Review Article

DIABETIC NEPHROPATHY- PATHOGENESIS AND NEWER TARGETS IN TREATMENT

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Accepted on: 06-12-2010; **Finalized on:** 12-02-2011.

ABSTRACT

Diabetic nephropathy has become the leading cause of end-stage kidney disease worldwide and is associated with an increased cardiovascular risk. The earliest clinical manifestation is microalbuminuria. Tight blood glucose and blood pressure control reduce the risk of microalbuminuria. Once microalbuminuria is present, the rate of progression to end stage kidney disease and cardiovascular disease can be delayed by aggressive management of blood pressure, glucose, and lipids. Inhibition of the reninangiotensin system is important in reducing intraglomerular pressure but other classes of antihypertensive agents may also be needed to obtain adequate control of systemic blood pressure. Such measures can at least reduce by half the rate of progression of nephropathy and cardiovascular disease.

Keywords: Diabetes, nephropathy, microalbuminuria, proteinuria.

INTRODUCTION

Diabetic Nephropathy (DN) is the major single cause of End Stage Renal Disease (ESRD) in developing countries[1] and extrapolations suggest that this number will multiply in the future[2]. End stage renal disease require dialysis and is becoming a staggering challenge to public health care systems due to the prohibitive costs of renal replacement therapy that could become unaffordable even for developed countries[3]. Advance diabetic nephropathy is also the leading cause of glomerulosclerosis and end-stage renal disease worldwide[4,5]. Between 20 to 40 % of all diabetic patients are prone to developing kidney failure, and family-based studies suggest that a significant genetic component confers risk for DN[6-8].

DN manifests as a clinical syndrome that is composed of albuminuria, progressively declining GFR, and increased risk for cardiovascular disease[9,10]. And it is a late complication of diabetes, occurring progressively in susceptible people only after 15 to 25 year of diabetes[11,13].

Natural History of Diabetic Nephropathy

The earliest clinical evidence of nephropathy is the presence of microalbuminuria (Table 1). It occurs in 30% of type 1 diabetics 5 to 15 years after diagnosis but may be present at diagnosis in type 2 diabetics as the time of onset of type 2 diabetes is often unknown. Microalbuminuria progresses to proteinuria over the next 7 to 10 years (Fig. 1). Once overt proteinuria develops, renal function progressively declines and end stage renal disease is reached after about 10 years.

Hence, the elucidation of molecular pathogenesis of diabetic nephropathy is necessary for the development of effective treatment modalities to prevent onset of diabetic nephropathy to end stage renal disease (ESRD).

PATHOGENESIS OF DIABETIC NEPHROPATHY

Multiple mechanisms contribute to the development and outcomes of diabetic nephropathy, and in this review we have focused on the particular factors involved in the pathogenesis of diabetic nephropathy:

Hemodynamic Changes: Under conditions of sustained hyperglycemia, the Glomerular cells are affected by various mechanisms. These changes lead to altered structure and function in the glomerulus[15].

In the kidney, the first observed functional change is an abnormally increased GFR [16]. This change develops before any major histological change in the Glomerular structure. Later in the development of diabetic nephropathy, typical histologically visible changes are seen: thickened GBM, diffuse glomerulosclerosis, nodular glomerulosclerosis, and exudative lesions in the Bowman's capsule and podocyte loss[17] glomerular hyperperfusion and hyperfiltration[18]. The early signs of glomerular hyperperfusion and hyperfiltration result from decreased resistance in both the afferent and efferent arterioles of the glomerulus. The afferent arteriole seems to have a greater decrease in resistance than the efferent. Many factors have been reported to be involved in this defective autoregulation, including prostanoids, nitric oxide, vascular endothelial growth factor (VEGF, now known as VEGF-A), TGF-β1, and the renin-angiotensin system; specifically angitensin II[19]. These cyclic changes in glomerular volume lead to recurrent episodes of stretch and relaxation of the glomerular structural components, including mesangial cells and podocytes[20-22].

Mesangial cells, when exposed to continuous cycle of stretch/ relaxation, alter their morphology. Specifically, these cells change from their normal stellate to a straplike appearance, aligning with their long axis perpendicular to the direction of stress[23]. This leads to enhanced proliferation and increased production of extracellular

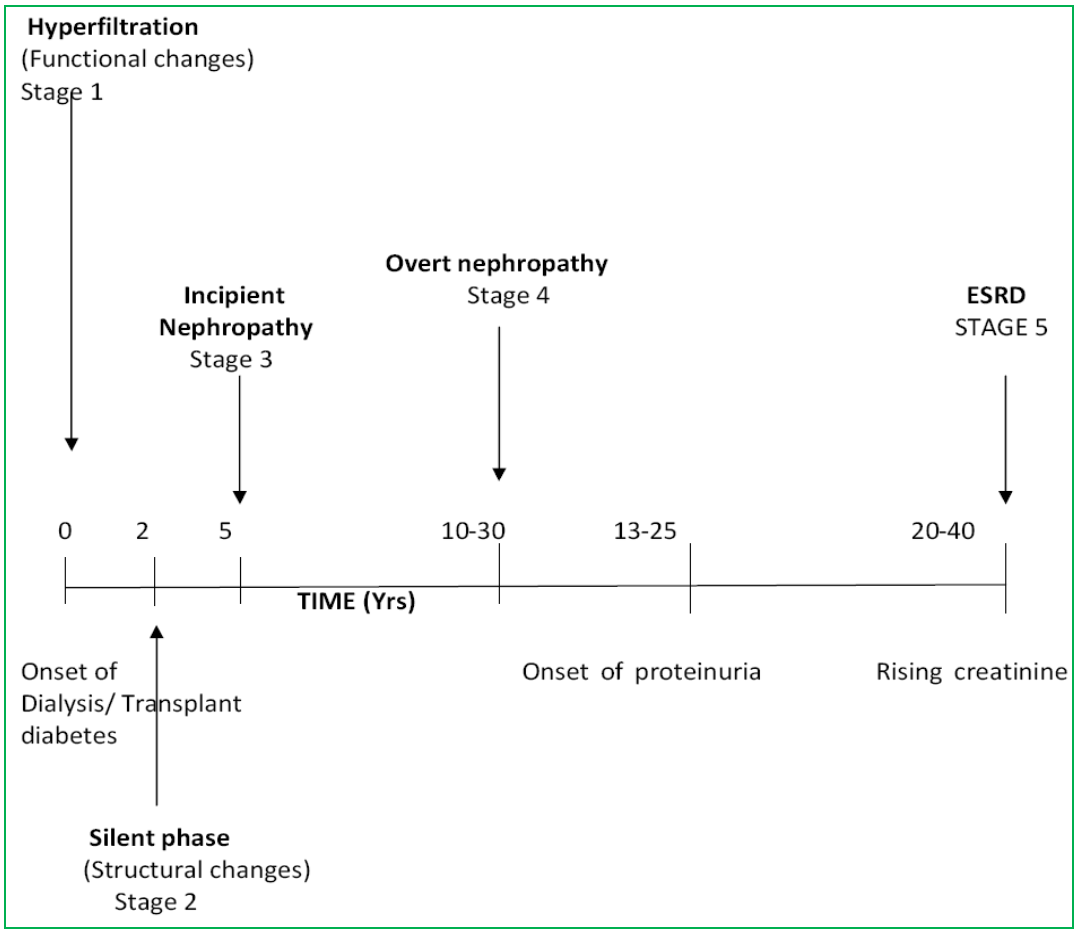
matrix components. This prosclerotic effect of stretch occurs partly as a result of increases in gene and/ or protein expression of extracellular matrix components, such as collagen I, III, and IV, fibronectin, and laminin [23-25]. These hemodynamic changes facilitative albumin leakage from the glomerular capillaries and overproduction of mesangial cell matrix, as well as thickening of the glomerular basement membrane and injury to podocytes. In addition, increased mechanical strain resulting from these hemodynamic changes can induce localized release of certain cytokines and growth factors [26, 27].

