Published in final edited form as:

Curr Opin Nephrol Hypertens. 2013 January ; 22(1): 17–25. doi:10.1097/MNH.0b013e32835b3766.

New Targets for Treatment of Diabetic Nephropathy: What We Have Learned from Animal Models

Frank C. Brosius III^a and Charles E. Alpers^b

^aDepartment of Internal Medicine, and Molecular and Integrative Physiology, University of Michigan Medical School

^bDepartment of Pathology, University of Washington

Abstract

Purpose of review—There has been an advance in our understanding of the mechanisms of diabetic nephropathy (DN) over the past few years, and much of that has occurred because of studies in animal models of DN.

Recent findings—Studies in animal models of DN, especially in mice, have underlined the multifactorial nature of the pathogenesis of the disease process and the recognition that these models only partly replicate changes found in human disease. Despite these limitations, recent animal model studies have identified a number of new, specific molecular abnormalities that point to pathways and specific molecules as potential targets for preventative or therapeutic intervention. These specific targets include the DN related decreases in eNOS activity and renal dopamine production and the increases in Nrf-2, JAK/STAT and mTORC1 signaling. These and other altered signaling pathways are described in this review. We emphasize the use of a unique investigative resource, Nephromine, to utilize a library of mRNA expression data obtained from kidney biopsies of humans with DN, to compare and validate findings in mouse models with human disease.

Summary—Several new pathways have been implicated in the progression of DN through studies of animal models. Some of these appear to be altered in human DN and may be targets for therapy.

Keywords

Diabetic kidney disease; transgenic mouse; signaling

Introduction

Diabetes mellitus is the major cause of End-Stage Renal Disease (ESRD) in the United States and elsewhere, and its incidence has increased by more than 50% in the last 10 years. (1) Despite this trend only a minority of individuals with either type 1 or type 2 diabetes develop nephropathy, indicating that specific environmental or genetic factors must contribute to its initiation and progression. While clear progress has been made in understanding the disease process, there has been limited success in identifying specific factors that cause human nephropathy and its progression. One of the reasons for the slow evolution in our understanding of DN is the lack of reliable animal models that mimic human disease. Over the past 15 years there has been a concerted effort by many individual

investigators and consortia address this deficiency (2, 3). While this effort has produced a number of important advances, there have been no new models of any diabetic complication that fully reproduce the human phenotype. This lack of a “perfect model” has led to a growing consensus that animal models are best used to provide clues about the pathogenesis of the human disease, but at present cannot serve as miniature replicas to plot the course of human complications. In addition it appears that most animal models described to date represent, at best, early changes of diabetic kidney disease (3, 4). Thus, most of the information derived from analysis of these models likely provides only limited or indirect information about the factors critical for progressive kidney disease in patients with diabetic kidney disease. That being said, many new pathways and potential targets for intervention have been suggested from animal models over the past several years. The purpose of this review will be to underline some of the more interesting new pathways and targets and to evaluate the evidence supporting their potential role in diabetic kidney disease in humans. We will concentrate on the data from mouse models as these have been the workhorses of diabetic complications research over the past few years.

Current state of mouse model research in diabetic kidney disease

Although no reported animal models fully replicate the human condition of progressive DN, some models are better than others. One clear-cut finding over the past 10 years has been the important effect of genetic background on development and severity of diabetic changes in the kidney of mouse models. Indeed, the mouse strain most commonly used for many experimental studies, the C57BL/6 strain, is remarkably resistant to diabetic injury (2, 3). Thus, most experts in the field have concluded that pathways identified in this model are suspect unless validated in another model (e.g., mouse strain with a more susceptible background) or in humans (3, 5). This is especially true for experiments that purport to mitigate disease. In the discussion below, therefore, we will emphasize the strain of mice that have been studied to aid in interpretation of results.

Changes in pathways that promote diabetic kidney disease in mice

The bulk of this review will focus on reports that have been published or presented in the last 2 years and have either identified new potential targets and pathways or altered our thinking about older ones. Thus, it is not a survey of important factors in the pathogenesis of DN. Although we organize this section based on the pathway or target being addressed by specific studies, we believe that the pathogenesis of DN is complex and multidimensional involving the interaction of many pathways and networks of abnormalities. With the advent of genome wide analytical capabilities it is now feasible to simultaneously analyze multiple regulatory biological systems at high precision and depth. We emphasize the utility of this strategy, especially in moving from animal model research to human studies. To help facilitate this analysis, we have utilized the online database, Nephromine (www.nephromine.org), which provides curated data from all published (and some unpublished) genome-wide expression studies in humans with any type of kidney disease, including DN. Nephromine provides sophisticated analytical tools that we have utilized in the simplest way to interrogate the expression levels in human DN of target genes described in the mouse models reported below. The human DN database used for this analysis is from a recently published Affymetrix expression array study(6) used to identify differentially regulated transcripts in microdissected human kidney samples, 22 of which were from patients with diabetes. DN samples were significant for their racial diversity and decreased glomerular filtration rate (~25-35 mL/min). Stringent statistical analysis was used to identify differentially expressed transcripts in control and diseased glomeruli and tubuli.

