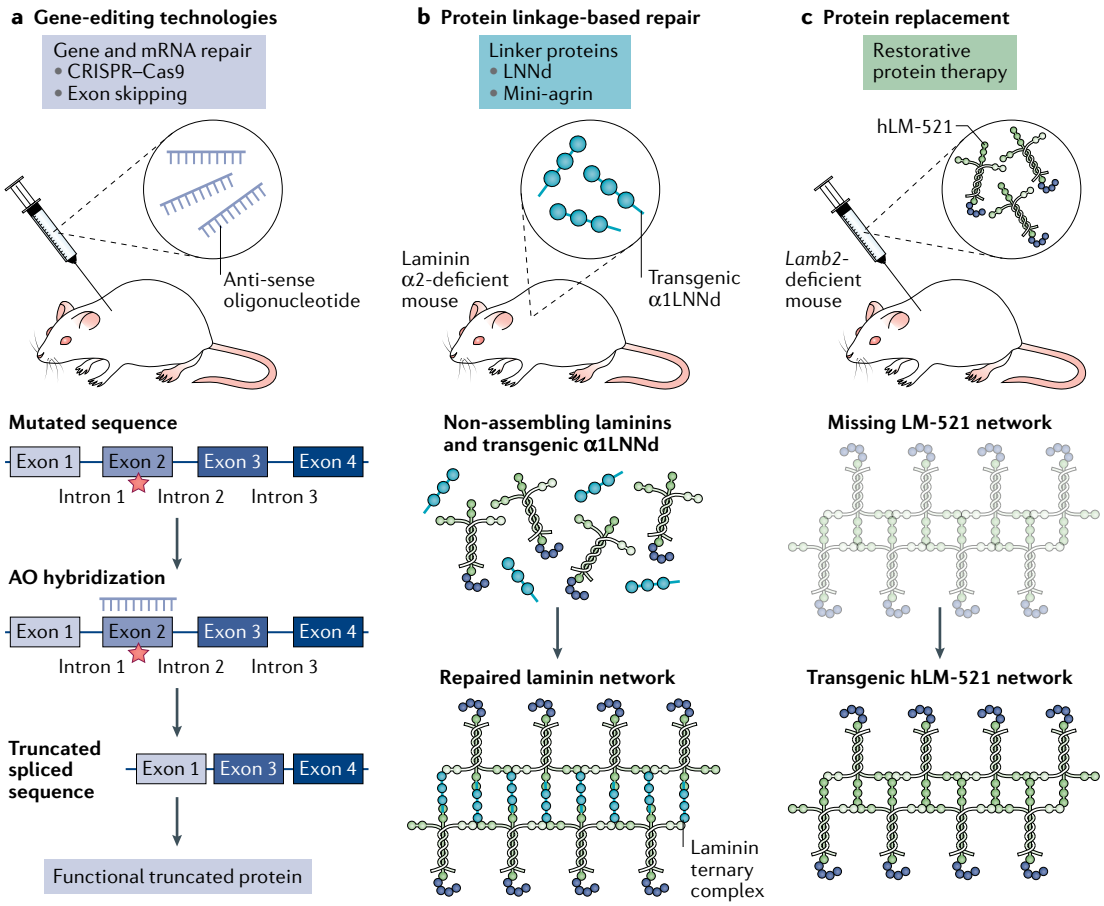

Fig. 5 | **New technologies to repair defective basement membranes. a** | Genetic approaches to repair damaged basement membranes include gene editing using systems such as CRISPR–Cas9 and exon skipping, which can be induced by CRISPR–Cas9 or with splice-blocking antisense oligonucleotides. For example, antisense oligonucleotide probes have been successfully used to remove mutated exons and splice sites during splicing and yield truncated mRNA that is translated into functional truncated proteins that partially restore basement membranes[181]. **b** | Protein linkage-based repair uses engineered linker proteins (such as α1LNNd and βLNNd and mini-agrin) to restore basement membrane assembly, cell-matrix anchorage and signalling. For example, transgenic expression of α1LNNd subunits in laminin α2-deficient mice (a model of LAMA2-related muscular dystrophy) can restore the polymerization of non-assembling laminin by providing laminin-α chains with laminin N-terminal domain binding sites to laminin-β and laminin-γ chains. **c** | Restorative protein therapy could also be used to replace missing basement membrane proteins. For example, injection of human recombinant laminin 521 (hLM-521) effectively replaced the missing laminin 521 network in the subendothelial aspect of the GBM in *Lamb2*$^{-/-}$ mice (a model of Pierson syndrome) and delayed the onset of proteinuria.

the design and development of strategic and efficient delivery systems.

One goal of future therapy for GBM-associated disease would be to exploit endogenous mechanisms of GBM repair; however, the regulated maintenance, turnover and potential for repair of basement membranes is currently poorly understood. The GBM undergoes turnover, although the exact timescale of this remodelling is unclear. One of the very few studies of GBM turnover published in 1972 assessed two patients following excessive exposure to silver. In one patient, silver was identified in the GBM 12 weeks after exposure to silver; in the other case, it was absent from the GBM 14 years following exposure[176] and is therefore inconclusive with regard to the timescale of GBM turnover. Current approaches to label endogenous matrix proteins[74,177] will ultimately improve our understanding of GBM turnover and the potential for basement membrane repair.

Conclusions

The GBM represents a highly specialized macromolecular structure that is uniquely designed to facilitate filtration. Studies of GBM composition using emerging imaging and proteomics technologies continue to build a picture of the complexity of the GBM and provide insights into how composition and structure relate to GBM function. GBM defects are seen across a wide spectrum of glomerular diseases. As our understanding of GBM assembly, maintenance and repair improves so will our ability to conceive new therapeutic strategies to stabilize or even repair defective basement membranes.

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