In a clinical setting, the most important sign of diabetic nephropathy is proteinuria which often worsens during the course of disease. Ultimately, end-stage renal disease may develop. The detection of very low but yet increased levels of albumin in urine have been used in predicting the development of overt diabetic nephropathy[28-30].

Table 1: Evolution of Diabetic Renal Disease

Stage 1	· Glomerular hypertension and hyperfiltration · Normoalbuminuria: urinary albumin excretion rate (AER) <20 ìg/min · Raised GFR, normal serum creatinine
Stage 2	· "Silent phase" (structural changes on biopsy but no clinical manifestations) · Normoalbuminuria
Stage 3	· Microalbuminuria: AER 20 – 200 ìg/min · Normal serum creatinine · There may be increased blood pressure
Stage 4	· Overt "dipstick positive" proteinuria (macroalbuminuria) : AER > 200 ìg/min · Hypertension · Serum creatinine may be normal · Increase in serum creatinine with progression of nephropathy
Stage 5	· End stage renal failure · Requiring dialysis or transplant to maintain life

Figure 1: Natural history of diabetic nephropathy[14]



HYPERGLYCEMIA AND ADVANCED GLYCOSYLATION END PRODUCTS

Hyperglycemia is a crucial factor in the development of diabetic nephropathy because of its effects on Glomerular and mesangial cells, but alone it is not causative. Mesangial cells are crucial for maintenance of Glomerular capillary structure and for the modulation of Glomerular filtration via smooth-muscle activity. Hyperglycemia is associated with an increase in mesangial cell proliferation and hypertrophy, as well as increased matrix production and basement membrane thickening. In vitro studies have demonstrated that hyperglycemia is associated with increased mesangial cell matrix production [31, 32] and mesangial cell apoptosis [33-34]. Mesangial cell expansion seems to be mediated in part by an increase in the mesangial cell glucose concentration, since similar changes in mesangial glucose milieu by overexpression of glucose transporters, such as GLUT 1 AND GLUT 4, thereby increasing glucose entry into the cells.

Hyperglycemia might also upregulate VEGF expression in podocytes, which could markedly increases vascular permeability [35, 36].

There are four mechanisms that explain how hyperglycemia causes tissue damage and diabetic nephropathy: Nonenzymatic glycosylation that generates advanced glycosylation end products, activation of protein kinase C (PKC), acceleration of the aldose reductase pathway[37, 38]. Oxidative stress seems to be a theme common to above three pathways[39] and activation of GPR 91 receptor in the kidney [40].

Non enzymatic glycosylation: Glycosylation of tissue proteins contributes to the development of diabetic nephropathy and other microvcascular complications [41]. In chronic hyperglycemia the AGEs formed by the result of a completely non-enzymatic process, in which protein glycation randomly takes place under abnormally high glucose concentrations. However, it has been shown that the intracellular dicarbonyl formation is likely a prerequisite of significant protein glycation [42]. These highly reactive dicarbonyls are formed as a result of autooxidation of glucose to glyoxal [43], degradation of glyceraldehydes-3-phosphate or dihydroxyacetone phosphate to methyglyoxal [44], and decomposition of the Amadori product to 3-deoxyglucosone. Reaction of dicarbonyls with amino groups in proteins leads to the formation of AGEs [45]. These advanced glycosylation end products (AGEs) can be involved in the pathogenesis of diabetic nephropathy by altering signal transduction via alteration in the level of soluble signals, such as cytokines, hormones and free radicals circulating levels of advanced glycosylation end products are raised in people with diabetic nephropathy; since they are normally excreted in the urine. The net effect is tissue accumulation of AGEs (in part by cross-linking with collagen) that contributes to the associated renal and microvascular complications [46]. It has now become evident that there is at least one major receptor for AGEs mediating the adverse effects [47, 48]. This receptor for AGE (RAGE) has signaling properties as well and it is found to be upregulated in human tissues susceptible to the complications of diabetes[49], hence RAGE is suggested to contribute to the pathogenesis of diabetes and its complications[50]. RAGE has also been found to be expressed in the podocyte and in a model of diabetic nephropathy; it has been shown to be required for the medication of the adverse effects of AGE [51-53].

Activation of protein kinase C

Intracellular hyperglycemia leads to increased de novo synthesis of diacylglycerol (DAG) which is a key intracellular metabolite and among others an activator of several protein kinase C (PKC) isoforms, most importantly the PKC-β isoforms [54]. Specifically; activation of this enzyme leads to increased secretion of vasodilatory prostanoids, which contributes to Glomerular hyperfiltration[55]. Activation of PKC-β leads to a great number of pathologic changes, such as increased production of endothelial nitric oxide synthase (eNOS), endothelin-1, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), plasminogen activator inhibitor 1 (PAI-1), nuclear factor kappa β (NF-Kβ), and the activation of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidases. The activation of angiotensin receptor in the mesangial cells is known to further stimulate TGF-β production and extracellular matrix (ECM) production[56].

Aldose reductase pathway/ polyol pathway

The polyol pathway is implicated in the pathogenesis of diabetic nephropathy. A number of studies have shown a decrease in urinary albumin excretion in animals administered aldose reductase inhibitors [57], but in humans these agents have not been studied widely and the results are inconclusive.