Reduction in eNOS (NOS3) activity predisposes to DN—It has become increasingly clear that one of the best mouse models of DN is the eNOS knockout mouse.

This appears to be true irrespective of the genetic background of the mice or the cause of experimental diabetes (3, 7-9). This has brought the role of eNOS in preventing disease as well as endothelial defects in DN to the fore. A recent study (10) explored the effect of experimental diabetes on eNOS activity in cultured endothelial cells and in a type 2 model of diabetes (db/db C57BLKS mouse). High glucose increased endothelial permeability in cultured cells and reduced eNOS dimer formation and phosphorylation both in the cultured cells and in glomeruli from diabetic mice. These abnormalities were reversed by supplementation with precursors of either the eNOS cofactor, tetrahydrobiopterin, or of nitric oxide itself. Importantly, these interventions also reduced some of the DN pathology and other findings in the mouse model, including reduced basement membrane thickening and albuminuria and reduced evidence of oxidative stress. (10) There were no changes in eNOS mRNA or monomer protein expression, as the defects were post-translational, but this study is important for showing that hyperglycemia and diabetes can reduce eNOS function. It seems reasonable that similar effects could be found in humans with DN since the murine model that lacks eNOS function replicates important features of advanced human DN and, in a manuscript in press, has been shown to have a better overlap in glomerular gene expression with that in human DN than the two other murine DN models that were studied (4). Finally, in a rat model of diabetic kidney disease and hypertension, eNOS uncoupling has been corroborated as a major factor in disease progression and in accumulation of reactive oxygen species (ROS) in kidney cortex. Interestingly, these abnormalities were largely reversed by green tea administration (11).

To analyze the role of eNOS gene expression in DN in humans, we explored the changes in DN collated in Nephromine. As expected there was not a significant change in eNOS mRNA levels in either glomeruli or tubulointerstitial regions from humans with progressive DN (data not shown). The data support scenarios by which a change in activity of eNOS, rather than a change in eNOS expression, is an important mediator of human and rodent DN.

Accordingly, a reduction in eNOS activity remains an attractive, but unproven, cause of DN in humans and a potential target for intervention. As an additional indicator of plausibility, Smithies' group has found increased nephropathy in mice with changes in eNOS (NOS3) expression that are similar to those associated with variants found in humans (12). Thus, normal variations in eNOS expression might alter the risk of progressive DN in humans, although statistically lower eNOS expression in kidney tissues was not identified in the Nephromine analysis.

Reduction in leptin/leptin receptor signaling may predispose to diabetic complications—A leading model of type 2 diabetes and DN, the *db/db* mouse, is both strain dependent and dependent on leptin deficiency due to a mutation in the leptin receptor. A recently described model of morphologically advanced DN is dependent on leptin deficiency via the *ob/ob* mutation of leptin and is also strain dependent, since the robust nephropathy develops in BTBR mice but not in other strains with the same mutation (e.g., C57BL/6). There is evidence that the susceptibility of this strain to develop diabetic complications results from a unique interaction between genomic features of the BTBR strain and disrupted leptin signaling. For example, BTBR wild-type mice with intact leptin signaling made diabetic by administration of streptozotocin (STZ) do not develop complications comparable to BTBR *ob/ob* mice. Also, BTBR *ob/ob* mice, treated with a PPAR agonist to lower blood glucose, do not show improvement in DN and leptin replacement reverses DN in this strain (13, 14). These studies have not clarified whether the salutary effects of leptin replacement are the result of leptin activity in the CNS, or the result of signaling in the kidney. A PET imaging study has shown leptin uptake in renal tubules via the megalin receptor, but did not report uptake within glomeruli (even though megalin is present in rodent glomeruli). There are few data at present that clarify how leptin acts on

kidney cells, and so the basis for the contribution of leptin deficiency to the development of DN in BTBR mice remains obscure.

In our Nephromine analysis of changes in leptin and the leptin receptor in human DN, there was no change in leptin levels but there was a significant 1.7-fold increase in leptin receptor expression in the tubulointerstitium of patients with progressive DN compared to controls.

