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Review Article

Mechanisms of podocyte injury and implications for diabetic nephropathy

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Albuminuria is the hallmark of both primary and secondary proteinuric glomerulopathies, including focal segmental glomerulosclerosis (FSGS), obesity-related nephropathy, and diabetic nephropathy (DN). Moreover, albuminuria is an important feature of all chronic kidney diseases (CKDs). Podocytes play a key role in maintaining the permselectivity of the glomerular filtration barrier (GFB) and injury of the podocyte, leading to foot process (FP) effacement and podocyte loss, the unifying underlying mechanism of proteinuric glomerulopathies. The metabolic insult of hyperglycemia is of paramount importance in the pathogenesis of DN, while insults leading to podocyte damage are poorly defined in other proteinuric glomerulopathies. However, shared mechanisms of podocyte damage have been identified. Herein, we will review the role of haemodynamic and oxidative stress, inflammation, lipotoxicity, endocannabinoid (EC) hypertone, and both mitochondrial and autophagic dysfunction in the pathogenesis of the podocyte damage, focussing particularly on their role in the pathogenesis of DN. Gaining a better insight into the mechanisms of podocyte injury may provide novel targets for treatment. Moreover, novel strategies for boosting podocyte repair may open the way to podocyte regenerative medicine.

The kidneys filter approximately 180 l of fluids everyday; however, there is no loss of proteins into urine as the glomerular filtration barrier (GFB) retains 99.99% of plasma proteins. Alterations in the GFB result in the development of proteinuria, ranging from albuminuria to massive nephrotic syndrome. Albuminuria is the hallmark of both primary and secondary proteinuric glomerulopathies, including focal segmental glomerulosclerosis (FSGS), diabetic nephropathy (DN), obesity-related nephropathy, and an important feature of chronic kidney diseases (CKDs). In addition, in patients with CKD, albuminuria is a predictor of progression towards end-stage renal disease (ESRD) and is associated with an increased risk of cardiovascular diseases. Glomerular podocytes are a component of the GFB and podocyte injury is the main cause of albuminuria development. Herein, we will describe patterns of podocyte damage and review underlying mechanisms, focussing particularly on DN.

Podocytes

Podocytes are highly specialised glomerular epithelial cells that form the GFB together with the fenestrated endothelium and the glomerular basement membrane (GBM) [1,2]. The podocyte cell body bulges into the urinary space and gives rise to long primary processes that branch into foot processes (FPs), enwrapping the glomerular capillaries [3]. FPs of neighbouring podocytes interdigitate, leaving between them long filtration slits that are bridged by a junction, named slit diaphragm (SD). Nephrin, NEPH1, FAT, podocin, and ephrin B1 form the transmembrane/extracellular portion of the SD, connecting adjacent FPs, while the cytoplasmic tails of these proteins interact with scaffold proteins, adapters, and signalling molecules to regulate the podocyte cytoskeleton [4]. FPs contain a dense cytoskeletal network of actin filaments connected with an array of linker proteins not only to the SD, but also to the GBM anchor proteins, such as $\alpha 3\beta 1$ integrin and dystroglycan. These interactions are essential to maintain the highly ordered structure of the FPs [2] (Figure 1). The SD is considered the major restriction site to protein

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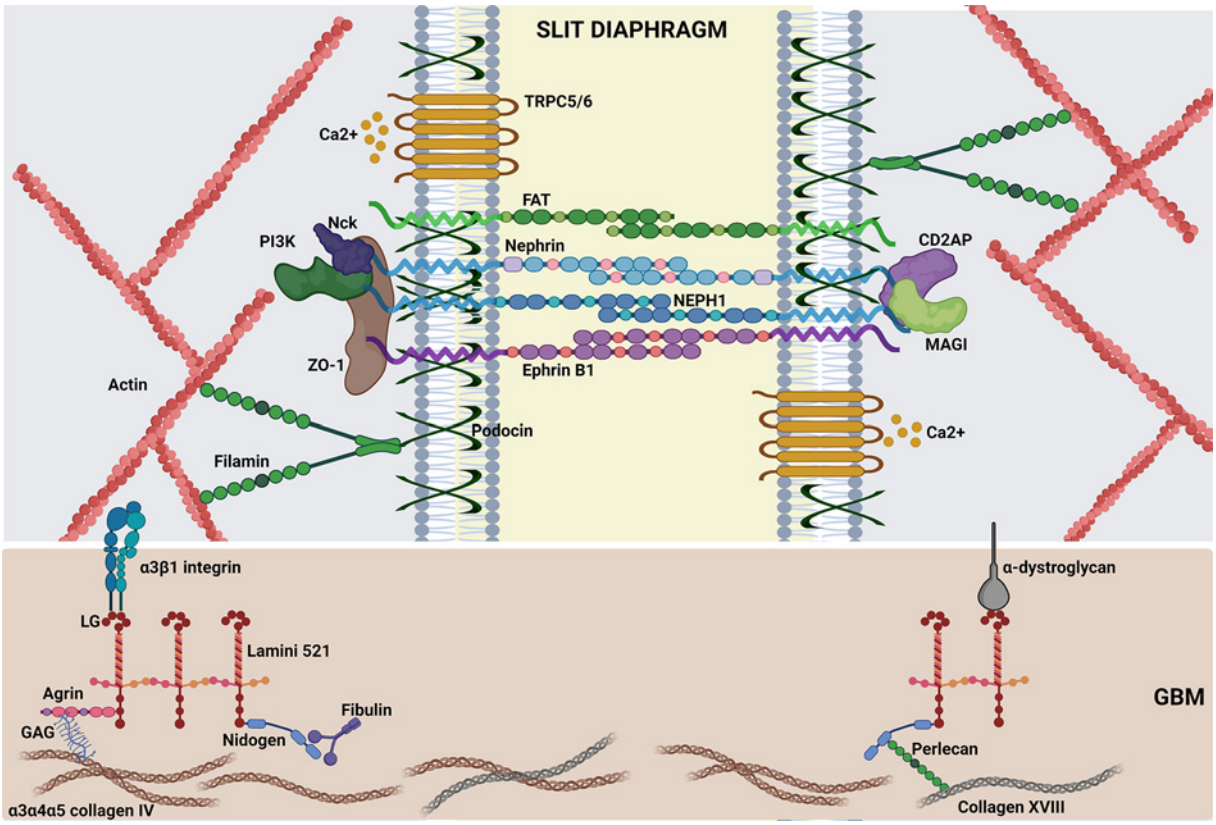

Figure 1. Schematic representation of podocyte FPs

The actin cytoskeleton of podocyte FPs is connected to both the SD, a specialised junction bridging the slit between FPs of neighbouring podocytes, and the GBM. Abbreviations: CD2AP, CD2-associated protein; GAG, glycosaminoglycan; LG, laminin G-like domain; MAGI, membrane-associated guanylate kinase; Nck, non-catalytic region of tyrosine kinase adaptor protein; PI3K, phosphoinositide 3 kinase; TRPC5/6, short transient receptor potential channel 5/6; ZO-1, zonula occludens-1.

filtration; however, the negatively charged sialoglycoproteins that cover the podocyte abluminal surface facing the GBM also contribute to the GFB by repulsing plasmatic anionic proteins. Moreover, flow-mediated compression of the GBM against the FPs changes the physical properties of the GBM, enhancing GBM permselectivity [5,6]. Finally, secretion of vascular endothelial growth factor (VEGF) by podocytes affects the permeability of the glomerular endothelium.

Patterns of podocyte injury

FP effacement

Podocyte injury alters both the SD and the FP cytoskeleton. Specifically, FPs undergo retraction with FP widening and shortening. This simplified architecture is named FP effacement and is associated with proteinuria even in the absence of podocyte loss. Early FP effacement is reversible, but if the underlying injury does not resolve, FP effacement progresses until podocytes deprived of their FPs attach to the GMB exclusively through their cell bodies [3,7].

Podocyte loss

Podocyte loss, a key feature of progressive proteinuric glomerulopathies, is due to either apoptosis or podocyte detachment. Dedifferentiation can protect podocytes from death, but alters their function/structure, possibly resulting in detachment from the GBM [8]. Moreover, injury leads to activation of integrin $\alpha v\beta 3$ that favours podocyte detachment [9]. The entity of damage is also important and a podocyte loss above the threshold level of 30–40% is required to trigger the development of glomerulosclerosis in FSGS [10]. In the adult kidney, podocytes have a limited capacity of proliferation and lost podocytes cannot be adequately replaced [11]. However, remaining podocytes can adapt by increasing their size to cover the denuded GBM.

Podocyte density

In glomerulopathies characterised by glomerular hypertrophy, including DN, podocytes undergo hypertrophy to cover the increased GBM surface area. This can result in reduced podocyte density without changes in podocyte number [12].