Activation of GPR 91 receptor in the kidney

High glucose levels led to increased accumulation of metabolic intermediate in the kidney tissue, which activated GPR 91 signaling in vascular endothelial cells. Secretion of nitric oxide (NO) and prostaglandin E_2 **(**PGE_2**)** by these same cells then triggered release of renin by adjacent juxtaglomerular cells. This leads to increased levels of renin and chronic RAS activation, with resulting long-term hypertension, edema and high levels of serum protein in the urine (proteinuria). This condition can lead to diabetic nephropathy to end-stage renal disease (ESRD).

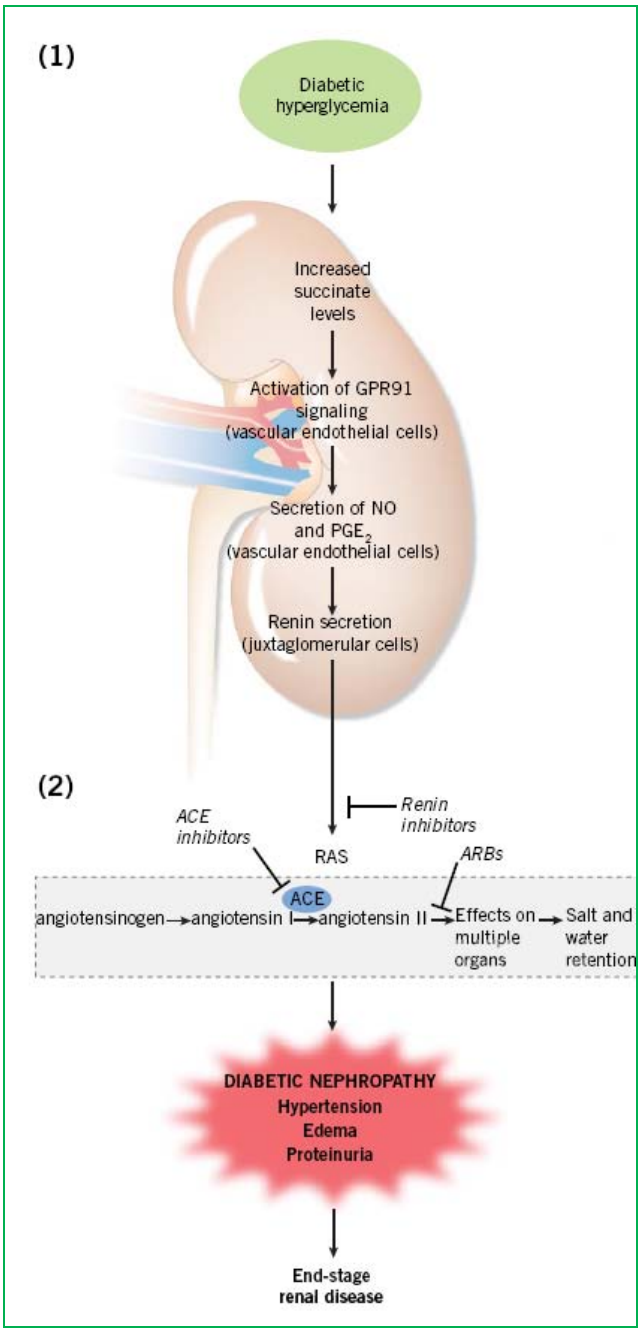
Research published in *The Journal of Clinical Investigation* 3 suggests that G protein–coupled receptor 91 (GPR91), which is upstream of the renin-angiotensin system (RAS), could be a key mediator of hyperglycemia-induced renal hypertension in diabetes—and thus could be a potential target for preventing onset or slowing progression of diabetic nephropathy.

(1) In kidney preparations isolated from mice, increased glucose levels triggered release of renin by the following mechanism:

High levels of glucose resulted in increased accumulation of the metabolic intermediate succinate in kidney tissue, which activated GPR91 signaling in vascular endothelial cells. Secretion of nitric oxide (NO) and prostaglandin E2 (PGE2) by these same cells then triggered release of renin by adjacent juxtaglomerular cells.

(2) In diabetic nephropathy, aberrantly high levels of renin lead to chronic RAS activation, with resulting long-term hypertension, edema and high levels of serum protein in the urine (proteinuria). This condition can lead to end-stage renal disease (ESRD).

Figure 2: Linking diabetic hyperglycemia to kidney diseases



PRORENIN

A recent study provided quantitative, functional and in vivo visual analysis of (pro)renin (a term denoting both renin and its precursor prorenin) in the diabetic rat kidney [58]. In diabetes there are two sites for the synthesis of (pro) renin; which are juxtaglomerular apparatus (JGA), and principal cells of the collecting duct (CD). The two most important intra-renal locations of pro(renin) synthesis and release and the *in vivo* imaging of these two sites by multiphoton microscopy are shown in figure.3.

Initial clinical studies in children and adolescents suggest that increased plasma prorenin activity is a risk factor for the development of diabetic nephropathy [59, 60]. The prorenin receptor in the kidney is recently identified and characterized [61-64]. The prorenin receptor in the kidney is located in the mesangium and podocytes, and its blockade has a beneficial effect on kidneys in animal models of diabetes. This effect is mediated by intracellular signals that are both dependent on and independent of the renin-angiotensin system. Prorenin binds to a specific tissue receptor that promotes activation of P44/ P42 MAPK [65].

A possible pathogenic role for prorenin in the development of diabetic nephropathy was noted in an experimental model of diabetes-mice with streptozotocin-induced diabetes. Sustained prorenin-receptor blockade abolished MAPK activatoin and prevented the development of nephropathy despite an unaltered increase in angitensin II activity [66].

ANGIOGENIC AND PROINFLAMMATORY FACTORS

Recent studies suggest that an inflammatory mechanism mediated by macrophages and angiogenesis may play important roles in the pathogenesis of diabetic nephropathy. Relatively recent reports [67-69] described that the degree of neovascularization was significantly increased in patients with diabetic nephropathy and correlates with the expression VEGF and angiopoietin which likely contribute to diabetic nephropathy by promoting vessel leakage and reducing transendothelial electrical resistance. The angiogenic growth factor VEGF induces the activation of matrix-degrading protease represented by matrix metalloproteases and migration and proliferation of endothelial cells[70]. Recent animal studies utilizing a neutralizing anti-VEGF antibody further demonstrated the involvement of this factor in early Glomerular hypertrophy and mesangial matrix accumulation in the progressive stage of diabetic nephropathy [71-73].

