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Review

Diabetic nephropathy: An update on pathogenesis and drug development

Vikram Rao A/L B Vasanth Rao [a], Sean Hong Tan [b], Mayuren Candasamy [c], Subrat Kumar Bhattamisra [c, *]

[a] School of Postgraduate Studies, International Medical University, No 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000, Kuala Lumpur, Malaysia
[b] School of Pharmacy, International Medical University, No 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000, Kuala Lumpur, Malaysia
[c] Department of Life Sciences, School of Pharmacy, International Medical University, No 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000, Kuala Lumpur, Malaysia

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ABSTRACT

Diabetic nephropathy (DN) is a major cause of end-stage renal disease and affects a large number of individuals with diabetes. However, the development of specific treatments for DN has not yet been identified. Hence, this review is concisely designed to understand the molecular pathways leading to DN in order to develop suitable therapeutic strategies.

Extensive literature search have been carried in regard with the pathogenesis and pathophysiology of DN, drug targets and updates on clinical trials, the consequences associated with DN and the potential biomarkers for diagnosis and prediction of DN are discussed in this review.

DN is characterised by microalbuminuria and macroalbuminuria, and morphological changes such as glomerular thickening, interstitial fibrosis, formation of nodular glomerulosclerosis and decreased endothelial cell fenestration. Besides, the involvement of renin-angiotensin-aldosterone system, inflammation and genetic factors are the key pathways in the progression of DN. In regard with drug development drugs targeted to epidermal growth factor, inflammatory cytokines, ACTH receptor and TGFβ1 receptors are in pipeline for clinical trials whereas, several drugs have also failed in phase III and phase IV of clinical trials due to lack of efficacy and severe adverse effect.

The research on DN is limited with respect to its pathogenesis and drug development. Thus, a more detailed understanding of the pathogenesis of DN is very essential to progress in the drug development process.

1. Introduction

Diabetic nephropathy (DN), also known as diabetic kidney disease, affects the proper functioning of kidneys chronically and can appear in people with Type I and Type II diabetes. DN affects approximately 40% of all diabetics and statistics show that the prevalence of diabetes is up to 382 million as of 2013, which represents 8–10% of the global population [1]. DN can be defined by increased urine albumin excretion (>300 mg/day), decreased glomerular filtration rate (GFR), diabetic glomerular lesions and raised arterial blood pressure [2]. The cause of DN is due to the high blood sugar that destroys the blood vessels in kidneys resulting in its dysfunction [3]. DN can be divided into 5 stages of kidney deterioration and symptoms are often only apparent in stage 4. Given that the symptoms only appear in the later stages, it is recommended that diabetics be screened for kidney complications yearly. Symptoms of DN that occur during stage 4 include swelling of ankles, legs and hands due to water retention, blood in urine, fatigue due to low oxygen levels in blood and nausea. If left untreated, this can lead to stage 5, end-stage renal disease (ESRD) where the kidneys can no longer function to meet the daily requirements and the only possible treatments are dialysis or kidney transplant. The risk factors of DN include dyslipidaemia, hypertension, poor glycaemic control and smoking along with a person's

* Corresponding author. Department of Life Sciences, School of Pharmacy, International Medical University, No 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000, Kuala Lumpur, Malaysia.
E-mail addresses: Vikram.rao@studentimuedu.onmicrosoft.com (V.R. A/L B Vasanth Rao), Tan.SeanHong@student.imu.edu.my (S.H. Tan), MayurenCandasamy@imu.edu.my (M. Candasamy), subratkumar@imu.edu.my (S.K. Bhattamisra).

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genetic profile also playing a major part in contracting DN as this disease is prone to develop in individuals with a family history of DN [4]. DN is currently reported to be the primary cause of ESRD and is responsible for 30–40% of ESRD cases in the US [5]. Specific treatments for DN have not yet been identified hence, it is of great importance to understand the molecular pathways leading to DN in order to develop suitable therapeutic strategies which are further discussed in this review.

2. Pathophysiology and pathogenesis of diabetic nephropathy

DN can be characterised by several morphological changes that involve all sections of the kidney, which affect the function of the organ. The series of alterations include the thickening of the basement membrane and the formation of nodular glomerulosclerosis within the glomeruli. The transformation in nature of the glomerular basement membrane (GBM) from its normal collagen chains, α1(IV) and α2(IV) to more restricted collagen chains, α3(IV) and α4(IV) results in the accumulation of type IV collagen in the lamina rara interna of the GBM [6]. As the disease progresses, more layers of the GBM are filled with these extracellular matrix components, which further expand the GBM by approximately twice the normal size. This affects the compositional quality and function of the GBM leading to proteinuria or macromolecular leakiness [7]. Besides that, the build-up of extracellular mesangial matrix components and to some extent, mesangial cell proliferation causes mesangial expansion within the glomerulus. This along with collagen deposition subsequently leads to glomerulosclerosis, which is the formation of eosinophilic nodules known as Kimmelsteil-Wilson nodules and finally tubulointerstitial fibrosis [8].