The Nephromine findings fit well with the PET imaging studies in suggesting activity of leptin in the tubulointerstitium, whether via megalin or other receptors in mice or by distinct leptin receptors in humans. Both the Nephromine and PET structures pose an as yet unexplained discrepancy between the striking glomerular changes and the very modest tubulointerstitial changes that develop in leptin deficient BTBR mice.

The role of NADPH oxidase in reactive oxygen species (ROS) mediated pathology in DN—Given the clear role of ROS in the pathogenesis of DN, much effort has focused on identifying the sources of enhanced oxidative stress in kidney cells and to determine which are most important in disease progression. Given the complexity of the disease process, it is not surprising that multiple sources including mitochondrial, cytoplasmic and membrane associated mediators of ROS accumulation have been identified and shown to play potential roles in experimental DN. One area of relatively recent focus is the potential role of NADPH oxidases in DN progression. Recent and promising observations about the role of these enzymes have come from the studies that modulate expression or activity of the NADPH oxidase catalytic subunit, NOX4, that is expressed in the kidney at high levels. These studies have shown that NOX4 levels and activity are increased in rat and mouse models of DN (15-17) including in the robust OVE26 FVB mouse model (15) and that inhibition of NOX4 reduces glomerular changes of DN in mice. A recent study found that a novel NOX1/4 inhibitor also reversed early DN changes in db/db BLKS mice but that the effects were not sustained. Worrisomely, in this study, albuminuria ultimately increased to a higher level than that seen in untreated diabetic animals (18).

Confounding the findings in rodent models is the observation that NOX4 levels were not changed in glomeruli or tubulointerstitium of humans with DN in our Nephromine analysis, although NOX2 levels were significantly increased around 2-fold in both compartments. There is a relative paucity of experimental data in experimental DN that would explain the basis for the elevated NOX2 identified in humans.

ROS generated by NADPH oxidases clearly accentuated progressive DN by stimulating tissue damage, inflammation and fibrosis. Given its high expression in kidney cells, NOX4 studies have predominated, but since NOX2 is also expressed in kidney (19) and vascular cells (20), and its expression appears to be altered in human DN, studies of the role of NOX2 in the pathogenesis of DN are in order.

Activation of NFE2-related factor 2 (Nrf2) and downstream effectors reduces DN—Another important regulator of oxidative stress in cells is Nrf2. This molecule regulates the expression of numerous genes including antioxidant enzymes, such as NADPH quinone oxidoreductase 1, glutathione S-transferase, heme oxygenase-1, glutathione peroxidase, glutamate cysteine ligase and peroxiredoxin I, which can counteract both pro-inflammatory signals as well as oxidative stress (21). Thus, Nrf2 and its negative regulator, Keap1, are attractive targets to modulate in DN. A recent report of a phase 2 clinical trial with an agent that activates Nrf2, bardoxolone methyl, has prompted even greater attention to this pathway as potentially protective in DN (22). The animal data supporting the role of Nrf2 in counteracting diabetic kidney damage include a study showing that Nrf2 knockout mice on a C57Bl/6 background developed greater nephropathy (expanded mesangium) after

being made diabetic with low dose STZ injections than did wild-type diabetic mice. Albuminuria increased approximately 3-fold in the knockout diabetic animals compared to a roughly 2-fold increase in the wild-type diabetic animals, compared to their respective non-diabetic counterparts (23). As in other studies on C57BL/6 animals the degree of nephropathy, even in the Nrf2 knockout animals, was modest. A more recent study from the same group showed that Nrf2 activation by exogenous dietary compounds, such as sulforaphane and cinnamic aldehyde, can reduce DN in the same models and is effective in both Nrf2 knockout and wild-type animals, suggesting that at least some of the helpful effects of the dietary compounds were independent of Nrf2. (although the compounds also had a major glucose lowering effect which could have accounted for much of this protection (24). In the same study, humans with DN were found to have high endogenous glomerular Nrf2 activity, which raises the question of whether activating Nrf2 more completely or earlier will substantially ameliorate DN progression.

In our Nephromine analysis, Nrf2 gene expression was not altered, but several of its downstream targets such as peroxiredoxin I, glutathione peroxidase 1, and heme oxygenase 1 (HO-1) showed increases in expression (or trends in that direction) in both glomerular and tubulointerstitial compartments in progressive human DN (Fig. 1). HO-1 itself is a potential target for modulation of DN (25). As is the case for Nrf2, induction of HO-1 activity can prevent at least some aspects of DN in rodent models (26).