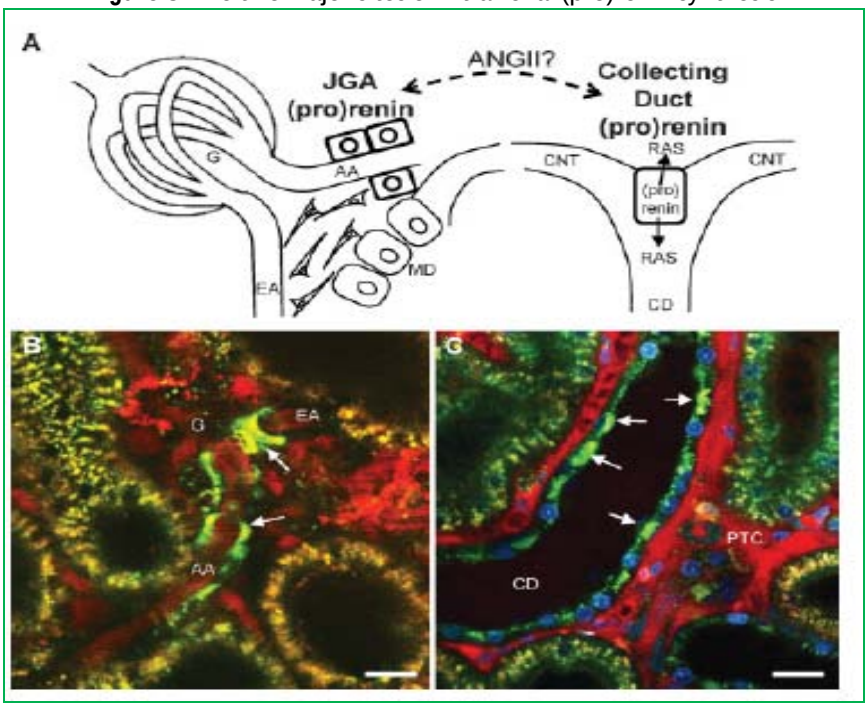
The therapeutic efficacy of endostatin peptide, a potent inhibitor of VEGF, which ameliorates renal alterations in the early stage of type 1 diabetic nephropathy, has been reported. Increased accumulation of monocytes/ macrophages in glomeruli has been reported in the diabetic nephropathy [74-76]. Although some evidence suggests that VEGF increases permeability of the Glomerular filtration barrier to proteins, levels of this

growth factor can be low in patients with diabetic nephropathy. Thus the role of VEGF in the pathophysiology of nephropathy is unclear.

Further evidence to support a pathogenic role for VEGF in diabetic nephropathy is the observation that VEGF blockade improves albuminuria in an experimental model of the disorder[77]. High glucose levels, TGF-β1, and angiotensin II stimulate VEGF expression, which leads to the synthesis of endothelial nitric oxide. This action promotes vasodilation and hyperfiltration, which are the early processes in the diabetic nephropathy. VEGF also stimulates the production of the Glomerular basement membrane. Indirect evidence suggests that increased production of this collagen chain contributes to the thickening of the Glomerular basement membrane observed in diabetic nephropathy. In animal studies, administration of an antibody to VEGF decreased urinary albumin excretion compared with that in untreated diabetic controls.

Findings from some studies refute causative role for high VEGF levels in diabetic nephropathy. Instead, results imply that low levels are harmful. Eremina et al [78] showed in a mouse model that VEGF is produced by podocytes and is necessary for Glomerular endothelial cell survival and differentiation as well as for mesangial cell development and differentiation. Gene expression of VEGF is decreased in humans with diabetic nephropathy [79], although whether this effect is due to podocytes loss, leading to reduced production of VEGF has been questioned. Baelde et al [80] showed that VEGF messenger RNA concentrations were decreased in the glomeruli of patients with diabetic nephropathy and correlated with reduction in the number of podocytes and progression of renal disease.

Figure 3: The two major sites of intra-renal (pro)renin synthesis



The juxtaglomerular apparatus (JGA) and the collecting duct (CD). Schematic drawing (**A**) and *in vivo* multi-photon fluorescence images (**B, C**) of these two sites. Direct *in vivo* visualization of quinacrine-labeled renin granules (green) in the JGA (**B**) and CD (**C**) in the intact kidney using multi-photon confocal microscopy. Control rat (**B**) and diabetic mouse kidney (**C**) are shown. A dextran-rhodamine B conjugate (70 kDa) labeled the intravascular space (plasma) red. (**B**) Quinacrine identified JGA renin granules in the terminal afferent arteriole (AA). The efferent arteriole (EA) is seen leaving the glomerulus (G). (**C**) Note the abundance of quinacrine staining in the bulging apical aspects of CD principal cells in diabetes (arrows). Nuclei are labeled blue with Hoechst 33342. Bars are 20 µm. MD: macula densa; CNT: connecting tubule; PTC: peritubular capillaries.

Another angiogenesis-associated factor, angiopoietin-1, is involved in the attachment of mesenchymal cells to endothelial tubes and in the differentiation to mature pericytes, so called 'nonleaky' blood vessels [81]. Angiopoietin-2 is the natural antagonist of angiopoietin-1 and loosens the attachment of pericytes, resulting in the promotion of sprouting angiogenesis In the presence of VEGF. Angiopoietin-1 is an apparent endogenous VEGF inhibitor, and angiopoietin-2 synergizes with VEGF and is upregulated in diabetic microvascular complications [82]. However, the involvement of angiopoietin-1 and angiopoietin-2 in the progression of diabetic nephropathy has yet to be elucidated.

TGF-β has been recognized as a profibrotic growth factor involved in the expansion of mesangial matrix and renal hypertrophy in diabetic nephropathy [83]. Elevated levels of TGF-β have been measured in the glomeruli of strptozotocin diabetic rats [84]. It was reported [85] that neutralizing TGF-β antibody prevented diabetic renal atrophy, mesangial matrix expansion, and the development of renal insufficiency in type 2 db/ db mice. In addition, connective tissue growth factor and heat

shock proteins, which are encoded by TGF-β, inducible genes, have fibrogenic effects on the kidneys of patients with diabetes. However, diabetes is associated with decreased expression of renal bone morphogenetic protein 7, which in turn seems to counter the profibrogenic actions of TGF-β1[86]. Mechanical stretch induces both gene and protein[87] expression of transforming growth factor (TGF-β1). Stretch, via the intracellular signaling molecule protein kinase C, causes early activation of p38 mitogen-activated protein kinase, which induces TGF-β1 and fibronectin production [88]. Evidence clearly shows that TGF-β1 contributes to the cellular hypertrophy and increased synthesis of collagen, both of which occur in diabetic nephropathy [89, 90]. Further evidence for these actions is provided by studies in which the combination of an antibody to TGF-β1 plus an angiotensin-converting-enzyme inhibitor normalized levels of protein in the urine of rats with diabetic nephropathy; proteinuria was only partly resolved with the use of an angiotensin-converting enzyme inhibitor alone [91].