These structural changes cause glomerular hyperfiltration, increased proteinuria and albumin excretion, and reduced GFR. There are multiple pathways leading to DN: hemodynamic alterations such as imbalanced arteriolar resistance, metabolic factors causing oxidative stress, cell signalling and transcription factors, and pro-inflammatory molecules [1]. All these pathways lead to apoptosis, podocyte loss and reduced GFR, which are key characteristics of DN.

2.1. Involvement of pro-inflammatory cytokines in diabetic nephropathy

Studies have shown that tumour necrosis factor (TNF)-α is increased in serum and urine of patients affected by DN as compared to control (diabetic and non-diabetic patients), which are associated with the progression of renal injury [9]. Moreover, the association between TNF-α IL-6, IL-1 and pathogenesis of DN, which are known to participate in the impairment of the interglomerular haemodynamic had been reported [10] (Fig. 1).

JAK: Janus kinase; STAT: signal transducer and activator of transcription; IL-6: Interleukin-6; IL-6R: IL-6 receptor; Gp130: glycoprotein 130; IL-1: Interleukin-1; IL-1R: IL-1 receptor; NFκB: Nuclear factor κB; IKK: IκB kinase; TNF-α: Tumour necrosis factor-α; TNFR: TNF-α receptor; TRADD: TNFR-associated death domain; TRAF2: TNF receptor associated factor 2; NIK: NF-κB inducing kinase.

2.1.1. TNF-α

As shown in Fig. 1 above, some of the early changes that occur in the kidney are renal hyperfiltration and hypertrophy, which is evidently due to inflammation particularly by TNF-α. The two mediators responsible for TNF-α to exert its actions are its receptors, TNFR1 and TNFR2. As TNF-α binds to these membrane-bound receptors, this results in the formation of the TNFR-associated death domain (TRADD). From here, there are two possible pathways. Should the Fas-associated protein with death domain (FADD) bind to TRADD, the apoptotic signalling pathway is activated. However, when the TNF receptor associated factor 2 (TRAF2) binds to TRADD followed by the binding of the death domain serine/threonine kinase pathway to TRAF2, NF-κB inducing kinase (NIK) is recruited. Subsequently, the IκB complex is formed which is commonly known as the enzyme complex formed in response to inflammation. This triggers the downstream activation of NF-κB, which results in the production of cytokines [11]. The TRAF2-RIP complex can also activate the p38 mitogen activated protein kinase (MAPK) signalling pathways in mesangial cells via NADPH oxidase resulting in the production of reactive oxygen species (ROS). This increased oxidative stress can alter the glomerular capillary wall to reduce barrier function causing increased permeability to albumin [10].

2.1.2. Interleukin 1 (IL-1)

IL-1 is represented by two main ligands, IL-1α and IL-1β, which are activated by their precursors, pro-IL-1β and caspase-1. These IL-1s are released through micro vesicle shedding and cell lysis and subsequently bind to its receptor, IL-1RI to initiate a signalling transduction. The myeloid differentiation primary response protein 88 (MyD88) will then bind to the IL-1RI and its neighbouring receptor, IL-1 receptor accessory protein resulting in the binding of IL-1 receptor associated kinase (IRAK1) and IRAK2 to the MyD88. This recruits TRAF6 to this complex causing the activation of NF-κB leading to accumulation of extracellular matrix (ECM), thickening of the glomerular basement membrane (GBM) and glomerulosclerosis [12].

2.1.3. Interleukin 6 (IL-6)

IL-6 was found to be associated with DN as studies have reported increased serum levels of IL-6 in patients with type 2 DN [13]. Kidney biopsies were taken from DN patients also showed infiltrated mesangial cells were positively expressing mRNA coding for IL-6 [14]. Once released, IL-6 binds to the membrane-bound IL-6R and gp130 signal-transducing chain. This result in the autophosphorylation and change in the conformational structure of Janus kinase (JAK) which brings both JAKs closer to each other to phosphorylate one another inducing an intracellular signal. This further phosphorylates and activates transcription factors, signal transducer and activator of transcription (STAT)-3. Consequently, this leads to the growth and proliferation of mesangial cells and subsequently a similar outcome as IL-1 as described previously [15].

2.2. Genetic mechanisms leading to diabetic nephropathy

2.2.1. Angiotensin-converting enzyme (ACE)

Identifying the genetic mechanisms involved in DN can provide vast clinical significance especially in terms of prevention and early diagnosis. Genome-wide association studies (GWAS) have been conducted to determine the mutations associated with DN with most results pointing towards the angiotensin-converting enzyme (ACE) gene. Ng and colleagues (2005) conducted a meta-analysis of 47 studies between 1994 and 2004 to investigate the genetic association of DN. In this study, it was found that an insertion/deletion polymorphism of ACE was common amongst patients (8663 individuals) as compared to controls (6064 individuals) [16]. This was later further substantiated based on the meta-analysis conducted by Mooyaart and colleagues (2011). They identified that a deletion of the rs179975 polymorphism of ACE gene was associated with DN based on 5721 patients and 7798 controls [17].