These studies suggest that humans with DN already have substantial upregulation of antioxidant responses by the time nephropathy is evident, and underline the question of whether super-induction of antioxidant regulators or enzymes will further ameliorate the disease process.

Podocyte mammalian target of rapamycin complex 1 (mTORC1) activity contributes to DN—

While a number of studies have suggested that enhanced mTOR activity contributes to glomerular hypertrophy and proteinuria, the most compelling data supporting the role of mTOR in DN have come from 2 recent papers (27, 28). These papers used parallel but complementary methods to assess the role of podocyte mTORC in DN. Critically, they separated out the effects of the 2 different mTOR complexes, mTORC1 and mTORC2 (28). Activation of mTORC1 specifically in podocytes by genetic deletion of an upstream negative regulator led to many changes of DN including albuminuria, glomerular basement membrane widening, podocyte loss, mesangial expansion and glomerular mesangial accumulation of fibronectin and collagen IV. All of these alterations were at least partly prevented by treatment with the mTORC1 inhibitor, rapamycin, which also produced similar changes in the *db/db* model of DN (27). These changes were prevented by a podocyte-specific genetic reduction in mTORC1 expression reduction via heterozygous deletion of raptor, a specific component of mTORC1. Although these studies indicate that targeting mTORC1 for therapy of DN is attractive, the difficulty of such a strategy was underlined by the finding that elimination of raptor expression in podocytes resulted in significant proteinuria (28).

Nephromine analysis did not show a significant increase in mTOR expression in either glomeruli or tubulointerstitium in progressive human DN but did suggest that downstream targets, based on the canonical signaling pathway found in Biocarta Pathways (www.biocarta.com), might be generally increased in expression in the tubulointerstitium (not shown). This finding would require further analysis to confirm.

An increase in mTORC1 activity in podocytes likely plays a significant role in glomerular changes of DN. However, reduced mTOR activity and mTOR inhibitors in humans can also lead to glomerular pathology and proteinuria (27-29). Added to this complexity is the

incompletely understood interaction of mTORC1 and mTORC2 and the apparent age and condition-specific responses to manipulation of mTORC1, which make it a less attractive target in DN until more is learned about potential benefits and risks of long-term mTORC1 inhibition.

Enhanced Janus kinase/signal transducer and activator of transcription (JAK/STAT) expression and signaling appears to contribute to progressive DN—

Over the past few years, the role of the JAK/STAT pathway in DN, and other chronic kidney diseases, had garnered attention. These studies began with Marrero's work a decade ago showing that JAK2 activation resulted in diabetic glomerulopathy (30) and was expanded by human data showing substantial upregulation of multiple genes in this system that, in the tubulointerstitium, tightly correlated with a decline in GFR (31). In addition, increased expression of endogenous STAT inhibitors, the suppressors of cytokine signaling proteins, SOCS-1 and SOCS-3, inhibited early changes of DN in a STZ-diabetic rat model (32). In an article in press, we performed a cross-species comparison of glomerular transcriptional networks in DN. Glomerular gene expression was profiled in patients with early type 2 DN as well as in three of the better current mouse DN models (STZ-diabetic DBA/2 mice, db/db C57BLKS, and eNOS-deficient C57BLKS db/db mice). Species-specific transcriptional networks were generated and compared using a novel network-matching algorithm. Shared genes across all networks reflected the important role of JAK-STAT signaling pathways, as the largest number of conserved changes were in these pathways(4).

Nephroline analysis showed that JAK1 (Fig. 2), JAK2, STAT1 and STAT3, but not STAT5a or 5b, SOCS-1 or SOCS-3 were significantly increased in the tubulointerstitium in human progressive DN.

The activation of JAK/STAT signaling appears to trigger both inflammatory and pro-fibrotic responses and may modulate angiotensin II signaling in the kidney. In addition, in recent unpublished data we have found that specific overexpression of JAK2 in podocytes augments mesangial expansion and albuminuria in a mouse diabetic model (H. Zhang, F. C. Brosius, unpublished data). A phase 2 clinical trial of a JAK1/JAK2 inhibitor (ClinicalTrials.gov identifier: NCT01683409) will help determine whether this pathway is a viable one for targeting in progressive DN.