The administration of hepatocyte growth factor, which specifically blocks the profibrotic actions of TGF-β1, ameliorates diabetic nephropathy in mice [92]. The platlet derivced growth factor beta (PDGF-β) is also involved in the histological alterations in the glomerulus. Under high glucose concentrations the PDGF-β growth factor and the corresponding receptor are upregulated in the mesangial cell leading to later increase in TGF-β expression [93].

IMMUNOLOGIACAL STUDIES ON MECHANISMS OF DIABETIC NEPHROPATHY

Inflammation: The hall-mark of type 1 diabetes is the selective destruction of insulin-producing islet beta cells by activated T cells. It has been reported that activated T cells are also associated with diabetic nephropathy [94, 95]. T cell accumulation is found in the juxtaglomerular apparatus of patients with type 1 diabetes. T cell influx would become the factor to exacerbate diabetes and correlates with Glomerular filtration surface and albumin excretion rate[96]. Recently increased levels of inflammatory markers such as C-reactive protein and interleukin-6 were found to be present in type 1 diabetic patients with micro-or macroalbuminuria[97]. Other inflammatory cytokines also contribute to the development and progression of diabetic nephropathy, specifically interleukin-1 (IL-1), IL-8 and tumor necrosis factor. Concentrations of all these cytokines were increased in models of diabetic nephropathy and seemed to affect the disease via multiple mechanisms[98]. This finding suggests that the pathogenetic mechanisms of diabetic nephropathy also involve the immunological and inflammatory process. This mechanism is further supported by the finding that diabetic mice deficient in intercellular adhesion molecule 1 (ICAM-1), a molecule mediating macrophages to diabetic kidney, are protected against renal injury[99]. It is also known that the activation of PKC the mesangial cell induces the expression of ICAM-1[100]. This links the metabolic disturbances and alterations in intracellular signaling in diabetes to inflammation[101].

Each cytokines has several different effects. IL-1 alters the expression of chemotactic factors and adhesion molecules, alters intraglomerular hemodynamics (by affecting mesangial cell prostaglandin synthesis), and might increase vascular endothelial cell permeability and epithelial cells (which in turn could increase Glomerular cellularity)[102]. IL-6 has a strong association with the development of Glomerular basement membrane thickening as well as possible relationships with increased endothelial permeability and mesangial cell proliferation. IL-18 induces the production of other inflammatory cytokines, such as IL-1, interferon-γ and tumor necrosis factor, and might be associated with endothelial cell destruction in type 1 diabetes. Tumor necrosis factor has the widest variety biological activities and effects that contribute to development of diabetic nephropathy. Importantly, though, it causes direct renal injury as a cytotoxin, as well as affecting apoptosis, Glomerular hemodynamics, endothelial permeability, and cell-cell adhesion. It also seems to play an important part in the early hypertrophy and hyperfunction of diabetic nephropathy[103, 104]. TGF-β is an important pleiotropic cytokine associated with the development of Tregs and Th17 cells. The progression to overt diabetes resulting in significant beta cell destruction is triggered by the development of a more aggressive T cell phenotype and a change in the Th1-to-Th2 balance towards a more proinflammatory milieu (Th1 dominant). Furthermore, evidence demonstrating the association of the Th17 subset, the recently discovered CD4+ effector T cell lineage distinct from Th1 and Th2, with pathogenesis of type1 diabetes is rapidly accumulating [105-107].

T-cell mediated beta cell destruction is induced by the release of cytotoxic molecules, including cytokines, granzyme B, or perforin, or by direct delivery of cell death signals via the Fas pathway [108, 109]. Activated CD4 + and CD8+ T cells act in unison to activate beta cell death via apoptosis. Apoptosis is introduced by activation of the Caspase pathway which, in turn, is activated by a number of alternative mechanisms such as Fas interaction with Fas ligand, action of nitric oxide and oxygen-derived free radicals, and membrane disruption by perforin and granzyme B produced by cytotoxic T cells. T cell cytokines, including IL-1, INF-γ and TNF-α exacerbate beta cell death by upregulation of Fas and Fas ligand and stimulation of nitric oxide and free radical production. Various cytokines are involved in the enhancement beta cell damage in type1 diabetes[110]. Beta cell destruction is enhanced by the Th1 and Th17 subsets of CD4+ T cells and cytokines, such as INF-γ, TNF-α and IL-2, IL-12, IL-17 and IL-18 (figure. 4). In patients with type1 diabetes, infiltration of mononuclear cells consisting of CD4+ and CD8+ and T cells, B cells and macrophages is observed in islets of pancreas biopsy specimens[111].

In contrast, there are mechanisms involved in the maintenance of peripheral tolerance by a specialized

subject of regulatory T cells (Tregs). CD4+ Tregs that constitutively co express the IL-2Rα chain (CD4+, CD25+) have been shown to play a critical role in controlling undesired immune responses to self-antigens [112]. A number of the forkhead family of transcription factors, FOX P3, has been shown to be expressed in murine and human CD4+, CD25+ Tregs and appears to be a master gene controlling CD4+, CD25+ Treg development [113]. CD4+, CD24+ Tregs with a reduced in vitro suppressive function were found in some studies performed on patients with type1 diabetes [114,115].

Oxidized LDL auto antibodies: Increased oxidative stress due to hyperglycemia is suggested to be involved in the pathogenesis of diabetic nephropathy. It is known that oxidized low-density lipoprotein (LDL) is immunogenic and forms immunocomplexes with corresponding antibodies and that these immunocomplexes are atherogenic and promote inflammation [116]. Therefore, the possible role of oxidized LDL in development of diabetic complications has been studied and several independent authors have reported that there seems to be no relation between oxidized LDL autoantibodies and albuminuria [117-119]. However, in 2002 it was found out that a high concentration of these immunocomplexes formed of antibodies with high avidity doses indeed relate to proteinuria in diabetic patients [120].

Other factors: Activation of the T-Cell-mediated immune system in genetically susceptible individuals leads to a lymphocytic infiltration within the islets (insulitis) as well as to a humoral (B cell) response with production of antibodies against one or more beta cell autoantigens. The model of the natural history of type1 diabetes suggests that there is a long prodromal phase preceding the onset of clinical symptoms in type1 diabetes (fig. 5). Overt diabetes clinically manifests only destruction of approximately 90% of the beta cells [121]. The initial interactions of genes and environmental factors, such as viral infections, trigger an immune response to islet autoantigens, with the emergence of autoantibodies as the first sign of beta cell destruction, followed by progressive loss of the first-phase insulin secretion [122]. To date, the best autoantibody predictor of a high type1 diabetes risk is the expression of multiple anti-islet autoantibodies. Among autoantibodies against insulin, GAD65, and IA-2, expression of a single autoantibody was associated with an approximate 20% risk of diabetes within 10 years of follow-up. In contrast, expression of multiple autoantibodies was associated with a very high risk of progression. The 'combinatorial' analysis allowing more than two autoantibodies are expressed, gives approximately 80% sensitivity for progression to diabetes with a very high specificity [123, 124].