The renin-angiotensin-aldosterone system (RAAS) plays a major

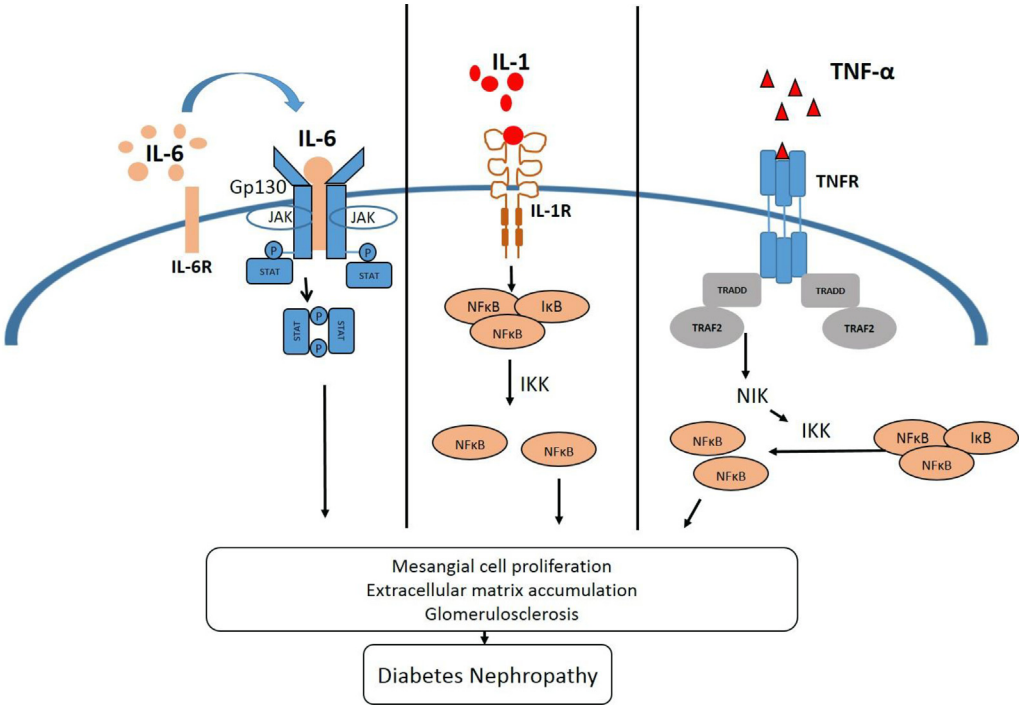

Fig. 1. Role of pro-inflammatory cytokines in DN.

role in regulating renal hemodynamic and its dysfunction is one of the hallmarks of DN (Fig. 2) [18]. The ACE gene is located on chromosome 17q23 containing an ALU repeat sequence in the 16th intron of the 287th base pair. A deletion of the ALU repeat sequence can lead to an insertion/deletion of ACE polymorphism [19]. The increase in aldosterone, caused by the dysfunctional ACE gene can cause toxicity and fibrosis of blood vessels [20]. It was found that aldosterone aids in the production of the extracellular matrix protein, fibronectin by glomerular mesangial cells via the activation of the Smad2-dependent TGFβ1 pathway. *In vitro* studies showed that TGFβ1 increased with aldosterone and in contrast, by inhibiting TGFβ1, aldosterone-induced fibronectin generation is suppressed [21]. Aldosterone also activates the ERK1/2-dependent pathways to produce types I, III and IV collagens in a dose-dependent manner leading to increased collagen deposition and severe tubulointerstitial fibrosis [22]. Furthermore, the presence of aldosterone enhanced phosphorylation of serum and glucocorticoid-inducible protein kinae-1 (SGK1) and SGK1-