GBM abnormalities

Podocytes produce most of the GBM components and secrete matrix metalloproteinases (MMPs) important in extracellular matrix remodelling [13,14]. Therefore, podocyte injury can alter the balance between GBM synthesis and degradation, leading to changes in GBM thickening [13].

Podocytopathies

Podocyte injury is the unifying mechanism of proteinuric glomerulopathies, regardless of both the triggering cause of podocyte damage (genetic, immune, infective, toxic, metabolic, haemodynamic) and the presence of other associated histological abnormalities. Therefore, these diseases have been recently renamed 'podocytopathies' [8].

The key role of podocytes in proteinuric conditions was highlighted by studies on genetic causes of proteinuria. More than 20 years ago, mutations in the nephrin gene (*NPHS1*) were identified as the cause of congenital nephrotic syndrome of the Finnish type [15]. Since then more than 50 mutations have been discovered, affecting predominantly components of the SD and the podocyte actin cytoskeleton [16–19]. These findings prompted research into podocyte physiopathology and led to the recognition that podocyte injury is also the underlying mechanism of most acquired proteinuric diseases, including DN.

Podocyte damage in DN

The term 'diabetic kidney disease' (DKD) encompasses all types of renal injury occurring in patients with diabetes. The classical albuminuric form of DKD is predominantly due to glomerular/podocyte injury and is characterised by both increased glomerular permeability to proteins and relentless renal function decline. DKD can also occur in the absence of albuminuria and the non-albuminuric form is now the prevailing phenotype in patients with type 2 diabetes. However, the non-albuminuric phenotype appears predominantly associated with atypical vascular and/or tubulointerstitial lesions rather than with podocyte injury [20].

Most of the available histological data in diabetes are from patients with the classical albuminuric phenotype. Nephrin down-regulation is observed in early human DN and correlates with both albuminuria and enhanced FP width [21–23]. Another early feature of DN is thickening of the GBM, resulting from overproduction of matrix components, reduced turnover, and diminished MMP expression/activity [24–26]. Thickening of the GBM precedes the development of albuminuria and is a predictor of renal survival [27]. Studies on kidney biopsies from patients with established DN showed FP effacement [28] and podocyte loss/reduced density that strongly correlate with albuminuria [12,29,30]. Besides podocyte loss, podocyte detachment from the GMB is also involved and viable podocytes are found in the urine of patients with DN [31].

Hyperglycaemia is a key determinant in the pathogenesis of the podocyte injury in diabetes. In the last decades, a large number of studies on podocytes exposed to a high glucose milieu have proven that hyperglycaemia can alter the phenotype of podocytes by inducing nephrin loss, changes in the production/degradation of extracellular matrix components, enhanced prosclerotic cytokine transforming growth factor β1 (TGF-β1) signalling, remodelling of the actin cytoskeleton, α3β1 integrin down-regulation, and both podocyte hypertrophy and apoptosis [32–39]. Excess glucose entry into podocytes via the glucose transporters GLUT1 and GLUT4 increases flux through the polyol pathway, accumulation of advanced glycation-end products (AGEs) precursors, activation of protein kinase C (PKC), and increased hexosamine pathway activity that have long been considered the predominant mechanisms of glucotoxicity [40]. Binding of extracellular AGEs to the advanced glycation-end product receptor (RAGE) expressed by podocytes also contributes to podocyte injury and has been proposed as a druggable target [39,41–43]. These mechanisms of diabetes-induced injury have been extensively described in previous reviews [39,44] and herein we will focus specifically on emerging new mechanisms of podocyte injury that are shared by most proteinuric glomerulopathies and discuss their implications for DN (Figure 2).

Mechanisms of podocyte injury

Haemodynamic insult

When the number of nephrons is reduced because of low nephron mass/nephron loss, there is a compensatory increase in glomerular capillary pressure/filtration of single remnant nephrons. This makes it possible to keep total

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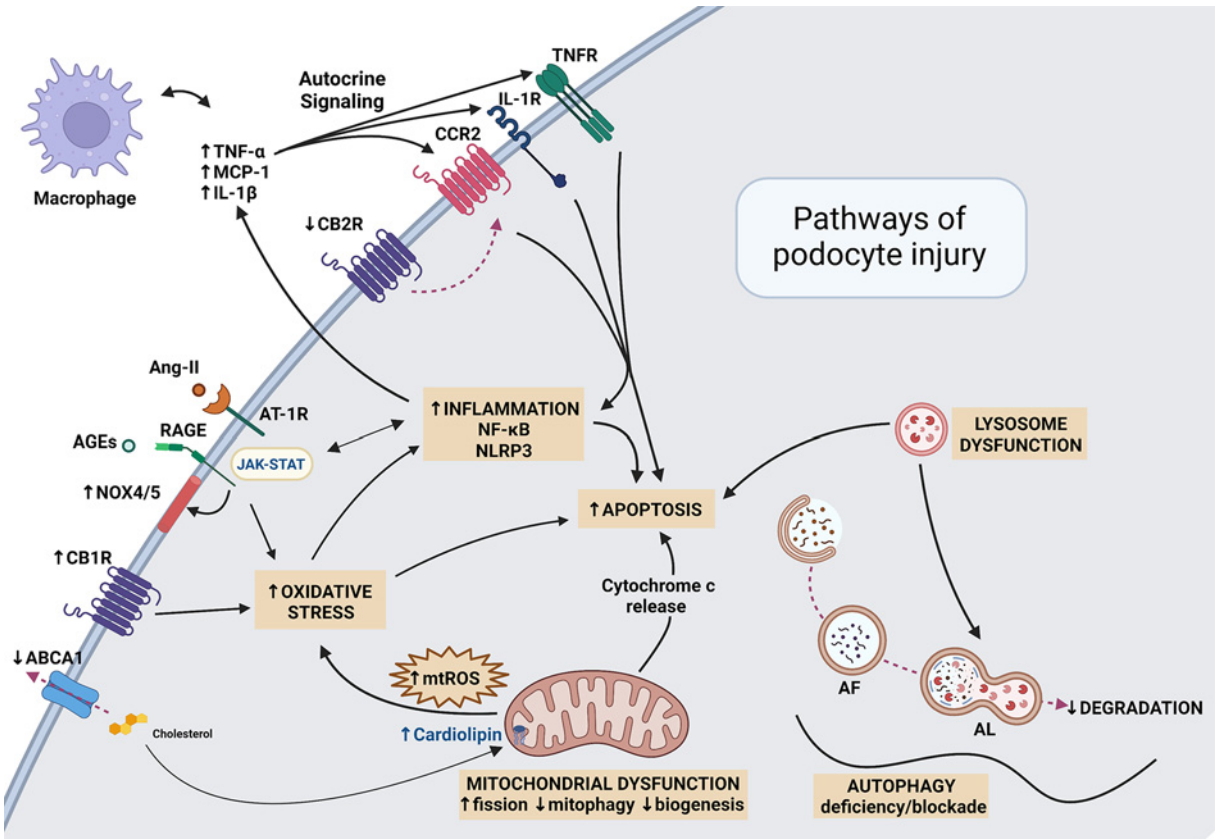

Figure 2. Pathways of podocyte injury

The picture shows selected mechanisms leading to podocyte damage such as inflammation, oxidative stress, organelle dysfunction as detailed in the text. Abbreviations: ABCA1, ATP-binding cassette transporters A member 1; AF, autophagosome; AL, autolysosome; Ang-II, angiotensin-II; AT-1R, angiotensin II type 1 receptor; CB1R, endocannabinoid receptor of type 1; CB2R, endocannabinoid receptor of type 2; CCR2, C–C chemokine receptor 2; IL-1β, interleukin-1β; IL-1R, interleukin 1 receptor; MCP-1, monocyte chemoattractant protein 1; mtROS, mitochondrial reactive oxygen species; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; NOX4/5, NADPH oxidase 4/5; TNF-α, tumour necrosis factor-α; TNFR, tumour necrosis factor receptor.

glomerular filtration rate (GFR) unchanged for a long time [45–47], but causes podocyte damage and represents an important mechanism of CKD progression. In addition, in obesity and diabetes, both glomerular capillary hypertension and single nephron hyperfiltration are early events, occurring prior to nephron loss [48–50]. In diabetes, glomerular hypertension is driven by both an overactive renin–angiotensin system (RAS), causing efferent arteriole vasoconstriction, and enhanced proximal tubular glucose/sodium reabsorption, leading to afferent arteriolar vasodilation. The mechanism whereby mechanical forces contribute to podocyte injury is poorly understood, but both shear stress and mechanical stretching are important [49,50].