Bone morphogenic proteins (BMPs) modulate DN—Reams of evidence point to TGF- activation as a critical inducer of the glomerular and tubulointerstitial fibrosis that is a hallmark of progressive DN. A major downstream target of TGF- is Connective Tissue Growth Factor (CTGF) which can act independently and sometimes oppositely to TGF- and itself mediates many of the effects critical for DN progression (33). BMPs, particularly BMP-7, have been found to be protective growth factors that antagonize the effects of TGF- and CTGF. Conversely, CTGF can bind to BMP-7 and antagonize its signaling, thereby promoting downstream effects consistent with worsening DN (34). Recently, a single group has published evidence suggesting a role for BMP-4 in aggravating, not ameliorating, DN. The investigators studied a C57BL/6 mouse model of inducible BMP-4 overexpression which, though not specific for mesangial cells, promoted BMP-4 expression primarily in these cells in the kidney (35). While the mice were not completely characterized in terms of systemic effects they did develop glomerular changes consistent with early DN including albuminuria, mesangial expansion, glomerular basement membrane thickening (35). Conversely, modest changes of DN found in STZ-diabetic C57BL/6 mice were attenuated in BMP-4 heterozygote knockout mice, though this latter finding is unconvincing given the limited nephropathy that develops in this mouse background. The same group then implicated BMP-4 in inducing chondrocyte markers as an aspect of its DN-inducing

properties. One of these markers was SRY-related HMG Box 9 (SOX-9) which itself induced chondrocyte changes in cultured mesangial cells (36). In type 1 diabetic BMP-4 transgenic mice, large amounts of SOX-9, collagen II as well as DN changes were documented.

Interestingly, although there were no changes in BMP-4 or BMP-7 gene expression in our Nephromine analysis of human DN there were substantial increases in SOX-9 in both glomeruli and tubulointerstitium of these DN patients when compared to controls (Fig. 3).

While preliminary, the data implicating BMP-4 and its effects on chondrocyte markers in diabetic glomeruli are intriguing and these reports should stimulate more interest in this signaling pathway as one potentially relevant to the progression of human DN.

Dopamine signaling may ameliorate DN—A relatively new concept in DN research is that reduced dopamine handling and signaling by the proximal tubule may lead to worsening DN through a number of mechanisms. L-DOPA is filtered and taken up by the proximal tubule epithelia and converted therein to dopamine which in turn is largely metabolized by catechol-O-methyl-transferase (COMT). A report this spring studied diabetic COMT knockout mice (on a nephropathic 129J/sv background) as a way to enhance proximal tubule dopamine levels and signaling (37). The knockout diabetic mice showed a substantial amelioration of early hyperfiltration (probably via reduced cyclooxygenase 2 activity) as well as a reduction in albuminuria, mesangial expansion, and markers of renal fibrosis and oxidative stress in diabetic knockout mice with diabetes induced either by STZ or by crossbreeding with Akita mice compared to wild-type diabetic mice. Similar improvement was found in diabetic wild-type mice transplanted with a COMT knockout kidney providing good evidence that it was the reduced COMT activity, and increased dopamine levels, in the kidney that specifically led to improved outcomes. Conversely, a model of selective proximal tubule intrarenal dopamine depletion was produced with a proximal tubule specific knockout of aromatic amino acid decarboxylase (AADC), the enzyme that converts L-DOPA to dopamine. These mice, made diabetic with STZ, developed a marked increase in albuminuria as well as increases in mesangial expansion, renal macrophage infiltration, and renal nitrotyrosine levels (37). This reduction in dopamine signaling would result in a primary increase in proximal tubular salt and water reabsorption, in part via SGLT2, that could lead to hyperfiltration (38) as well as hypertension (39).

On Nephromine analysis, COMT levels were significantly increased by 1.9-fold in the tubulointerstitium in DN patients. There were no changes in COMT levels in the glomerulus or in AADC levels in either compartment. If all other processes were equivalent, the increased COMT levels could be associated with decreased tubulointerstitial dopamine levels.

Intrarenal dopamine may counteract some of the salt reabsorptive, hemodynamic and profibrotic changes of progressive DN. If these changes can be pathophysiologically confirmed in humans, it may open up another new target for intervention.

Conclusion

Small animal research in DN, though hobbled by the lack of ideal models of progressive disease, has still been able to identify a number of new potential targets for intervention that can be investigated more fully in other models as well as in humans when appropriate. Targets identified in animal models require confirmation in humans. The increasing availability of annotated databases of human disease specific expression data represents one approach to enable such confirmation. While adding to the complexity of the interconnected

networks responsible for the disease process, recent research has opened up more possibilities where therapies may already exist or be available for clinical trials. A largely arbitrary (and alphabetical) list of those targets to be considered for targeting include: dopamine (augment), eNOS (augment), JAK1/2 (inhibit), mTORC-1 (inhibit), Nrf-2 (inhibit).

Acknowledgments

FCB has accepted travel costs, but no honoraria, for consulting trips for Merck and Lilly and Co. As part of his University of Michigan appointment, he is principal investigator of a Phase 2 clinical trial of a JAK1/2 inhibitor being conducted by Lilly and Co.