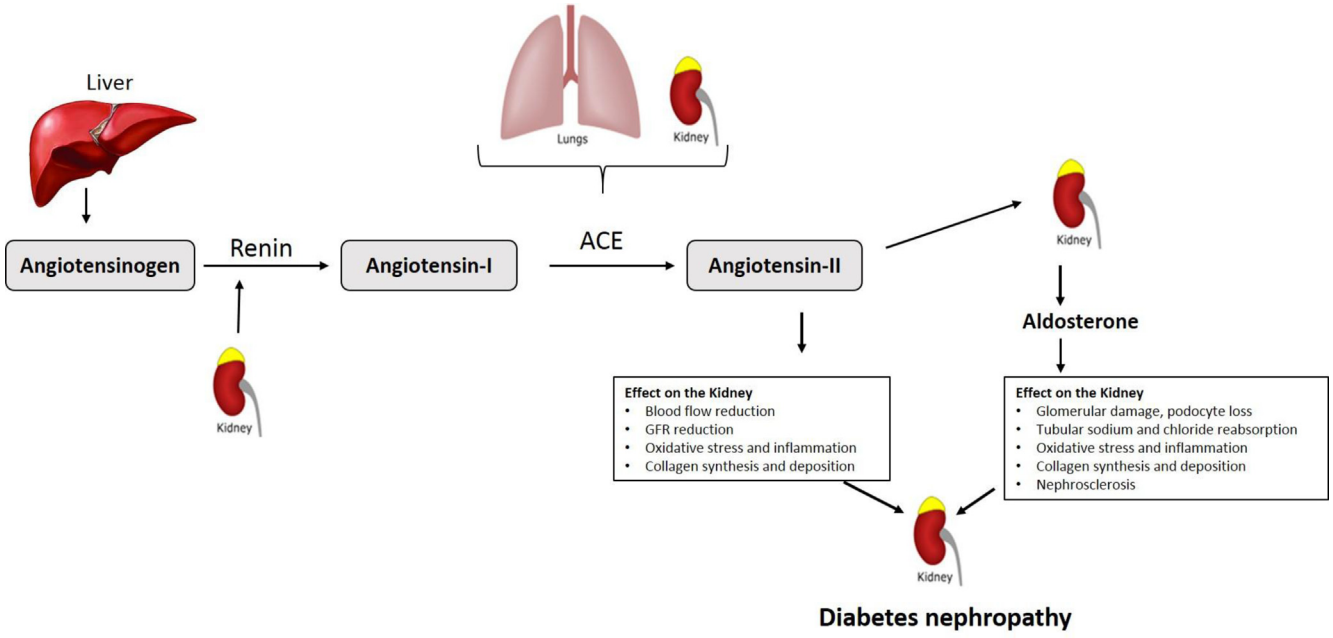

Fig. 2. RAAS pathway and the pathological conditions that occur during its dysfunction; ACE: angiotensin-converting enzyme.

dependent NF-κB activity. Both these mechanisms increase the transcription and expression of the intercellular adhesion molecule-1 (ICAM1) and the profibrotic cytokine connective tissue growth factor resulting in mesangial fibrosis and inflammation [23].

2.2.2. FRMD3

Another gene associated with DN is the FRMD3 (4.1 protein ezrin, radixin, moesin [FERM] domain containing 3) gene which was identified in a GWAS conducted by the US Genetics of Kidneys in Diabetes [24]. Amongst 820 case subjects and 885 control subjects, a strong association between FRMD3 and DN was observed. The FRMD3 gene is located on chromosome 9 of the band q21.32. FRMD3 codes for a structural protein that is part of the 4.1 cytoskeletal protein family and responsible for maintaining cell shape and integrity in various cells including nephron cells [25]. An SNP, rs1888747 located on chromosome 9 in the promoter region of FRMD3 is strongly associated with DN [26]. Martini and colleagues (2013) suggested that FRMD3 operates in conjunction with the bone morphogenetic protein (BMP)—signalling pathway [27]. Palmer and Freedman (2013) analysed renal biopsies obtained from patients with DN and reported that FRMD3 and BMP signalling pathways were co-expressed and shared transcription factor binding sites suggesting a co-regulation between the FRMD3 gene and the BMP family [28]. BMP is part of the TGFβ1 superfamily plays a part in kidney development, chemotaxis, cell differentiation and regulates apoptosis of various adult cell types such as hematopoietic, epithelial, neuronal and mesenchymal cells [29]. The rs1888747 SNP in the promoter region of FRMD3 is believed to suppress the activity of FRMD3, which prevents the activation of BMP and the depletion of BMP-mediated renal protection in patients with diabetes [30]. FRMD3/BMP inhibition can result in albuminuria and increase in fractional mesangial area. For example, BMP7 agonists and antagonists like kielin/chordin-like protein and gremlin respectively, have been reported in patients with DN and suppressed the expression of BMP7 and its agonists have shown to increase profibrotic activity in DN [31].

BMP7 is endogenously expressed in the epithelial cells of collecting ducts and distal tubules, while it is over-expressed in the kidneys during kidney injury to facilitate repair of renal injury. Complete knockout of BMP7 in mice models results in death due to diffuse renal dysplasia indicating the importance of BMP7 for kidney development [32]. During renal injury, BMP7 is synthesized together as a BMP7-prodomain complex in cells of the collecting duct and distal tubules. This complex is bound to fibrillin-1 in the extracellular space of the kidney. Inhibitory proteins, USAG-1, Gremlin and Noggin modulate the BMP7-prodomain complex bound to fibrillin thus freeing the BMP7 to bind to its receptors. Signal transduction of BMP7 is activated by binding to its receptor complex, comprising of three type 1 receptors, activin receptor-like kinase (ALK2, ALK3 and ALK6). This phosphorylates the type 1 receptors, which results in the phosphorylation of Smad1, Smad5 and Smad8. The BMP7 together with Smad1, Smad5 and Smad8 forms a heteromeric complex with Smad4 which translocate from the cytoplasm to the nucleus to induce renal repair [29,32]. In patients with chronic kidney disease, gremlin, a BMP7 antagonist is upregulated indicating the loss of function of the endogenous BMP7 activity. A loss of function of BMP7 will lead to tubulointerstitial fibrosis [33].