Shear stress

The filtration flow produces shear stress that affects predominantly the lateral site of the FPs. Shear stress is very high and further enhanced by hyperfiltration. Increased shear stress tends to detach the podocytes from the GBM, dragging the FPs in the direction of flow. Beyond a certain limit, the SD–FP connection is disrupted, leaving an empty space [51,52]. According to Kriz et al., FP effacement may represent the attempt of podocytes to cope with the increased shear stress and to avoid detachment. Indeed, replacement of the SD with a tight junction would close the slit, abolish shear stress, and allow the system to correct the underlying defect [51]. Lack of FPs and SD in cultured podocytes is an important limit to the study of shear stress *in vitro*; however, there is evidence that podocytes undergo both cytoskeleton reorganisation and apoptosis in response to fluid shear stress [53–56].

Mechanical stretch

Glomerular capillary hypertension increases the pressure gradient between the glomerular capillary and the Bowman space. This expands the glomerular capillaries leading to podocyte stretching. *In vitro* studies have shown that podocyte stretching induces changes in both actin cytoskeleton and cell morphology, down-regulation of α3 integrin, increased fibronectin production, down-regulation of nephrin, and apoptosis [57–64], proving that stretching can dramatically alter the podocyte phenotype. Moreover, a recent study provided the first *in vivo* evidence that podocytes are mechanosensitive by showing that glomerular capillary hypertension can increase podocyte intracellular Ca^{2+} concentration in living mice [65].

Therapeutic relevance

Blockade of the RAS is a well-established therapy for DN. A local RAS is present within the glomeruli and RAS inhibition can limit the direct deleterious effects of Angiotensin-II (Ang-II) on podocytes. However, RAS blockade is believed to provide benefit predominantly by lowering glomerular capillary pressure through efferent arteriole vasodilation [66,67].

Recently, sodium/glucose cotransporter 2 (SGLT2) inhibitors and glucagon-like peptide-1 receptor agonists (GLP1-RAs) were proven to reduce albuminuria/GFR decline in patients with DKD. Moreover, SGLT2 inhibitors also showed efficacy in patients with non-diabetic proteinuric glomerulopathies [68–72]. The underlying mechanism is still unclear; however, amelioration of the haemodynamic insult is likely implicated. Inhibition of SGLT2 blocks sodium/glucose reabsorption in the proximal tubules, while activation of the GLP-1 receptor reduces tubular Na^+ reabsorption [73] by inhibiting the sodium–hydrogen exchanger 3 [74]. The resulting enhanced delivery of Na^+ to the macula densa reactivates the tubular–glomerular feedback. This leads to afferent arteriole vasoconstriction and reduces glomerular capillary pressure/filtration [73–75]. Several other mechanisms of renoprotection have been proposed for both classes of drugs [75,76]; however, it is unclear whether they can have direct effects on podocytes. There is no evidence that podocytes express the GLP-1 receptor *in vivo* [77], while preliminary data in a model of protein-overload proteinuria and a small group of patients with membranous nephropathy [78] suggest that podocytes may express SGLT2 and be a direct target of SGLT2 inhibitors.

Oxidative stress

Reactive oxygen species (ROS) play a key role in the activation of various intracellular signalling pathways. However, increased ROS production and/or insufficiency of antioxidant systems can lead to ROS accumulation and cause oxidative stress. In kidney cells, oxidative stress has several deleterious effects, including lipid peroxidation, DNA damage, protein modification, activation of both pro-inflammatory and pro-fibrotic pathways, and apoptosis [79]. Consistently, mice knockout for superoxide dismutase are more susceptible to adriamycin, Ang-II, and protein overload glomerulopathies [80]. Moreover, *in vitro* studies have shown that a variety of insults (adriamycin, puromycin, Ang-II, TGF-β, high glucose, AGEs) cause podocyte damage by increasing oxidative stress [81–84]. The predominant source of ROS in renal cells is still a matter of debate; however, both mitochondria and NADPH oxidases (NOXs) are believed to be important [85,86].

Mitochondrial ROS

Electrons leaking from the mitochondrial electron transport chain (ETC) react with oxygen to form superoxide anions [87]. Mitochondrial ROS (mtROS) cause inflammation via nuclear factor kB (NF-kB)/inflammasome activation and apoptosis through the release of mitochondrial cytochrome *c* into the cytosol [88,89]. Accumulation of mtROS has been shown in various CKD, including DN and FSGS, and enhanced mtROS production recently confirmed in diabetic db/db mice by real-time mitochondrial redox assessment [90]. Moreover, podocyte-specific overexpression of the antioxidant metallothionein ameliorates experimental DN [91], while podocyte-restricted deletion of mitochondrial glycerol 3-phosphate dehydrogenase exacerbates mitochondrial oxidative stress, podocyte loss, and proteinuria in animal models of both DN and FSGS [92]. However, most of the studies did not identify the glomerular cell type in which mtROS exert their deleterious effect [93–95]. Furthermore, it is unclear whether alterations of the ETC are either the cause or the consequence of oxidative stress.

NOXs

NOXs are transmembrane proteins that transfer electrons across biological membranes, generating anion superoxide. Among NOX isoforms, NOX4 and NOX5 are of particular relevance. In diabetic mice, podocyte-specific induction of NOX4 resulted in GBM thickening, albuminuria, and podocyte loss [96], while both global and podocyte-specific NOX4 deletion attenuated albuminuria, nephrin down-regulation, and FP effacement [97–99]. In cultured podocytes,

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both high glucose and TGF-β1 induce NOX4 expression, resulting in enhanced ROS production [90,97,100]. Moreover, NOX4-derived ROS induce inflammation, fibrosis, and podocyte apoptosis [97,100,101]. Despite these promising preclinical findings, treatment with the specific NOX1/4 inhibitor GKT137831, given on the top of RAS inhibition, failed to show efficacy in patients with DKD [102]. However, rodents do not express NOX5 and, in transgenic animals expressing humans NOX5, NOX4 deletion is no longer protective, suggesting a predominant role of NOX5 over NOX4 in humans [103]. Consistent with this, NOX5 is overexpressed by podocytes in human DN and in podocytes exposed to high glucose. Moreover, transgenic mice overexpressing NOX5 exclusively in podocytes develop albuminuria and FP effacement [104,105]. Ongoing studies will clarify if targeting NOX5 may be beneficial in patients with DN. A role for NOX in podocyte injury has also been demonstrated in other CKD. Exogenous advanced oxidation protein products (AOPPs), which are both markers and triggers of oxidative stress, are increased in the circulation in patients with CKD. Moreover, AOPPs induce podocyte injury both *in vivo* and *in vitro* through activation of the RAGE-NOX-ROS-Wnt/β-catenin pathway [106–108].

Inflammation

A low-grade glomerular inflammation is believed to contribute to the pathogenesis of non-immune proteinuric glomerulopathies. Injured podocytes can release chemokines, inducing recruitment of monocytes, and inflammatory cytokines, contributing to glomerular inflammation. Besides fuelling local inflammation, podocytes also express the receptors for various inflammatory cytokines and are thus potential targets of the deleterious effects of a proinflammatory environment [109]. Indeed, exposure of podocytes to macrophage-conditioned medium induces cell shrinkage, disorganisation of F-actin microfilaments, loss of cell processes, and down-regulation of both nephrin and podocin [110]. Moreover, abrogation of macrophage infiltration ameliorates podocyte injury and albuminuria in DN [111,112], indicating that infiltrating macrophages play a critical role in podocyte dysfunction/injury. Of particular relevance to DN and other proteinuric diseases are the inflammatory systems/pathways described below.

MCP-1/CCR2

Podocytes secrete the chemokine monocyte chemoattractant protein 1 (MCP-1) and binding of MCP-1 to the cognate receptor, C–C chemokine receptor 2 (CCR2) on monocytes drives glomerular monocyte recruitment/activation. Overexpression of MCP-1 prominently by podocytes is observed in several proteinuric diseases, including diabetic and hypertensive glomerulopathies [109,113,114]. Consistently, high glucose, AGEs, and Ang-II induce MCP-1 expression in cultured podocytes via activation of NF-kB [115–117]. The transcription factor, Twist family BHLH transcription factor 1 (TWIST1) is an important negative regulator of MCP-1 expression in podocytes. TWIST1 is overexpressed in both human proteinuric glomerulopathies and animal models of proteinuria and podocyte-specific TWIST1 deletion increases MCP-1, which then promotes glomerular macrophage accumulation, podocyte injury, and proteinuria [118]. Besides inducing podocyte injury indirectly by attracting macrophages, MCP-1 has also direct effects on podocytes as CCR2 is exposed by podocytes and overexpressed in both DN and crescent glomerulonephritis [119,120]. *In vitro* studies have clarified that binding of MCP-1 to CCR2 can induce/mediate nephrin down-regulation [120], podocyte migration, and TGF-β1-induced podocyte apoptosis [119,121].