Antisulphide antibodies have been suspected to be involved in the pathogenesis of diabetic complications but the available data do not give further support to this hypothesis. Recently, the immunological pathogenetic aspect has been supported by the finding that circulating immune complexes of IgG antibodies are associated with early diabetic nephropathy [125].

LIPID MEDIATORS

Small lipids derived from arachidonic acid have been implicated in the pathogenesis of diabetic nephropathy. Cyclo-oxygenease-2 breaks down arachidonic acid into several different prostanoids. In a rat model of streptozotocin induced diabetes, levels of inflammatory prostanoids, such as proprostaglandins E2 and I2 were raised [126]. Furthermore, increased expression of cyclo-oxygenase-2 has been reported in animal studies of diabetes and in the macula densa of kidneys from humans with diabetes[127]. In diabetic rats, inhibition of cyclo-oxygenase-2 is associated with decreased Glomerular hyperfiltration[128]. High glucose and succinate-induced GPR91 activation trigger paracrine signaling (fig 6) from the (juxta)glomerular endothelium to the adjacent renin-producing JG cells to increase renin synthesis and release [129], the rate limiting step of RAS activation. Elements of the signal transduction cascade involve succinate and GPR 91- dependent elevations in vascular endothelial $[Ca^{2+}]i$ as well as the synthesis and release of NO and PGE_2, classic mediators of renin release [130]. Endothelial NO and prostaglandin production also directly causes vasodilation of the afferent arteriole, which may be important in the development of Glomerular hyperfiltration.

Arachidonic acid can also be oxidized by lipoxygenases [131]. Evidence is accumulating that some of the products derived from the actions of lipoxygenases contribute to diabetic nephropathy. Specifically, levels of lipoxygenases 12 and 15 are increased in diabetic animals. In addition, high glucose levels increase expression of lipoxygenases 12 and 15 in cultured mesangial cells. To conclude, this pathway has a key mediatory role in the critical processes of mesangial cell hypertrophy and extracellular matrix accumulation mediated by TGF-β1 and angiotensin II.

OXIDATIVE STRESS

Generally, metabolic activity within the nephron produces a large amount of reactive oxygen species that are counterbalanced by a large number of antioxidant enzymes and free radical scavenging systems. Reactive oxygen species mediate many negative biological effects, including peroxidation of cell membrane lipids, oxidation of proteins, renal vasoconstriction and damage to DNA. Unfortunately, hyperglycemia tips the balance towards production of reactive oxygen species, most of which seem to be generated in the mitochondria [132]. The metabolism of glucose through harmful alternate pathways such as via PKC activation and advanced glycosylation end product formation, in the setting of hyperglycemia also seems partly dependent on reactive oxygen species [133,134].

Hyperglycemia specifically induces oxidative stress, even before diabetes becomes clinically apparent. Concentrations of markers of DNA damage induced by reactive oxygen species are higher in patients with more-

severe nephropathy (i.e proteinuria versus microalbuminuria). Furthermore, histological analysis of human kidney biopsy specimens has detected products of glyco-oxidation (combined products of glycation and protein oxidation) and lipoxidation in the mesangial matrix and glomeruli, whereas these lesions are much less common in specimens from individuals without diabetes [135].

ENDOTHALIAL CELL DYSFUNCTION (ECD)

Diabetic nephropathy is preceded by Glomerular hyperperfusion and hyperfiltration; which occur early in type1 and in some 15-44% of type2 diabetic patients at diagnosis, and these play a pathogenetic role in ECD, the early stage of diabetic nephropathy [136-139]. Endothelial cell dysfunction (ECD) is therefore defined as decreased synthesis, release, and/ or activity of endothelial-derived nitric oxide [140]. The pathophysiology of ECD expressed in various degrees is emerging as a hallmark of several highly prevalent renal as well as cardiovascular diseases and other chronic diabetic complications [141].

What triggers ECD in diabetes mellitus?

A casual relationship between oxidative stress, ECD and diabetic nephropathy has been established [142] by observations that:

a. High glucose can directly cause ECD and increases oxidative stress in glomeular mesangial cells, a target cell of diabetic nephropathy.

b. Lipid peroxides and 8-hydroxy deoxyguanosine, indices of oxidative tissue injury, were increased in the kidneys of diabetic rats with albuminuria.

c. Oxidative stress induces mRNA expression of NFKB genes which in turn promotes production of proinflammatory proteins-TGF-β, fibronectin, laminin, elastin, IL-1, IL-6, and PDGF, and

d. Inhibition of oxidative stress ameliorates all the manifestation associated with ECD and diabetic nephropathy.

PODOCYTE DAMAGE AND NEPHRIN LOSS

Recent data suggest that the podocytes, specialized visceral epithelial cells, are important for the maintenance of the dynamic functional barrier [143], and the number of podocytes may be reduced in the glomeruli of both type1 and type2 diabetic patients [144,145]. Furthermore, it has been reported that nephrin, a recently found podocyte protein, is crucial for maintaining the integrity of the interpodocyte slit membrane structure and for maintenance of an intact filtration barrier. In diabetic nephropathy, the protein level of nephrin decreases, possibly via loss into the urine due to synthesis of splice variant isoforms of the nephrin lacking a transmembrane domain [146,147]. Patients with diabetic nephropathy have markedly reduced renal nephrin expression and fewer electron-dense slit diaphragms compared with patients without diabetes and minimal nephropathic changes or controls [148].

Furthermore, nephrin excretion is raised 17-30% in patients with diabetes (with and without albuminuria) compared with that in individuals without diabetes. Thus, nephrin excretion could be an early finding of podocyte injury, even before the onset of albuminuria. Several studies have been performed on the angitensin II activity which is involved in podocyte injury in diabetes. Angiotensin-converting enzyme inhibitors prevented loss of podocytes and podocyte injury in the streptozotocin-induced diabetic rat. In addition to angitensin-converting enzyme inhibitors, angitensin II type1 receptor antagonism attenuated podocyte foot process broadening in the streptozotocin-induced diabetic rat [149, 150].