3. Conventional drug targets for diabetic nephropathy

Patients with DN are exposed to a higher risk of morbidity, mortality and pain, all amounting to a poor quality of life. Both proteinuria and hypertension result in glomerular hypertrophy, fibrosis and renal function deterioration. RAAS activation by high glucose and mechanical stress is the main culprit for this, whereby, angiotensin II elevates proteinuria and intraglomerular pressure and proteinuria as well as inducing the production of cytokines and further stimulating inflammatory pathways [34]. The current focus in clinical therapy for DN constitutes of mostly antihypertensive and antiproteinuric means, which is dependent upon RAAS inactivation by either ACE inhibitors or angiotensin receptor-1 blockers. These interventions inhibit the production and formation angiotensin II, thus reducing intraglomerular pressure and slowing-down progression of DN. There are multiple studies evaluating the effect of RAAS blockades with most of them bearing positive results such as ameliorating the effects angiotensin II (oxidative stress and inflammatory reactions), lowering blood pressure and proteinuria as well as improving glomerular permeability [35,36]. However, ACE inhibitors and ARBs are only able to partially resolve the problem as these agents slow-down the progression of DN rather than prevent, while many patients receiving these sorts of interventions may eventually progress to ESRD [37]. Positive clinical data on the efficacy of RAAS blockades are only shown in patients with significant proteinuria (>300 mg/day). Additionally, patients with a glomerular filtration rate (eGFR) below 30 mL/min/1.73 m^2, RAAS blockades are not recommended due to risk of hyperkalaemia [38,39].

Other therapies for DN include dipeptidyl peptidase-4 (DPP-4) and sodium-glucose cotransporter 2 (SGLT2) inhibitors, both of which are hypoglycaemic agents [40]. DPP-4 inhibitors lower blood glucose levels by inhibiting the DPP-4 enzyme, normally increased in DN patients, causing an increase in endogenous plasma glucagon-like peptide-1, which is responsible for enhancing insulin secretion and suppressing glucagon secretion [41,42]. In addition to lowering blood glucose, DPP-4 inhibitors also suppress TGFβ1 induced by high glucose in the proximal tubular cells. This results in the downstream reduction of Smad2 phosphorylation as well as the transcription and expression of fibronectin thus ameliorating kidney fibrosis [43].

On the other hand, SGLT2 inhibitors work by decreasing renal glucose re-absorption and increasing urinary glucose excretion, which further slowdown the progression of diabetic kidney disease [44]. SGLTs glucose/co-transporters are members of a structural class of membrane proteins translated from unrelated gene families of H^+ and Na^+ symporters and antiporters. SGLT2 is present in the brush-border membrane of the early proximal tubule and it regulates the bulk of tubular glucose uptake as compared to SGLT1, which has a lower capacity for glucose reabsorption [45]. As SGLTs aid in the increase of Na^+ and glucose reabsorption in the kidneys, the Na—Cl—K concentrations at the macula densa is reduced causing diabetic glomerular hyperfiltration resulting in an increase in GFR [46]. The introduction of a SGLT inhibitor normalizes the concentration of Na—Cl—K in the macula densa thus suppressing glomerular hyperfiltration. Dapagliflozin, a SGLT2 inhibitor that was used in hyperglycaemic streptozotocin-induced diabetic rats lowered proximal reabsorption of glucose and a great reduction of single nephron GFR via the normalisation of the Na—Cl—K concentration in the macula densa [47]. To further support this mechanism, a SGLT2 gene knockout in streptozotocin diabetic mice was successful in preventing glomerular hyperfiltration, which is a precursor to DN [46].

Various drugs for DPP-4 and SGLT2 inhibition are available in the market that has slight differences in efficacy and mechanism of action as compared to many different ACE inhibitors and ARBs drugs, which essentially function in the same manner. An overview of the renoprotective functions of ACE inhibitors, ARBs, DPP-4 inhibitors and SGLT2 inhibitors are shown in Table 1.

Table 1
Functions and possible mechanisms of ACE inhibitors, ARBs, DPP-4 and SGLT2 inhibitors.

Class	Drug	Function	Possible Mechanism	References
DPP-4 and SGLT2 inhibitors	Linagliptin	Reduction of albuminuria; Reduction of urinary cystatin C; Normalisation of podocalyxin	Amelioration of podocyte injury; Reduction of TGF-β	[48]
	Vildagliptin	Reduction of albuminuria; Reduction of fibrosis; Reduce thickening of GBM	Anti-inflammatory; Anti-apoptosis; Anti-oxidative	[49]
	Gemigliptin	Reduction of albuminuria; Reduction of fibrosis; Reduce thickening of GBM	Reduction of Smad3 phosphorylation	[50]
	Empagliflozin	Reduction of albuminuria; Reduction of renal growth; Reduction in GFR	Reduction of renal growth marker; Prevent glucose reabsorption by inhibiting Na-glucose cotransporter	[51]
	Saxagliptin	Reduction of fibrosis	Anti-inflammatory	[52]
	Sitagliptin	Reduction of fibrosis; Reduction of lipid peroxidation	Anti-inflammatory; Anti-oxidative; Anti-apoptosis	[53]
ACE Inhibitor	Lisinopril, Fosinopril, Ramipril, Captopril, Benazepril, Perindopril	Improve renal function; Reduction of hypertension	Blockade of RAAS; Reduction of proteinuria; Reduction of urinary monocyte chemoattractant protein-1 (MCP-1); Prevents production of angiotensin II resulting in vasodilation	[54]
ARB	Irbesartan	Reduction of hypertension	Reduction of high blood pressure; Blocks angiotensin II (AT1 receptor) resulting in vasodilation	[35]
	Telmisartan	Reduction of fibrosis	Activation of peroxisome proliferator activator (PPAR)-γ	[55]