Intervention studies in experimental animals have provided convincing evidence of a pathogenic role of the MCP-1/CCR2 system in DN. In experimental DN, blockade/deletion of either MCP-1 or CCR2 reduces monocytes/macrophages infiltration, proteinuria, and podocyte injury [120,122–128]. Moreover, in diabetic mice lacking CCR2, re-expression of CCR2 exclusively in podocytes exacerbated DN [129]. In humans, a selective inhibitor of CCR2 (CCX140-B) added to standard care and the Spielgelmer NOX-E36 showed benefit in patients with DKD in phase II clinical trials [130,131], though an improvement of glucose control was also observed in these studies. A phase II clinical trial of CCX140-B in FSGS is underway.

Tumour necrosis factor-α/tumour necrosis factor-α receptor

Podocytes produce tumour necrosis factor (TNF)-α and also express the TNF-α receptors (TNFRs) [132,133]. TNF-α infusion induces albuminuria and podocyte apoptosis *in vivo* and exposure of cultured podocytes to TNF-α causes nephrin loss and apoptosis [134–138]. However, TNF-α deletion specifically in podocytes does not affect albuminuria in proteinuric glomerulopathies, suggesting that TNF-α derived from either infiltrating monocytes or other resident glomerular cells plays a major role in the podocyte injury [118]. Both activation of the phosphoinositide 3 kinase (PI3K)-Akt pathway and alterations of the podocyte actin cytoskeleton are considered the predominant mechanisms of TNF-α-induced podocyte injury [135,136,139]. Recently, additional pathways of damage have been described. For instance, TNF-α induces in podocytes the expression of the transmembrane protein retinoic acid receptor responder 1 (RARRES1), which is cleaved by MMPs into soluble RARRES1. Soluble RARRES1 is then endocytosed by podocytes

and induces apoptosis via a serine/threonine-protein kinase (RIOK1)-p53-dependent mechanism [140,141]. In the context of DN, high glucose and Ang-II have been shown to induce TNF-α expression [142,143]. Moreover, blocking of TNF-α by etanercept, a soluble TNFR2 fusion protein, reduces albuminuria in diabetic mice [144]. However, evidence that this benefit was due to abrogation of TNF-α deleterious effects specifically in podocytes is lacking.

Inflammasome/IL-1β

The inflammasome is a molecular complex that can cleave the precursors of the interleukins (IL)-1β and IL-18, resulting in the production of mature inflammatory cytokines. Moreover, the inflammasome induces pyroptosis, which is a special form of inflammation-driven apoptosis [145]. In cultured podocytes, Ang-II, aldosterone, high glucose, and AGEs activate the inflammasome NLR family pyrin-domain containing 3 (NLRP3) [146–149] and this leads to both nephrin loss and podocyte apoptosis [147]. Moreover, NLRP3 activation is observed in podocytes in high fat-diet (HFD)-induced nephropathy, DN, and following Ang-II/aldosterone infusion. Genetic/pharmacological blockade of NLRP3 ameliorates both albuminuria and podocyte injury in these models [146–151]. Studies in chimeric animals have proven that the NLRP3 inflammasome expressed by renal resident cells, particularly podocytes, rather than in inflammatory cells is the main contributor to the pathogenesis of DN [149]. Data in humans are still lacking; however, two recent randomized controlled trials (RCTs) have proven that treatment with the selective mineralocorticoid receptor antagonist finerenone has renal and cardioprotective effects in patients with DKD [152,153] and NLRP3 blockade may be implicated.

Janus kinase-signal transducer/activator of transcription protein pathway

The Janus kinase (JAK)-signal transducer/activator of transcription protein (STAT) signalling pathway is a major transducer of inflammatory signals. In podocytes this pathway is activated not only by inflammatory cytokines, but also by diabetes-related insults, such as high glucose, AGEs, mechanical stretch, and Ang-II [154–156]. Moreover, an increased expression of JAK-STAT genes was found in kidney glomerular cells, including podocytes, of patients with early DN [157] and renal activation of the JAK-STAT pathway reported in patients with FSGS [158]. Overexpression of JAK2 specifically in podocytes exacerbated both albuminuria and podocyte loss in experimental DN [159]. In addition, exposure of podocytes to AGEs enhanced STAT3 acetylation and blockade of STAT3 acetylation attenuated proteinuria in db/db mice [160]. Recently, a phase II RCT in patients with DKD showed a 40% reduction in albuminuria in subjects receiving the JAK1/2 inhibitor baricitinib for 6 months, though no benefit was observed on renal function [161].

Cytoskeleton and glycocalyx

The actin cytoskeleton is important in preserving the complex podocyte structure and is believed to play a key role in FP effacement. Several mutated genes causing FSGS regulate the actin cytoskeleton and/or its attachment at GBM [162,163]. Mutations of genes encoding for cytoskeleton components/modulators (*ACTN4*, *INF2, AHRGAP24*, *AHRGDIA*) alter podocyte FPs in experimental animals [164–166] and deletion of actin-related protein 3 (ARP3), which mediates the formation of branched actin networks, leads to podocyte detachment [167].

The Rho family of small GTPases (Cdc42, RhoA, Rac1) is the master regulator of the actin cytoskeleton in podocytes and both excessive and insufficient Rho activity can be detrimental [168]. Podocyte-specific Cdc42-deficient mice develop congenital nephrotic syndrome and mice with constitutively active RhoA/Rac1 in podocytes show both FP effacement and proteinuria [169–174]. Nephrin phosphorylation triggers actin cytoskeletal remodelling via Rac1 [175] and Rac1 activation induces internalisation of β1-integrin, reducing podocyte adhesiveness to the GBM [173]. TPCR5, a non-selective channel that promotes influx of Ca^{2+} into podocytes, induces podocyte migration by forming a complex with Rac1 [176] and deletion of short transient receptor potential channel 5 (TRPC5) has protective effects in experimental models of podocyte injury [177]. In FSGS, soluble urokinase plasminogen activator receptor (suPAR) activates β3-integrin and promotes both Cdc42/Rac1 activity and FP effacement [178].

The actin cytoskeleton of podocytes has also been implicated in the pathogenesis of DN. Podocyte-specific Rac1 deficiency ameliorates both podocyte damage and proteinuria in experimental DN by suppressing the Rac1/P21 RAC1-activated kinase 1 (PAK1)/p38/β-catenin signalling cascade [179]. SSLIT-ROBO Rho GTPase-activating protein 2 (SRGAP2a), which suppresses podocyte motility by inactivating RhoA and Cdc42, is down-regulated in both DKD patients and db/db mice. A transcriptional profile of renal biopsy from DKD patients showed that SRGAP2a is one of the genes strongly associated with proteinuria and increasing podocyte SRGAP2a levels ameliorates both podocyte injury and proteinuria in db/db mice [180].

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The cross-talk between podocytes and other glomerular cells can also affect the podocyte actin cytoskeleton. For instance, glomerular endothelial cells (GECs) and mesangial cells release slit guidance ligand 2 (SLIT2) and binding of SLIT2 to the roundabout receptor for SLIT2 (ROBO2) receptor on podocytes inhibits actin polymerisation and reduces podocyte adhesion [181]. Endothelin 1 (ET-1) has also been involved. Podocytes and GECs can secrete ET-1 and they both express the ET-1 A (ET_A) and B (ET_B) receptors. In podocytes, protein overload induces the release of ET-1 through Rho kinase activation and actin cytoskeleton rearrangement [182]. Moreover, in both DN and FSGS, binding of ET-1 secreted by podocytes to the ET_A receptor exposed by GEC induces mitochondrial oxidative stress and dysfunction in GECs, which in turn lead to the release of factors inducing podocyte injury [183–185].

Recent studies have highlighted the importance of the glycocalyx in the ET-1-mediated cross-talk between podocytes and GECs. Glomerular glycocalyx is formed by proteoglycans, glycoproteins, glycolipids, and glycosaminoglycans. Increased expression of proteolytic enzymes (MMP9, hyaluronidase, heparanase), which degrade the glycocalyx, was observed in diabetic patients and implicated in the pathogenesis of albuminuria [186,187]. In experimental DN, GECs-derived ET-1 induces podocyte production of heparanase, which in turn promotes heparan sulphate degradation and glycocalyx disruption on the surface of both podocytes and GECs [188]. In non-diabetic models, degradation of the GECs glycocalyx was instead due to increased GECs expression of heparanase and hyaluronoglucosaminidase in response to podocyte-released factors and ET-1 [189]. Treatment with the ET_A receptor antagonist atrasentan prevented glycocalyx degradation in patients with DKD through reduction in glomerular and endothelial heparanase expression [190]. In line with these experimental studies, treatment with ET_A receptor antagonists showed anti-proteinuric/renoprotective effects in patients with DKD and FSGS [191,192].