CEA has been a consultant on the use of diabetic models for Bristol Myers Squibb, Omeros, Reata, and ChemoCentryx. He has received research support to study endothelin receptors in diabetic nephropathy from Abbott, Inc.

This work was supported by the Applied Systems Biology Core of the George O'Brien Kidney Core Center (NIH P30 DK081943) at the University of Michigan and by NIH grants R24 DK082841 (FCB), RO1 DK083391 (CEA), and DP3 DK094311 (CEA, Co-I). Access to Nephromine was provided by the University of Michigan Kidney Core Center and is a product of Compendia Bioscience, Ann Arbor, MI.

References

1. U.S. Renal Data System. USRDS 2008 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; Bethesda, MD: 2008.
2. Breyer MD, Bottinger E, Brosius FC 3rd, Coffman TM, Harris RC, Heilig CW, et al. Mouse models of diabetic nephropathy. *J Am Soc Nephrol*. Jan; 2005 16(1):27–45. [PubMed: 15563560]
3. Brosius FC 3rd, Alpers CE, Bottinger EP, Breyer MD, Coffman TM, Gurley SB, et al. Mouse models of diabetic nephropathy. *J Am Soc Nephrol*. Dec; 2009 20(12):2503–12. [PubMed: 19729434]
4. Hodgins JB, Nair V, Zhang H, Randolph A, Harris RC, Nelson RG, et al. Identification of Cross-Species Shared Transcriptional Networks of Diabetic Nephropathy in Human and Mouse Glomeruli. *Diabetes*. 2012;61. [PubMed: 22124464]
5. Alpers CE, Hudkins KL. Mouse models of diabetic nephropathy. *Curr Opin Nephrol Hypertens*. May; 2011 20(3):278–84. [PubMed: 21422926]
6. Woroniecka KI, Park AS, Mohtat D, Thomas DB, Pullman JM, Susztak K. Transcriptome analysis of human diabetic kidney disease. *Diabetes*. Sep; 2011 60(9):2354–69. [PubMed: 21752957]
7. Nakagawa T, Sato W, Glushakova O, Heinig M, Clarke T, Campbell-Thompson M, et al. Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. *J Am Soc Nephrol*. Feb; 2007 18(2):539–50. [PubMed: 17202420]
8. Kanetsuna Y, Takahashi K, Nagata M, Gannon MA, Breyer MD, Harris RC, et al. Deficiency of endothelial nitric-oxide synthase confers susceptibility to diabetic nephropathy in nephropathy-resistant inbred mice. *The American journal of pathology*. May; 2007 170(5):1473–84. [PubMed: 17456755]
9. Zhao HJ, Wang S, Cheng H, Zhang MZ, Takahashi T, Fogo AB, et al. Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. *J Am Soc Nephrol*. Oct; 2006 17(10):2664–9. [PubMed: 16971655]
10. Cheng H, Wang H, Fan X, Pauksakon P, Harris RC. Improvement of endothelial nitric oxide synthase activity retards the progression of diabetic nephropathy in db/db mice. *Kidney international*. Jul 11.2012
11. Faria AM, Papadimitriou A, Silva KC, Lopes de Faria JM, Lopes de Faria JB. Uncoupling endothelial nitric oxide synthase is ameliorated by green tea in experimental diabetes by re-establishing tetrahydrobiopterin levels. *Diabetes*. Jul; 2012 61(7):1838–47. [PubMed: 22586583]
12. Wang CH, Li F, Hiller S, Kim HS, Maeda N, Smithies O, et al. A modest decrease in endothelial NOS in mice comparable to that associated with human NOS3 variants exacerbates diabetic