DPP-4: Dipeptidyl peptidase-4; SGLT2: Sodium-glucose co-transporter 2; ACE: Angiotensin-converting enzyme; ARB: Angiotensin receptor blocker; RAAS: renin angiotensin aldosterone system.

4. Future prospects of drug targets for diabetic nephropathy

In recent years, there has been increasing evidence relating to autophagy and DN, due to its cytoprotective activity in the kidney [41]. Autophagy can be defined as the intracellular degradation of damaged organelles, proteins and macromolecules in order to maintain intracellular homeostasis. Autophagy in the kidneys is often activated during stressful condition such as hypoxia and oxidative stress. However, it is found to be suppressed in obese type 2 diabetes patients. Diet-induced obesity was found to down-regulate proteinuria-induced autophagy, which eventually aggravates proteinuria-induced tubular cell damage. To show the renoprotective activity of autophagy, Yamahara and colleagues created proximal tubule-specific autophagy deficient mice by knockout of the Atg5 gene and subjected them to an overload of free fatty acid-albumin causing them to develop severe proteinuria-induced tubular damage. It was also found that mTORC1, a potent autophagy suppressor was stimulated in the proximal tubules of the obese mice. Subsequent treatment with an mTORC1 inhibitor improved the autophagy insufficiency. This was supported by observing the p62 protein expression, a marker for impaired autophagy in renal biopsies from obese and non-obese patients [56]. Obesity and diabetes can disrupt basal and stress-responsible renal autophagy in podocytes and proximal tubular cells. This causes impaired cellular homeostasis resulting in proteinuria and tubular cell damage [57] (Fig. 3).

Research showed that calorie restriction or short-term starvation causes the activation of autophagy in proximal tubular cells, indicating that these cells respond to energy depletion by inducing autophagy [57]. Calorie restriction was found to have benefits in protecting kidneys from renal injury. Kume and colleagues (2010) investigated the effect of calorie restriction on the protection against hypoxia in aged kidneys of mice. Calorie restriction had promoted the expression of NAD-dependent deacetylase sirtuin 1 (Sirt1) in aged kidneys and ameliorated hypoxia-induced mitochondrial and renal damage by stimulating expression of BCL2/adenovirus E1B 19 kDa interacting protein 3-dependent (Bnip3-dependent) autophagy. Proximal tubular cells isolated from mice subjected to calorie restriction induced deacetylation of Sirt1-mediated forkhead box O3 (Foxo3), which is important for Bnip3 and p27Kip1 expression, and for subsequent autophagy and survival of proximal tubular cells under hypoxia [58]. Additionally, deficiency in Sirt1 is linked to podocyte injury proximal tubular cell damage in DN patients. In contrast, improvements in symptoms of DN were observed upon reactivation of Sirt1 [59].

AMPK on the other hand, is activated during stressful conditions such as energy depletion, and is found to be suppressed in patients with DN, suggesting a renoprotective activity of AMPK-induced autophagy [57]. Research on type 1 and type 2 diabetes in animal models have shown that AMPK is deactivated in the glomeruli and tubules due to dephosphorylation. In the study conducted by Kume and colleagues (2007), mice that were fed a high-fat diet for 16 weeks developed albuminuria, glomerulosclerosis and interstitial fibrosis. Renal lipolysis as shown by the decrease in mRNA expression of carnitine palmitoyl acyl-CoA transferase 1 (CPT-1), a lipolytic enzyme, and AMPK were found to be suppressed in the

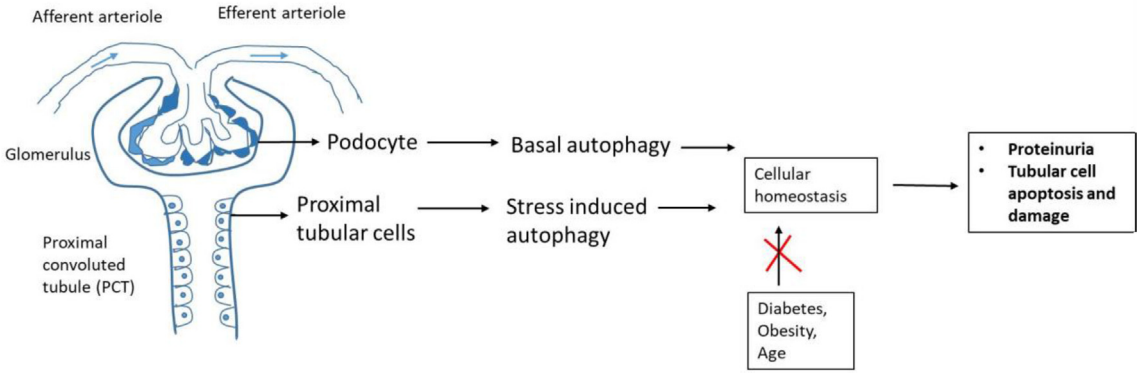

Fig. 3. Overview of autophagy in podocytes and proximal tubular cells.