The endocannabinoid system

The endocannabinoid system (ECS) comprises the cannabinoid receptors of type 1 (CB1R) and of type 2 (CB2R), the two endocannabinoids (ECs), anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and the enzymes involved in their synthesis and degradation [193]. The ECS plays an important role in multiple aspects of neural functions [194,195] and is involved in the control of both glucose and lipid metabolism in the liver and adipose tissue [196–198]. In non-metabolic organs, the EC receptors are expressed at low levels, but may undergo up/down-regulation in pathological conditions. In addition, in various chronic degenerative diseases an increased CB1R signalling has been causally linked to the development of oxidative stress, apoptosis, and fibrosis, while an overactive CB2R appears beneficial as it down-regulates inflammatory processes [199].

The hypothesis of a possible relevance of the ECS in podocytes originates from a bulk of work showing that podocytes have structural and molecular similarities with neurons [200–202]. Consistent with this, studies performed in both experimental animals and human renal biopsies have shown that normal podocytes express the CB2R at high levels and have all the enzymatic armamentarium for the synthesis and catabolism of the two ECs [203]. The physiological role of CB2R signalling in normal podocytes is unknown; however, given the importance of CB2R in suppressing inflammation, a high CB2R tone may be required to keep inflammation at bay and to prevent inflammation-driven podocyte injury [203,204].

Most of the studies exploring the role of the ECS in podocyte injury were performed in the context of haemodynamic/metabolic-induced renal diseases, such as obesity-induced nephropathy, Ang-II-nephropathy, and DN. Overall this work has demonstrated that the ECS is altered in these pathological conditions and that correction of these ECS abnormalities ameliorates SD protein loss, albuminuria, and podocyte apoptosis, proving that the ECS is implicated in the pathogenesis of the podocyte injury [205–207]. Specifically, in both human and experimental DN, podocytes show both CB1R overexpression and CB2R down-regulation [203,205,206,208,209]. Studies on cultured podocytes suggest that hyperglycaemia is the likely mechanism of CB1R up-regulation, while mechanical stretching is involved in podocyte CB2R loss [205,206,210]. The causal link between altered CB1R/CB2R signalling and podocyte injury have been proven by studies of both pharmacological and genetic manipulation of the ECS. Transgenic mice overexpressing CB1R spontaneously develop both albuminuria and nephrin loss [211]. Moreover, in diabetic animals, treatment with CB1R blockers and/or CB2R agonists as well as selective CB1R deletion in podocytes ameliorates both albuminuria and loss of SD proteins [203,205,206,208,212,213]. Importantly, the efficacy of CB1R blockade was also proven in diabetic animals with established albuminuria and in combination with RAS inhibition to mimic the clinical scenario [214]. *In vitro* studies have clarified that activation of the CB1R on podocytes induces nephrin loss via a Gi/o-cAMP-NOX4-dependent pathway and that both high glucose- and AGE-induced nephrin down-regulation are mediated by CB1R [205]. In addition, CB1R blockade reduces Ang-II-induced SD protein loss, suggesting that signalling through CB1R may be the final common pathway whereby not only hyperglycaemia, but also Ang-II causes podocyte damage [205]. Although CB2R signalling has potent anti-inflammatory effect, studies in

chimeric animals and cultured podocytes clarified that activation of the CB2R expressed on podocytes plays a major role in preventing both nephrin loss and albuminuria. This occurs via CB2R-mediated CCR2 down-regulation that prevents MCP-1-induced nephrin loss [210].

In view of potential future clinical applications, it is important to underscore that novel specific and peripheral-restricted CB1R blockers, such as AM6545 and JD5037, that do not cross the blood–brain barrier and are thus devoid of undesired central side effects are now available and are currently under testing in humans in the context of metabolic liver diseases [215]. Therefore, pharmacological manipulation of the ECS may represent a novel strategy for podocyte protection.

Autophagy

Autophagy is a conserved process by which cytoplasmic components, including organelles, are sequestrated within double-membraned vesicles termed autophagosomes, and then delivered to the lysosomes for degradation and recycling. Autophagy is under the control of the nutrient-sensing mTOR complex 1 (mTORC1), AMP-activated protein kinase (AMPK), and NAD-dependent deacetylase Sirt-1 pathways. mTORC1, which is activated by amino acids and growth factors, suppresses autophagy, whereas AMPK and Sirt-1, which are activated by energy depletion and increased NAD^+ levels, respectively, promote autophagy [216].

Podocytes have high levels of basal autophagy [217,218] and at variance with other cell types basal autophagy is primarily regulated by AMPK, rather than by mTORC1 signalling [219]. Autophagy allows the removal of both dysfunctional organelles and misfolded proteins, and serves as an energy-saving mechanism through recycling. Therefore, the maintenance of an intact basal autophagic flux is crucial for both integrity and function of podocytes. Moreover, under conditions of cellular stress, autophagy is activated in podocytes to preserve cell homoeostasis.

Among proteinuric conditions, autophagy is of particular relevance in DN given the close link between autophagy and cell metabolism. The effect of diabetes on autophagy is complex as excess of nutrients and/or energy would inhibit autophagy, whereas enhanced diabetes-induced cellular stresses would activate autophagy [220]. Studies in both streptozotocin (STZ)-induced diabetes and culture podocytes exposed to high glucose have shown an early induction of autophagy, followed by inhibition [221]. β-arrestins, which are up-regulated by high glucose in podocytes, have been proposed as a mechanism of autophagy inhibition via suppression of autophagy-related protein (Atg) 7 (Atg7) [222]. Moreover, AGEs inhibit autophagy in podocytes by activating mTORC1 and blocking transcription factor EB (TFEB) [223]. On the other hand, studies in both patients with DN and podocytes exposed to AGEs suggest that autophagy blockade due to lysosomal dysfunction rather than autophagy deficiency is the major abnormality in DN [224]. Consistent with the notion that lysosomal dysfunction is important in altering podocyte autophagy, mice with podocyte-specific deletion of lysosomal enzymes, such as prorenin receptor and cathepsin D, show autophagosome accumulation and FP effacement [225,226].

The importance of autophagy is highlighted by studies of genetic/pharmacological modulation of autophagy in animal models of proteinuria. Podocyte-specific deletion of the autophagic gene *Atg5* dramatically increases the susceptibility to the development of puromycin-, diabetes-, and HFD-induced nephropathy [218,221]. Global deletion of SIRPα, which positively controls autophagy, exacerbates adriamycin, puromycin, and DN [227]. Conversely, pharmacological induction of autophagy, using either the mTORC1 inhibitor rapamycin [228–231] or a variety of AMPK/SIRT1 activators [232–234] ameliorates podocyte injury in DN. However, the complete ablation of mTORC1 activity in podocytes by Raptor deletion led to the early development of proteinuria in diabetic mice [235,236], indicating that while excessive mTORC1 activation is detrimental, basal mTORC1 activity is required for maintaining physiological functions of podocytes. Taken together, these findings indicate that autophagy in podocytes may represent a promising target in DN and other glomerulopathies. Of interest, a highly effective oral inhibitor of activated AMPK degradation has been recently developed [237] and will be tested in humans. However, given the high degree of complexity of autophagy, fine-tuning of podocyte autophagy is required to achieve clinical benefit. Of interest, among current anti-diabetic drugs both metformin and SGLT2-i have pro-autophagic properties that are believed to contribute, at least in part, to their renoprotective effects in DN [238,239].

Mitochondria

The most important function of mitochondria is the generation of ATP through oxidative phosphorylation (OXPHOS). Moreover, mitochondria are also involved in a vast array of other cellular functions, including calcium homoeostasis, cell metabolism, and apoptosis [240]. Injured mitochondria not only produce less ATP, but also release

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dangerous molecules, such as mtROS, cytochrome *c*, and free mitochondrial DNA (mtDNA) [241,242]. Highly orchestrated processes of mitochondrial quality control enable cells to avoid the dangers of mitochondrial injury. Mitochondrial fusion reduces mitochondrial stress by mixing the content of both damaged and healthy mitochondria, thus diluting stress [243]. Fission segregates damaged portions of the mitochondria that are then removed by mitophagy [244]. Defective mitochondria are replaced by mitochondrial biogenesis [245]. Although mice with podocyte-specific deletion of crucial components of the mitochondrial quality control do not have a renal phenotype [246], these processes may become important in podocytes exposed to stressful conditions and/or having enhanced metabolic needs.