- nephropathy. Proceedings of the National Academy of Sciences of the United States of America. Feb 1; 2011 108(5):2070–5. [PubMed: 21245338]
13. Pichaiwong, W.; Hudkins, KL.; Wietecha, T.; Askari, B.; Kobayashi, T.; Shankland, S., et al. Reversibility of Diabetic Nephropathy and Podocyte Loss in the BTBR ob/ob Mouse.. 43rd annual meeting of the American Society of Nephrology; Denver, CO. November 2010; 2010.
 14. Wietecha, T.; Hudkins, KL.; Fox, EJ.; Nguyen, TQ.; Loeb, LA.; Alpers, CE., et al. Leptin-dependent effects of CP900691, a highly and potent PPAR α agonist on diabetic nephropathy in the BTBR ob/ob Mouse.. 72nd annual meeting of the American Diabetes Association; Philadelphia. June 2012; 2012. p. A132
 15. Gorin Y, Block K, Hernandez J, Bhandari B, Wagner B, Barnes JL, et al. Nox4 NAD(P)H oxidase mediates hypertrophy and fibronectin expression in the diabetic kidney. The Journal of biological chemistry. Nov 25; 2005 280(47):39616–26. [PubMed: 16135519]
 16. Eid AA, Ford BM, Block K, Kasinath BS, Gorin Y, Ghosh-Choudhury G, et al. AMP-activated protein kinase (AMPK) negatively regulates Nox4-dependent activation of p53 and epithelial cell apoptosis in diabetes. The Journal of biological chemistry. Nov 26; 2010 285(48):37503–12. [PubMed: 20861022]
 17. Sedeek M, Callera G, Montezano A, Gutsol A, Heitz F, Szyndralewiez C, et al. Critical role of Nox4-based NADPH oxidase in glucose-induced oxidative stress in the kidney: implications in type 2 diabetic nephropathy. American journal of physiology. Dec; 2010 299(6):F1348–58. [PubMed: 20630933]
 18. Sedeek M, Gutsol A, Montezano AC, Burger D, Nguyen Dinh Cat A, Kennedy CR, et al. Renoprotective effects of a novel Nox1/4 inhibitor in a mouse model of type 2 diabetes. Clin Sci (Lond). Aug 24; 2012
 19. Fu Y, Zhang R, Lu D, Liu H, Chandrashekar K, Juncos LA, et al. NOX2 is the primary source of angiotensin II-induced superoxide in the macula densa. Am J Physiol Regul Integr Comp Physiol. Mar; 2010 298(3):R707–12. [PubMed: 20053956]
 20. Montezano AC, Touyz RM. Oxidative stress, Noxs, and hypertension: experimental evidence and clinical controversies. Annals of medicine. Jun; 2012 44(Suppl 1):S2–16. [PubMed: 22713144]
 21. Zhang DD. Mechanistic studies of the Nrf2-Keap1 signaling pathway. Drug Metab Rev. 2006; 38(4):769–89. [PubMed: 17145701]
 22. Pergola PE, Raskin P, Toto RD, Meyer CJ, Huff JW, Grossman EB, et al. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. N Engl J Med. Jul 28; 2011 365(4):327–36. [PubMed: 21699484]
 23. Jiang T, Huang Z, Lin Y, Zhang Z, Fang D, Zhang DD. The protective role of Nrf2 in streptozotocin-induced diabetic nephropathy. Diabetes. Apr; 2010 59(4):850–60. [PubMed: 20103708]
 24. Zheng H, Whitman SA, Wu W, Wondrak GT, Wong PK, Fang D, et al. Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy. Diabetes. Nov; 2011 60(11):3055–66. [PubMed: 22025779]
 25. Elmarakby AA, Faulkner J, Baban B, Saleh MA, Sullivan JC. Induction of hemeoxygenase-1 reduces glomerular injury and apoptosis in diabetic spontaneously hypertensive rats. American journal of physiology. Apr; 2012 302(7):F791–800. [PubMed: 22205229]
 26. Lee SC, Han SH, Li JJ, Lee SH, Jung DS, Kwak SJ, et al. Induction of heme oxygenase-1 protects against podocyte apoptosis under diabetic conditions. Kidney international. Oct; 2009 76(8):838–48. [PubMed: 19657327]
 27. Inoki K, Mori H, Wang J, Suzuki T, Hong S, Yoshida S, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. J Clin Invest. Jun; 2011 121(6):2181–96. [PubMed: 21606597]
 28. Godel M, Hartleben B, Herbach N, Liu S, Zschiedrich S, Lu S, et al. Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. J Clin Invest. Jun; 2011 121(6):2197–209. [PubMed: 21606591]
 29. Letavernier E, Legendre C. mTOR inhibitors-induced proteinuria: mechanisms, significance, and management. Transplant Rev (Orlando). Apr; 2008 22(2):125–30. [PubMed: 18631865]