kidneys of the mice indicating a blockade in the AMPK-induced autophagy pathway [60]. In a normal scenario, the AMPK is phosphorylated causing the inactivation of acetyl-CoA carboxylase (ACC) and thus, decreasing the intracellular levels of malonyl-CoA. Subsequently, up-regulating CPT-1 activity, which accelerates lipolysis [61]. Agents such as resveratrol and metformin are able to reactivate AMPK and ameliorate renal injury as shown in streptozotocin-induced diabetic mice. The treated group had increased expression and phosphorylation of AMPK compared to the non-treated groups [62,63].

Given that autophagy is regulated by nutrient-responsive intracellular signals like Sirt1, AMPK and mTORC1, which could pose as potential drug targets to treat DN. However, more research needs to be conducted to identify the potential risks of long-term usage, recommended dosage and whether it is safe and effective in all demographics with DN. It was reported by Inoki and colleagues (2011) that excessive inhibition of mTORC1 can lead to podocyte dysfunction [64]. Rapamycin or Sirolimus, two mTORC1 inhibitors have the potential as renoprotective agents but can also cause negative effects on renal function and proteinuria [65,66]. Complete inhibition of mTORC1 activity is associated with the worsening of podocyte function and renal medulla atrophy, which could result in tubular proteinuria and the further deterioration of kidneys. Hence, small doses of mTORC1 inhibitors that are designed to reduce, but not halt mTORC1 activity can attenuate podocyte injury and inhibit the progression of DN [64].

5. Updates on recent clinical trials for the treatment of diabetic nephropathy

Given the worldwide health concern for DN, there are a multitude of potential drugs being developed and currently undergoing clinical trials. However, the failure rate of new drugs in clinical trials is in excess of 90% with only a handful of these therapies breaching phase III trials. These unfavourable results are possible due to the diverse and complex pathogenic mechanisms associated with DN as well as the long-term safety of the potential drug. In this review, we summarise the outcomes of potential interventions for the treatment of DN, from recently completed clinical trials in the past 5 years (2013–2018) and it is shown in Table 2 [67].

The common primary endpoints of interventional trials for DN are proteinuria, albuminuria and GFR however, a majority of these interventions flailed at the clinical trial stage due to lack of efficacy or safety concerns. An overview of some of these failed or terminated trials and reasons for its failure can be seen in Table 3 [67,68].

As mentioned in Table 3 above, the drug, avosentan failed in the clinical trial phase due to increased risk of fluid overload and congestive heart failure. Regardless of its beneficial effect on reducing albuminuria, the intervention still caused these adverse events due to proximal tubular sodium reabsorption [69]. Bardoxolone methyl on the other hand, which was expected to have strong anti-inflammatory and antioxidative properties via the activation of Nrf2 failed due to the resultant side effects: muscle cramps, increased albuminuria, elevated blood pressure, heart failure, stroke, non-fatal myocardial infarction and cardiovascular associated mortality. Sulodexide is a highly purified blend of glycosaminoglycans that has shown to reduce TGFβ1 levels and albuminuria in experimental diabetic glomerulosclerosis [70]. However, sulodexide failed to show improvements in albuminuria and renal function in large scale randomised clinical trials.

6. Consequences associated with diabetic nephropathy

One of the complications in DN is anaemia. Joss and colleagues (2007) reported that approximately 50% of patients with DN are also affected by anaemia and explained that patients should be screened for anaemia as soon as they enter stage 3. Anaemia can be a suitable predictor of ESRD [71]. Some of the factors that associate DN with anaemia are iron deficiency, erythropoietin (EPO) deficiency, chronic inflammation and oxidative stress [72].

EPO is a hormone that is produced in the interstitial fibroblasts of the kidney and is responsible for regulating the proliferation, differentiation and maturation of erythrocytes in the bone marrow. The cause of EPO deficiency in DN patients is thought to be due to a reduction in renal mass causing decreased amounts of EPO. EPO is released during hypoxia and binds to EPO receptors on the erythroid progenitors in the bone marrow to activate the production of erythrocytes. In the absence of EPO, erythroid progenitors undergo programmed cell death resulting in anaemia. One of the proposed molecular pathways leading to anaemia is via the renin-angiotensin-aldosterone system (RAAS). Normally, angiotensin II increases EPO production, which acts as a growth factor in the bone marrow [73]. Patients with DN/ESRD have decreased plasma renin activity and this suppresses RAAS resulting in inhibition of erythropoiesis and anaemia [74].