OXPHOS

The importance of OXPHOS as an energy source in podocytes is a matter of debate. Podocytes have less mitochondria compared with other kidney cells and cultured podocytes rely predominantly on anaerobic glycolysis to meet their energy requirements [246]. However, OXPHOS components increase during podocyte differentiation [247], suggesting relevance in podocyte homoeostasis. Moreover, cultured podocytes lacking interdigitating FPs might have different energy requirements compared with podocytes *in vivo*. Finally, OXPHOS may become important in podocytes exposed to stress. Consistent with the notion that OXPHOS is relevant in podocytes, mutations of genes (*COQ2, PDSS2, COQ6 ADCK4, PDSS1*) involved in coenzyme Q10 biosynthesis cause the development of FSGS in humans [248–252]. A mouse with a spontaneous homozygous missense mutation of decaprenyl diphosphate synthase subunit 2 (PDSS2) develops proteinuria and FP effacement [253]. Podocyte-specific AarF domain-containing protein kinase 4 (ADCK4) ablation results in abnormally large and dysfunctional mitochondria and FSGS development [254,255]. Inhibition of mitochondrial OXPHOS machinery by selective deletion of CR6-interacting factor 1 (CRIF1) results in mitochondrial dysfunction, FP effacement, and proteinuria [256]. In DN, exposure of podocytes to high glucose reduces OXPHOS [247] and glomeruli from Pima Indians with early DN show dysregulation of genes encoding components of the respiratory chain [257]. Moreover, there is evidence that hyperglycaemia reduces OXPHOS in podocytes via Smad4-mediated inhibition of the degradation of the ATP synthase inhibitor [258].

mtDNA

In humans, mtDNA 3243 A>G mutation causes the development of FSGS. A large-scale proteomics analysis of urine samples from adult patients with mitochondrial diseases found this mutation in 75 out of 117 subjects [259], indicating that it is the most common genetic mitochondrial disorder with renal involvement. mtDNA is highly susceptible to oxidative stress because of lack of protective histone proteins and proximity to mtROS and there is renal accumulation of oxidative mtDNA lesions and loss of mtDNA copy number in experimental DN [260,261]. High glucose, aldosterone, and peroxide hydrogen reduce mtDNA in podocytes [262,263] and mtDNA-depleted podocytes show increased mtROS levels, reduced mitochondrial membrane potential, and nephrin down-regulation [262]. Mechanisms of mtDNA repair are highly relevant and podocyte-specific deletion of Mpv17, which is involved in mtDNA maintenance, results in mitochondrial dysfunction and severe glomerular disease under conditions of stress [264].

Fusion–fission

Enhanced mitochondrial fragmentation due to increased mitochondrial fission is observed in podocytes in both human and experimental DN [265]. Mitochondrial fission is under the control of dynamin-related protein 1 (Drp1), which, once activated through phosphorylation, translocates to the outer mitochondrial membrane and promotes fission [266]. In experimental DN, genetic strategies blocking Drp1 expression/phosphorylation specifically in podocytes reduce mitochondrial fission, ameliorate podocyte damage, and protect against DN progression [267–269]. In keeping with this, *in vitro* pharmacological inhibition/deletion of Drp1 protects podocytes exposed to high glucose, aldosterone, palmitate from mitochondrial dysfunction and injury [270–272]. By contrast, puromycin and adriamycin enhance podocyte mitochondrial fission by repressing mitofusin 1, which is a key driver of mitochondrial fusion [273].

Mitophagy

Mitophagy is a mechanism of selective degradation of mitochondria via autophagy. It is predominantly controlled by the PTEN-induced kinase 1 (PINK1)/Parkin pathway that is responsible for both tagging and delivery of mitochondria to autophagosomes [274]. Increasing evidence indicates impaired mitophagy in DN. PINK1 expression is reduced in both diabetic mice and podocytes exposed to high glucose [275]. The SIRT1-PGC1α-FOXO1 pathway is implicated in PINK1 down-regulation [276,277] and podocyte-specific forkhead box protein O1 (FOXO1) overexpression restores PINK1 expression and reduces both mitochondrial dysfunction and podocyte injury [278].

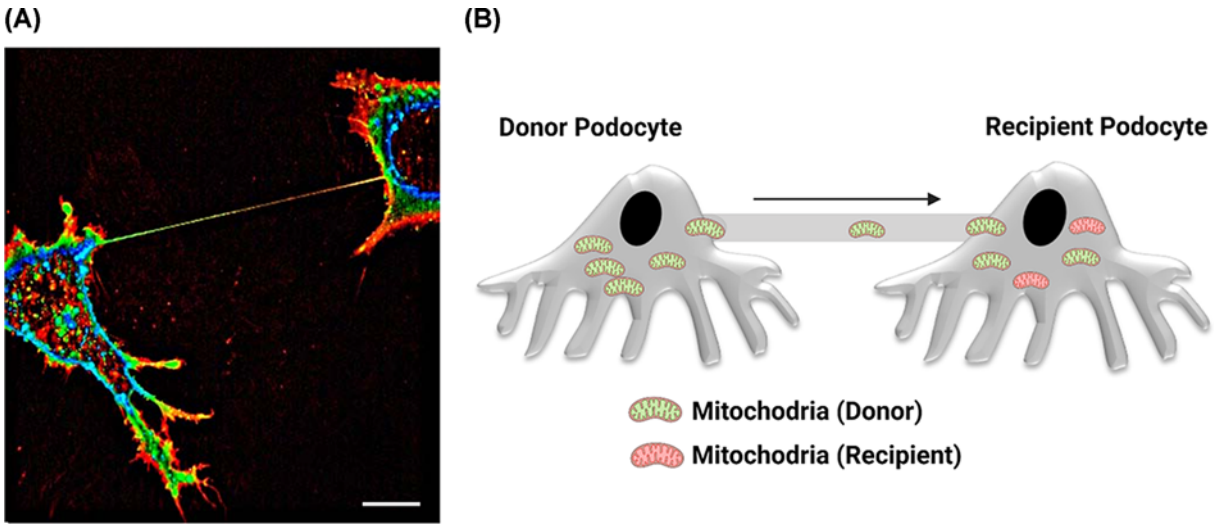

Figure 3. TNT formation between podocytes

(**A**) Representative image showing a TNT-like channel interconnecting two podocytes (magnification 630×, scale bar = 50 μm). Serial Z-stack images prove that the TNT does not adhere to the substrate. Colours represent the Z-depth (depth coding; red: bottom, blue: top). (**B**) Schematic representation of TNT-mediated mitochondrial transfer between podocytes.

Mitochondrial biogenesis

Peroxisome proliferator-activated receptor-γ co-activator (PGC-1α) is the master regulator of mitochondrial biogenesis and silencing of PGC-1α leads to podocyte injury by disrupting mitochondrial function. Decreased glomerular PGC-1α expression has been reported in both patients with DKD and experimental models of both FSGS and DN [279–281]. Sirt1, AMPK, and Tug1 are positive regulators of PGC-1α and they are likely implicated. Sirt1 activates PGC-1α via deacetylation and podocyte-specific deletion of Sirt1 not only decreases PGC-1α expression, but also increases the susceptibility to both adriamycin and DN [282]. Moreover, it exacerbates both albuminuria and podocyte damage in aged non-diabetic mice [283]. On the contrary, overexpression of Sirt1 in podocytes attenuates DN progression [284]. Activation of the AICAR-AMPK-PGC-1α pathway improves experimental DN [234]. Overexpression of Tug1 in podocytes rescues PGC-1α expression, improves mitochondrial bioenergetics, and reduces both podocyte injury and albuminuria in db/db mice [285,286]. Although PGC-1α may represent a potential target to improve podocyte health, a recent study has shown that PGC-1α overexpression in podocytes induces the formation of giant mitochondria and increases podocyte both proliferation and dedifferentiation, resulting in collapsing glomerulopathy. Therefore, the level of PGC-1α expression needs to be tightly regulated to maintain podocyte health.

Lipotoxicity

Growing evidence indicates that lipids can contribute to podocyte injury. Lipid accumulation in renal cells, including podocytes, may cause lipotoxicity and CKD has been proposed as a form of fatty kidney disease. Moreover, the podocyte SD is assembled in specialised plasma membrane domains (lipid rafts), which are enriched in both cholesterol and sphingolipids. Therefore, changes in podocyte lipid metabolism/composition may affect podocyte intracellular signalling.