30. Marrero MB, Banes-Berceli AK, Stern DM, Eaton DC. Role of the JAK/STAT signaling pathway in diabetic nephropathy. *American journal of physiology*. Apr; 2006 290(4):F762–8. [PubMed: 16527921]
31. Walsh DW, Roxburgh SA, McGettigan P, Berthier CC, Higgins DG, Kretzler M, et al. Co-regulation of Gremlin and Notch signalling in diabetic nephropathy. *Biochim Biophys Acta*. Oct 11.2007
32. Ortiz-Munoz G, Lopez-Parra V, Lopez-Franco O, Fernandez-Vizarra P, Mallavia B, Flores C, et al. Suppressors of cytokine signaling abrogate diabetic nephropathy. *J Am Soc Nephrol*. May; 2010 21(5):763–72. [PubMed: 20185635]
33. Mason RM. Connective tissue growth factor(CCN2), a pathogenic factor in diabetic nephropathy. What does it do? How does it do it? *J Cell Commun Signal*. Jun; 2009 3(2):95–104. [PubMed: 19214781]
34. Nguyen TQ, Roestenberg P, van Nieuwenhoven FA, Bovenschen N, Li Z, Xu L, et al. CTGF inhibits BMP-7 signaling in diabetic nephropathy. *J Am Soc Nephrol*. Nov; 2008 19(11):2098–107. [PubMed: 18632843]
35. Tominaga T, Abe H, Ueda O, Goto C, Nakahara K, Murakami T, et al. Activation of bone morphogenetic protein 4 signaling leads to glomerulosclerosis that mimics diabetic nephropathy. *The Journal of biological chemistry*. Jun 3; 2011 286(22):20109–16. [PubMed: 21471216]
36. Kishi S, Abe H, Akiyama H, Tominaga T, Murakami T, Mima A, et al. SOX9 protein induces a chondrogenic phenotype of mesangial cells and contributes to advanced diabetic nephropathy. *The Journal of biological chemistry*. Sep 16; 2011 286(37):32162–9. [PubMed: 21795715]
37. Zhang MZ, Yao B, Yang S, Yang H, Wang S, Fan X, et al. Intrarenal Dopamine Inhibits Progression of Diabetic Nephropathy. *Diabetes*. Jun 11.2012
38. Vallon V. The proximal tubule in the pathophysiology of the diabetic kidney. *Am J Physiol Regul Integr Comp Physiol*. Jan 12.
39. Zhang MZ, Yao B, Wang S, Fan X, Wu G, Yang H, et al. Intrarenal dopamine deficiency leads to hypertension and decreased longevity in mice. *J Clin Invest*. Jul; 2011 121(7):2845–54. [PubMed: 21701066]

Key points

1. Animal models, including mouse models, represent early changes, can be best used to provide clues about the pathogenesis of DN, but will not serve as miniature replicas of humans with the disease.
2. Changes in animal models need to be validated in human DN before assuming they are involved in the actual pathogenesis of the disease.
3. Alterations in multiple pathways are required to produce the changes found in human DN and systems biological methods can provide a window into this complexity.
4. A number of new pathways potentially involved in human DN have been recently described or more clearly elucidated in mouse model studies, including alterations involving dopamine, eNOS, JAK1/2, mTORC-1, Nrf-2.

Over-expression Gene Rank: 929 (in top 8%)

P-value: 0.011Z

Reporter: 203665_at

t-Test: 2.783

Fold Change: 2.418

NEPHROMINE

Figure 1.

Heme oxygenase-1 (HO-1) mRNA levels in glomeruli from humans with DN compared to those from living transplant donors. HO-1 levels were 2.4-fold higher in the DN subjects than in controls. The statistical comparisons are shown including the rank of HO-1 in terms of overexpression among all analyzed genes. Reporter indicates the DNA probe used for analysis. Nephromine™ (Compendia Bioscience, Ann Arbor, MI, supported by O'Brien Kidney Core Center at the University of Michigan) was used for analysis and visualization.

Figure 2.

Janus kinase 1 (JAK1) mRNA levels in tubulointerstitium from humans with DN compared to those from living transplant donors. JAK1 levels were 3.2-fold higher in the DN subjects than in controls. The statistical comparisons are shown including the rank of JAK1 in terms of overexpression among all analyzed genes. Reporter indicates the DNA probe used for analysis. Nephromine™ (Compendia Bioscience, Ann Arbor, MI, supported by O'Brien Kidney Core Center at the University of Michigan) was used for analysis and visualization.

Over-expression Gene Rank: 655 (in top 6%)

P-value: 0.002

Reporter: 202936_s_at

t-Test: 3.345

Fold Change: 2.845

Figure 3.

SRY-related HMG Box 9 (SOX-9) mRNA levels in tubulointerstitium from humans with DN compared to those from living transplant donors. SOX-9 levels were 2.8-fold higher in the DN subjects than in controls. The statistical comparisons are shown including the rank of SOX-9 in terms of overexpression among all analyzed genes. Reporter indicates the DNA probe used for analysis. Nephromine™ (Compendia Bioscience, Ann Arbor, MI, supported by O'Brien Kidney Core Center at the University of Michigan) was used for analysis and visualization.