Besides that, patients with DN/ESRD have elevated losses of iron due to chronic gastrointestinal bleeding and impaired intestinal iron absorption [75]. In relation to this, Ganz and colleagues (2012) identified hepcidin as the major hormone involved in iron homeostasis [76]. Hepcidin is produced by the liver, which causes the degradation of ferroportin, the iron exporter on hepatocytes and enterocytes thus inhibiting the entry of iron transport and absorption [77]. In patients with DN, the increased levels of pro-inflammatory cytokines like IL-6 and reduced renal clearance increase the production and secretion of hepcidin resulting in iron-restricted erythropoiesis and anaemia [74].

Table 2
Summary of completed clinical trials on potential interventions for the treatment of diabetic nephropathy within the past 5 years. NCT: National Clinical Trial; Nrf2: Nuclear factor erythroid 2-related factor 2; ACTH: adrenocorticotropic hormone; UACR: Urinary albumin creatinine ratio.

Drug	Target	NCT No.	Phase	Sponsor	Completion Date	Outcome
LY3016859	Epidermal growth factor ligand inhibitor	NCT01774981	II	Eli Lilly	August 2015	Reduced proteinuria and albuminuria
Acthar	ACTH receptor	NCT01601236	II	Mallinckrodt	March 2016	Decrease in serum creatinine; Reduced frequency of progression to ESRD/death
Lipo-prostaglandin E1	Cytokine and Angiopoietin inhibitor	NCT02628106	IV	West China Hospital	February 2016	Improves renal hypoxia
N-acetylcysteine and milk thistle	Antioxidant	NCT01265563	II	VA Office of R&D	December 2016	Reduced albuminuria
Baricitinib	Janus kinase inhibitor	NCT01683409	II	Eli Lilly	November 2014	Lower UACR
PF-04634817	Chemokine inhibitor	NCT01712061	II	Pfizer	September 2014	Reduction in UACR, blood pressure
Structured exercise plan (no intervention)	–	NCT01036490	–	VA Office of R&D	December 2014	No improvement in renal parameters
Febuxostat	TGFβ1 inhibitor and anti-inflammatory	NCT01350388	–	University of Utah	December 2013	Increase in adiponectin concentration Reduced TNF-α and IL-6 concentration

Table 3
Interventional drugs that failed the clinical trial phase and reasons for its failure.

Drug	Target	NCT No.	Phase	Sponsor	Sample Size	Reason for Termination
Sulodexide	Glycosaminoglycans	NCT00130312	IV	Keryx Biopharmaceuticals	1248	Lack of efficacy
Avosentan	Endothelin receptor antagonists	NCT00120328	III	Speedel Pharma	1402	Fluid overload and congestive heart failure
Bardoxolone methyl	Nrf2 activator	NCT01351675	III	Reata Pharmaceuticals	2185	Adverse cardiovascular events

[Nrf2- Nuclear factor erythroid 2-relater factor 2].

7. Biomarkers for diagnosing diabetic nephropathy

One of the most common ways used to diagnose the development of DN is by measuring albumin in urine (albuminuria). However, several contradictory factors emerge like urinary tract infections, exercise and cardiac failure, which can also cause albuminuria and the fact that most patients are affected by renal impairment prior to experiencing albuminuria [78]. Therefore, this provides room for improvement for discovering more accurate biomarkers for DN.

Al-Rubeaan and colleagues (2017) conducted a study to evaluate potential biomarkers for the diagnosis and prediction of DN. Among the 22 biomarkers tested in this study, urinary transferrin and retinol-binding protein (RBP) were discovered as potential biomarkers with excellent diagnostic accuracy. Transferrin is an iron-binding glycoprotein that regulates iron levels and is responsible for iron transport in serum. Urinary transferrin increases gradually as patients progress through the stages of DN [79]. Transferrin also shows a positive correlation with albumin: creatinine ratio (ACR) indicating that it is a specific and sensitive marker for DN. It was reported by Cheung and colleagues (1989) that 95% of patients with microalbuminuria and 100% of patients with macroalbuminuria had significantly increased urinary levels of transferrin [80].

RBP is found in skeletal muscles and white adipose tissues, and responsible for transporting retinol and transthyretin to tissues. Several studies have shown increased urinary excretion of RBP in the progression of DN [81]. RBP provides good diagnostic value in patients with macroalbuminuria but to a lesser extent in patients with microalbuminuria [79].

8. Conclusion

DN remains a significant problem in today's world as diabetic cases are rapidly increasing and can ultimately lead to ESRD or renal failure. There is no single treatment for curing DN whereas patients are required to take continuous medication for albuminuria and hypertension. Once patients reach the final stage of DN, the only two methods of treatment are regular blood cleansing by dialysis or kidney transplants. Therefore, further research on confirming the potential biomarkers are required in order to help prevent this morbid condition.

Conflicts of interest

All authors declare no conflict of interest to disclose.

Ethics statement

This study doesn't involve animals or human subjects.

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