Cholesterol

Most of the studies linking cholesterol to podocyte injury focused on the ATP-binding cassette transporters A member 1 (ABCA1) that mediates cholesterol efflux from podocytes. Both ABCA1 down-regulation and podocyte accumulation of lipid droplets are observed in patients with FSGS and DN [134,287] as well as in cultured podocytes exposed to Ang-II, TNF-α, and serum from patients with early DN and FSGS [134,257,287,288]. Furthermore, podocyte-specific ABCA1 deletion exacerbates albuminuria in both experimental DN and TNF-induced glomerulopathy [134,257]. However, accumulation of the mitochondrial phospholipid cardiolipin, leading to mitochondrial dysfunction, rather than accumulation of free cholesterol appears to be the predominant mechanism linking ABCA1 deficiency to podocyte apoptosis. Consistent with this, deletion of sterol-*o*-acyltransferase-1 (SOAT1), which converts free cholesterol into cholesterol esters, induces free cholesterol accumulation, but does not cause glomerular injury in

Table 1 Ongoing clinical trials on new drugs in FSGS and DKD

Mechanism of action	NCT number	Drug	Phase	Subjects (*n*)	RCT (Y/N)	Population	Primary outcome
Dual ET_AR and AT1R antagonist	NCT03493685	Sparsentan	III	371	Y	FSGS	Slope eGFR UP/C $\leq$ 1.5 g/g UP/C $\downarrow$ 40%
	NCT05003986	Sparsentan	II	57	N	FSGS, MCD, Alport Syndrome, IgA Nephropathy, IgA-Associated Vasculitis	Safety change in UP/C
ET_AR antagonist	NCT04573920	Atrasentan	II	80	N	FSGS, Alport Syndrome, IgA nephropathy, DKD on the top of RASi and SGLT2i	Change in UP/C (FSGS) Change in UA/C (DKD)
APOL1 antagonist	NCT04340362	VX-147	II	16	N	APOL1-mediated FSGS	Change in UP/C
CCR2 inhibitor	NCT03703908	CCX140-B	II	13	N	FSGS	Change in UP/C
FLAP	NCT04492722	AZD5718	II	632	Y	67% DKD; 33% non-DKD	Change in UA/C
SLIT2 antagonist	NCT03448692	PF-06730512	II	44	N	FSGS	Change in UP/C
TRPC5 inhibitor	NCT04387448	GFB-887	II	125	Y	FSGS, TR-MCD, DKD on top of RASi or ARB	Change in UP/C Change in UA/C
PDE4	NCT04755946	Roflumilast	III	48	Y	DKD	Change in UA/C Absolute change in eGFR
Non-specific PDE inhibitor	NCT03625648	Pentoxifylline	IV	2510	Y	DKD	Time to ESKD or death
Periferally CB1R inverse agonist	NCT04880291	GFB-024	I	56	Y	Healthy overweight and obese volunteer, T2DM treated with lifestyle modification or metformin	Safety and tolerability
IL-33 monoclonal Ab	NCT04170543	MEDI3506	II	565	Y	DKD on top of RASi or ARB and dapagliflozin	Change in UA/C
Monoclonal Ab-based inhibitor of VEGF-B	NCT04419467	CSL346	II	100	Y	DKD	Change in UA/C
PPARα activator	NCT04929379	Fenofibrate	II	40	Y	DKD (T1DM)	Baseline-adjusted eGFR Baseline-adjusted levels of 21 serum biomarkers of increased ESKD risk
TP	NCT04881123	SER150	II–III	100	Y	DKD on top of RASi or ARB	>30% change in UA/C
Selective MR modulator	NCT04595370	AZD9977	II	540	Y	HFrEF+CKD (40% DKD) on top of dapaglifozin	Change in UA/C
MSCs	NCT04125329	UC-MSCs	I	15	N	DKD	Safety
	NCT04562025	UC-MSCs	NA	38	Y	DKD	Safety
	NCT04216849	UC-MSCs	I–II	54	Y	DKD	Change in UA/C
	NCT02585622	MSCs	I–II	48	Y	DKD	Safety
	NCT03840343	Autologous MSCs	I	30	N	DKD	Safety

Trials were selected by using as key words, FSGS (focal segmental glomerulosclerosis), DN (diabetic nephropathy). Only trials that are yet to be completed have been included. Search on clinicaltrails.gov was performed on 27 October 2021. Abbreviations: APOL1, apolipoprotein L1; ARB, angiotensin receptor blocker; AT1R, angiotensin II receptor type 1; CB1R, cannabinoid receptor type 1; CCR2: C–C chemokine receptor type 2; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; ET_AR, endothelin receptor subtype A; FLAP, 5-lipoxygenase-activating protein; HFrEF, heart failure with reduced ejection fraction; MCD, minimal change disease; MR, mineralocorticoid receptor; PDE4, phosphodiesterase 4; PPARα, peroxisome proliferator-activated receptor α; RASi, renin–angiotensin system inhibitor; SGLT2i, sodium/glucose cotransporter-2 inhibitor; TP, thromboxane; TPCR5, transient potential channel receptor 5; T1DM, type 1 diabetes; T2DM, type 2 diabetes; UA/C, urine albumin/creatinine; UC-MSC, human umbilical cord MSC; UP/C, urine protein/creatinine; VEGF-B, vascular endothelial growth factor type B.

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mice with podocyte-specific deletion of ABCA1. Moreover, the cardiolipin peroxidase inhibitor elamipretide ameliorates both albuminuria and podocyte loss in mice with established DN [257]. Small molecule drugs that up-regulate ABCA1-dependent cholesterol efflux by targeting oxysterol efflux by targeting oxysterol-binding protein-like 7 (OSBPL7) normalise proteinuria and prevent renal function decline in both adtiamycin-induced nephropathy and Alport Syndrome [289].

Sphingolipids

Recent studies implicate sphingolipids and in particular ceramide in podocyte injury. This is not surprising given the central role of ceramide in promoting apoptosis, cell cycle arrest, and senescence [290,291]. Mice with podocyte-specific deletion of the acid ceramidase main catalytic subunit (ASAH1) show glomerular ceramide accumulation, FP effacement, and nephrotic proteinuria [292]. In these mice, further deletion of sphingomyelin phosphodiesterase 1 (Smpd1), which hydrolyses sphingomyelin to ceramide, prevents ceramide accumulation, and protects against podocyte injury. Sphingomyelinase-like phosphodiesterase 3b (Smpdl3b), which inhibits ceramide conversion into ceramide-1-phosphate (C1P), is increased in the glomeruli from patients with DN and db/db mice and Smpdl3b deletion specifically in podocytes protects from the development of albuminuria [293]. Ceramide is also metabolised by sphingosine kinase (SPHK) to sphingosine 1 phosphate (S1P), which is then degraded to phosphoethanolamine and hexadecenal by S1P lyase (SGPL1). The importance of S1P has been highlighted by the discovery that genetic SGPL1 deficiency, leading to S1P and ceramide accumulation, is associated with the development of FSGS in humans. Moreover, mice with global SGLP1 deletion develop both FP effacement and severe proteinuria [294–297], though evidence of a specific relevance of S1P in podocytes is still lacking.

Podocyte repair/regeneration

Given the poor capacity of podocytes to proliferate and thus to replace lost podocytes [11], strategies for podocyte repair/regeneration are of great interest.

Parietal epithelial cells

Recent studies suggest that glomerular parietal epithelial cells (PECs) covering the Bowman's capsule are podocyte precursors and can replace lost podocytes. Studies of single lineage tracing in experimental FSGS support this notion [298–300]. Recently, using dual lineage tracing of both podocytes and PECs, it was convincingly shown that PECs can transdifferentiate in adult podocytes in FSGS [301]. Cells of the renin lineage have also been proposed as podocyte precursors based on experiments of single/dual lineage tracing in models of both FSGS and nephron loss [302–304]. There is, thus, an increasing quest for novel approaches that may favour transdifferentiation of precursors into podocytes. In mice with FSGS, treatment with RAS inhibitors leads to partial podocyte replenishment after podocyte loss [305], suggesting podocyte regeneration. Inhibition of the chemokine, C–X–C motif chemokine ligand 12 (CXCL12) is another potential strategy as CXCL12 is released by healthy podocytes and suppresses PECs transdifferentiation [300]. Moreover, retinoic acid favours PECs differentiation into podocytes [306,307] and in experimental proteinuric glomerulopathies treatment with all-trans retinoic acid increases the number of both podocytes and cells with a mixed PEC/podocyte phenotype [307]. Evidence of replacement of lost podocytes by local precursors in DN is limited. However, in leptin-deficient BTBR ob/ob mice, leptin re-expression reverted advanced DN, indicating that restoration of lost podocytes is possible [308]. Moreover, cells expressing both podocyte and PEC markers were observed in both the glomerular tuft and the Bowman's capsule in kidney biopsies from patients with DN [309].

Stem cells

Mesenchymal stem cells (MSCs) have been proposed as a potential therapeutic strategy for regenerative medicine. Today, we know that the beneficial effects of exogenous MSCs are predominantly due to MSC release of both soluble factors and extracellular vesicles (EVs). MSC-derived EVs can horizontally transfer to podocytes miRNAs, mRNAs, and proteins, affecting the podocyte phenotype. In a 3D glomerular fluidic system, treatment with MSC-EVs decreases adriamycin-induced both podocyte apoptosis and albumin permeability [310]. In db/db mice, EVs released by adipose tissue-derived MSC (ADMSC-EVs) ameliorate both albuminuria and podocyte apoptosis by transfer of miR-486, miRNA-215-5p, miRNA-26a-5p [311–313]. Podocytes can also be obtained from induced pluripotent stem cells (iPSCs) [314]. Recent studies have shown that iPSC-derived podocytes transplanted into mouse kidneys can integrate within the glomeruli [315] and reconstitute the kidney glomerular–capillary wall function on a microfluidic chip [316]. Therefore, both healthy and patient-specific human podocytes can be generated from iPSCs to model podocyte diseases, screen drugs, and develop cell-based treatment. From pluripotent stem cells, it is also possible to

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generate kidney organoids [317]. Of interest, a recent study has identified gene expression signatures of podocytes during human nephrogenesis and organoid development and discovered that elements of these signatures were reactivated in progressive glomerular disease, including DN [318].