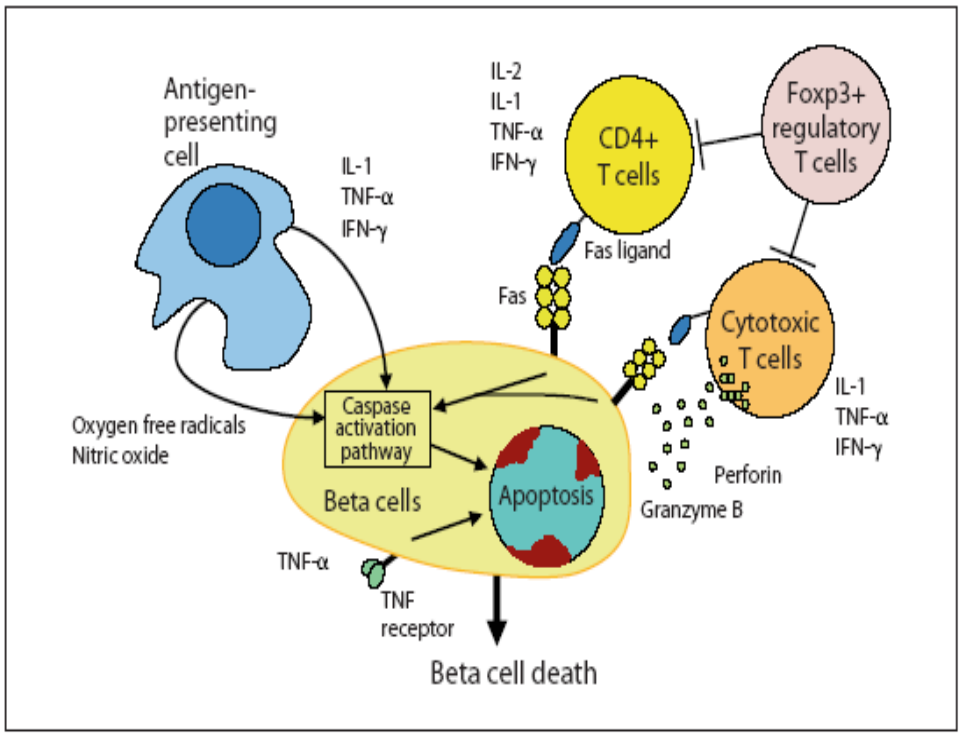
In a study of patients with type2 diabetes, treatment with an angiotensin-converting enzyme inhibitor for 2 years maintained nephrin expression at control levels compared with that in untreated patients with diabetes. By contrast, the expression of two other important podocyte and slit diaphragm proteins, podocin and CD2AP, was similar in the three groups. Comparable decreases in renal nephrin expression were reported in other studies of diabetic nephropathy [151].

GENETIC SUSCEPTIBILITY

Genotype seems to be an important determinant of both incidence and severity of diabetic nephropathy [152-154]. The increase in risk cannot be explained by the duration of diabetes or hypertension, or the degree of glycemic control. Environmental and genetic factors must, therefore, have roles in the pathogenesis of diabetic nephropathy. In patients with type1 or type2 diabetes, the likelihood of developing diabetic nephropathy is markedly increased in those who have a sibling or parent with diabetic nephropathy [155, 156]. There is also growing evidence that the genetic background determines the risk of nephropathy in patients with diabetes. Epidemiologic studies have shown that 35% of the patients with diabetes develop nephropathy, irrespective of glycemic control [157, 158].

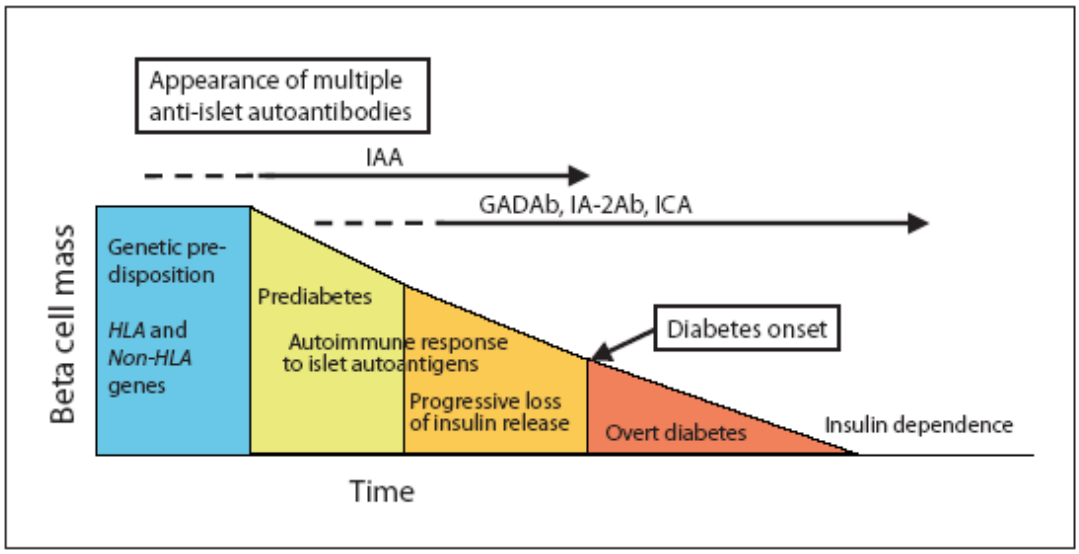
Studies of families of patients with diabetic nephropathy have shown an increased risk in the relatives [159]. Also, the differences between various ethnic groups in development of diabetic nephropathy support the genetic aspect. Examples of this are the Pima Indians, who in addition to having a high incidence of type2 diabetes, have an elevated risk for diabetic nephropathy as well. In families of African American patients with type2 diabetes-induced end stage renal disease the relatives have an eight fold risk of developing diabetic nephropathy, as compared to the relatives o a type2 diabetic control population without renal complications [160].

Figure 4: Mechanisms of beta cell destruction in type 1 diabetes



T-cell-mediated beta cell destruction is induced by the release of cytotoxic molecules, including cytokines, granzyme B, or perforin, or by direct delivery of cell death signals via the Fas pathway. Beta cells die through an apoptotic process which is activated by Fas interaction with Fas ligand and action of nitric oxide and oxygen free radicals, perforin, and granzyme B. T cell cytokines, including IL-1, IFN- γ, and TNF-α , exacerbate beta cell death. In contrast, CD4+CD25+ Foxp3+-regulatory T cells will suppress effector T cells via cell-cell contact.