Tunnelling nanotubes

Tunnelling nanotubes (TNTs) are nanosized open-ended membrane channels without contact with the substrates that interconnect cells. TNTs are a mechanism of cell-to-cell communication that allows horizontal intercellular transfer of various cellular components, including organelles as mitochondria and lysosomes [319,320] (Figure 3). Transfer of organelles from healthy to damaged cells via TNTs represents an important survival mechanism and TNT-mediated mitochondrial exchange is considered a mechanism of mitochondrial quality control [321]. Recent data show that podocytes exposed to injury can form TNTs via an M-Sec-dependent mechanism. In adriamycin-treated podocytes the M-Sec–TNT system allows mitochondria transfer, ameliorates mitochondrial bioenergetics, and partially reverts podocyte injury. Moreover, *in vivo* M-Sec is overexpressed by podocytes in both human and experimental FSGS and M-Sec deletion causes podocyte injury, mitochondrial abnormalities, and the spontaneous development of progressive FSGS [322].

Podocytes and personalised medicine

In the past decade, there has been growing interest in the use of omics techniques to study the kidney. Omics data derived from kidney biopsy tissue coupled with genetic, epigenetic, proteomics, metabolomics analyses and clinical data (phenotypes, longitudinal outcomes) have the potential not only to identify novel biomarkers and druggable targets, but also to clarify cellular responses to insults and disease mechanisms. Achieving a more comprehensive molecular understanding of proteinuric glomerulopathies through systems biology is instrumental for providing personalised care to people with CKD/DKD [323].

Transcriptomics data from human kidney biopsy samples can be integrated with genetic information to clarify the functional effect of genetic polymorphisms [324]. For instance in patients with FSGS comparison of the transcriptome of subjects with high-risk vs. low-risk ApoL1 variants showed differences in expression and/or coexpression of genes involved in mitochondrial regulation [325]. Differential gene expression profiling can help identify transcriptional changes that are involved in the pathogenesis of the renal injury or can predict outcomes. Integration of transcriptomic data from human kidney biopsies with data from animal models can identify shared pathways of injury and help select appropriate models for preclinical studies [326]. Transcriptional data can also be integrated with structural data in order to identify both markers and pathways of progression. Novel approaches, such as digital pathology, computational image analysis, and pattern recognition, can complement traditional histological assessment. The NEPTUNE digital pathology scoring system, which enables morphologic profiling of renal structures, including podocyte damage, has been validated and shows good reproducibility [327]. Using this scoring system, patients with FSGS/minimal change disease were grouped into clinically and biologically relevant subgroups that were associated not only with clinical outcomes, but also with molecular signatures, reflecting activation of immune/inflammatory pathways [328].

Single-cell RNA sequencing (scRNA-seq) technology can also be applied to kidney biopsy samples to obtain cell-specific data. scRNA-seq transcriptome analyses can redefine cellular types and subtypes within the glomeruli based upon their transcriptional profile and improve disease classification. In addition, this novel approach can give insight on the molecular processes at the cellular level [329] and identify podocyte-specific changes that occurs during disease onset, progression, and remission [330]. A recent study profiled 4332 individual glomerulus-associated cells isolated from human living donor renal biopsies and mouse kidney. The study identified genetic programmes for all glomerular cell types, including podocytes, and demonstrated remarkable species differences [331]. Although scRNA-seq is very promising for the study of podocyte pathophysiology, new strategies for tissue processing into single cells, such as microfluidic systems, are required [332]. Moreover, integration of large and complex scRNA-seq data into biological mechanisms is still very challenging [332]. However, rapid progress in statistical, computational, and artificial intelligence methods will enable mapping of very large datasets in the near future.

Conclusion and future perspective

Despite progress in our understanding of the mechanisms of podocyte injury, several questions remain unanswered. In particular, the study of mitochondria, lipid metabolism, and autophagy in podocytes is still in its infancy and awaits further investigation in both health and disease. The lack of podocytes forming a filtration slit in culture represents an

important limit to *in vitro* studies, given the importance of the SD for podocyte structure, intracellular signalling, response to haemodynamic stress, and likely cell metabolism. Moreover, renal biopsies are rarely performed in DKD and usually only in advanced stages of the disease, limiting our ability to relate mechanisms of hyperglycaemia-induced podocyte damage to early podocyte abnormalities. New high-resolution imaging systems and systems biology approaches are likely to significantly enhance our understanding of podocyte biology in the next future [333,334].

Novel drugs have recently become available for the treatment of diabetic and other proteinuric glomerulopathies. Dual inhibition of SGLT2 and RAS is highly effective in reducing both proteinuria and eGFR decline in patients with DKD [68] and other proteinuric forms of CKD [69]. RCTs have also proven benefit of both finerenone and atrasentan in DKD [152,153,191]. However, there is still a residual risk of progression to ESRD and stimulated emission depletion (STED) super-resolution microscopy has revealed a significant residual podocyte injury in db/db mice treated with metformin and both RAS and SGLT2 inhibitors [335]. Available drugs predominantly target the hemodynamic insults; therefore, non-hemodynamic mechanisms described in this review may represent additional promising targets. Drugs acting upon oxidative stress, inflammation, lipotoxicity, ECS, autophagy, and mitochondrial dysfunction, have been tested in experimental models of proteinuria and some are currently under clinical development (Table 1). However, in the last 20 years, drugs that were very effective in experimental animals failed to prove efficacy in humans. To improve translation of results from preclinical to clinical studies, it would be important to test novel drugs in animal models that closely replicate the clinical scenario, including treatment with standard-of-care therapy, and to apply study designs similar to that used in RCTs [336]. The discovery that patients with mutations of genes involved in coenzyme Q10 biosynthesis respond to oral coenzyme Q10 supplementation demonstrates that a personalised medicine approach, targeting specific mechanisms of podocyte injury, may be advantageous [337]. Moreover, culturing podocytes derived from iPSCs on the chip and kidney organoids represent new important tools in drug discovery [316]. Besides preventing and/or halting the progression of podocyte injury, an emerging approach is to favour podocyte repair/replacement by either activating podocyte precursors or modulating podocyte phenotype via both EVs and TNTs. In particular, genetically engineered EVs may represent next-generation cell-free therapeutic products for podocyte regenerative medicine.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ABCA1, ATP-binding cassette transporters A member 1; AGE, advanced glycation-end product; AMPK, AMP-activated protein kinase; Ang-II, angiotensin-II; Atg, autophagy-related protein; CB1R, cannabinoid receptor of type 1; CB2R, cannabinoid receptor of type 2; CCR2, C–C chemokine receptor 2; CKD, chronic kidney disease; CXCL12, C–X–C motif chemokine ligand 12; DKD, diabetic kidney disease; DN, diabetic nephropathy; Drp1, Dynamin-related protein 1; EC, endocannabinoid; ECS, endocannabinoid system; ESRD, end-stage renal disease; ETC, electron transport chain; ET-1, endothelin 1; EV, extracellular vesicle; FP, foot process; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; GEC, glomerular endothelial cell; GFB, glomerular filtration barrier; GFR, glomerular filtration rate; GLUT, glucose transporter; HFD, high fat-diet; IL, interleukin; iPSC, induced pluripotent stem cell; JAK, Janus kinase; MCP-1, monocyte chemoattractant protein 1; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; mtDNA, mitochondrial DNA; mTORC1, mTOR complex 1; mtROS, mitochondrial reactive oxygen species; NF-kB, nuclear factor kB; NLRP3, NLR family pyrin-domain containing 3; NOX, NADPH oxidase; PDSS2, decaprenyl diphosphate synthase subunit 2; PEC, parietal epithelial cell; PGC-1α, peroxisome proliferator-activated receptor-γ co-activator; PINK1, PTEN-induced kinase 1; RAGE, advanced glycation-end product receptor; RARRES1, retinoic acid receptor responder 1; RAS, renin–angiotensin system; RCT, randomized controlled trial; ROS, reactive oxygen species; SD, slit diaphragm; SGLT2, sodium/glucose cotransporter 2; SGPL1, S1P lyase; SLIT2, slit guidance ligand 2; Smpdl3b, sphingomyelinase-like phosphodiesterase 3b; SOAT1, sterol-*o*-acyltransferase-1; SRGAP2a, SLIT-ROBO Rho GTPase-activating protein 2; STAT, signal transducer/activator of transcription protein; STZ, streptozotocin; S1P, sphingosine 1 phosphate; TGF-β1, transforming growth factor β1; TNF, tumour necrosis factor-α; TNFR, tumour necrosis factor-α receptor; TNT, tunnelling nanotube; TWIST1, Twist family BHLH transcription factor 1.

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