Figure 5: Schematic representation of the natural history of type1 diabetes



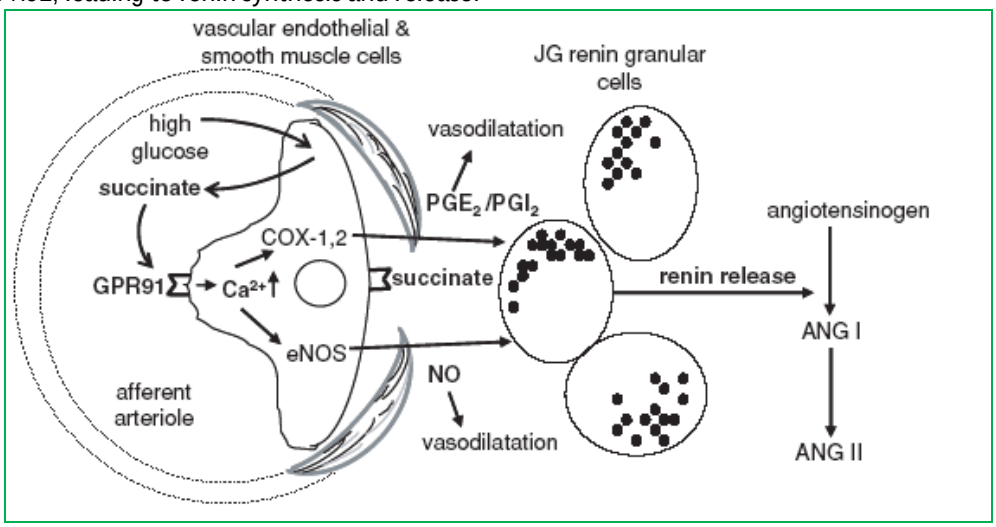
The initial interaction of *HLA* and *non-HLA* genes and environmental factors trigger an autoimmune response to islet autoantigens, with the emergence of multiple anti-islet autoantibodies, followed by the progressive loss of the insulin release. Over time, there is impaired glucose tolerance and ultimately overt diabetes. Several years after the onset of type 1 diabetes, the beta cell mass is completely or near completely lost. IAA = Insulin autoantibodies; GADAb = GAD65 autoantibodies; IA-2Ab = IA-2/ICA512 autoantibodies; ICA = islet cytoplasmic autoantibodies.

There are only a few descriptions of genome wide scans for the analyses would not be enough. In the genome wide scans for micro vascular complications in Pima Indians, four loci on chromosomes 3, 7, 9, and 20 were identified [161]. A candidate gene study of type1 diabetic nephropathy also identified a 63-CM region on chromosome 3q, containing the angiotensin II type1 receptor gene [162]. Furthermore, other linkage studies identified additional loci on chromosomes 7q21.3, 10p15.3, 14q23.1, and 18q22.3 as diabetic nephropathy susceptibility genes [163, 164]. Association studies of candidate genes have been the most common approach to identify genes involved in susceptibility to diabetic nephropathy. The greatest risks seems to be associated with genes encoding angiotensin-converting enzyme, angitensin II receptor, cytokines, proteins involved in

glucose or lipid metabolism, and extracellular matrix proteins.

The angiotensin-converting enzyme gene (ACE) polymorphism has been explored in several studies. The insertion-deletion polymorphism is responsible for the difference between individuals in plasma levels of angiotensin-converting enzyme. In patients with type2 diabetes, the DD polymorphism of the ACE gene has been associated with an increased risk of developing diabetic neohropathy, severe proteinuria progressive renal failure, and of mortality during dialysis [165-167]. In addition, an analysis of more than 1,000 white patients with type1 diabetes showed a strong correlation between genetic variation in the ACE gene and the development of nephropathy[168]. To date, a number of single-nucleotide polymorphisms are reported as diabetic nephropathy susceptibility genes [169].

Figure 6: Schematic illustration of the newly described (juxta) glomerular paracrine signaling cascade involving succinate activation of GPR91, leading to renin synthesis and release.



High glucose levels result in the accumulation of the metabolic intermediate succinate, which acts directly on the vascular endothelium and triggers cell-to-cell signaling to culminate in renin release from the juxtaglomerular apparatus. The chain of events involves elevating glucose levels, succinate accumulation in the plasma and local interstitium, GPR91 activation, increases in endothelial cytosolic calcium, nitric oxide (NO) and prostaglandin (at least PGE2) production and release from endothelium, PG actions on renin-producing JG cells, renin release, angiotensin (Ang I and Ang II) synthesis and RAS activation. Endothelial NO and PG production also causes vasodilatation that may be important in the development of glomerular hyperfiltration.

Table 2: Candidate genetic determinants for diabetic nephropathy

Symbol	Name	Locus	Rationale
SLC12A3 [91]	Solute carrier family 12 member (sodium/chloride) 3	16q13	The *SLC12A3* gene encodes a thiazide-sensitive Na^+-Cl^- cotransporter that mediates reabsorption of Na^+ and of Cl^- at the renal distal convoluted tubule [91, 92]
ELMO1 [55]	Engulfment and cell motility 1	7p14.1	ELMO1 contributes to glomerular injury through dysregulation of the ECM metabolism and reduction in cell-adhesive properties to ECMs [55, 93]
ICAM-1 [85]*	Intracellular adhesion molecule	19p13	Increased ICAM-1 expression accompanies progression of type 1 diabetes and diabetic nephropathy [94–96]
VEGF [97]*	Vascular endotherial growth factor	6p12	VEGF expression increased in glomeruli of diabetic animals; anti-VEGF therapy reduced AER and hyperfiltration [43, 63, 64]
MBL2 [98]*	Mannose-binding lectin	10q11.2-q21	The MBL pathway of complement activation may contribute to the development of diabetic microvascular complications [98, 99]
SUMO4 [83]*	Small ubiquitin-like modifier 4	6q25	SUMO mRNA is mainly expressed in kidneys and immune system [100]
TNF-α [101]*	Tumor necrosis factor-α	6p21.3	The level of expression of TNF-α correlated with obesity and hyperinsulinemia [102]
TGF-β [86]*	Transforming growth factor-β 1	19q13.1	TGF-β mRNA and proteins (and TGF-β receptor mRNA) identified in rodent glomerular cells [103–105]
MCP-1 [84]*	Monocyte chemotactic protein-1	17q11.2-q12	MCP-1 is produced in response to proinflammatory stimuli and high glucose level in mesangial cells [106]

* Common genetic determinants for the development of type 1 diabetes and diabetic nephropathy.

CONCLUSION

The pathogenesis of diabetic nephropathy has multiple facets and is the leading cause of glomerulosclerosis and end-stage renal disease. Though the pathology is well understood the treatment remains quite lucid. Interference in the heamodynamic changes, Nonenzymatic glycosylation that generates advanced glycosylation end products, activation of protein kinase C (PKC), acceleration of the aldose reductase pathway products may be some of the important targets that can be explored for its treatment. Probably drugs that inactivate GPR 91 receptor signaling in the kidney will be the most useful candidates for the treatment of diabetic nephropathy.

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