Published in final edited form as:

Rev Endocr Metab Disord. 2008 December ; 9(4): 267–274. doi:10.1007/s11154-008-9103-3.

Defining human diabetic nephropathy on the molecular level:

Integration of transcriptomic profiles with biological knowledge

Sebastian Martini, Felix Eichinger, Viji Nair, and Matthias Kretzler

Division of Nephrology, Dept. of Internal Medicine, University of Michigan, 1150 W. Medical Center Drive, 1552 MSRB II, Ann Arbor, MI 48109-0676, USA

Abstract

Diabetic nephropathy (DN) is the most common cause for end stage renal disease (ESRD). Next to environmental factors, genetic predispositions determine the susceptibility for DN and its rate of progression to ESRD. With the availability of genome wide expression profiling we have the opportunity to define relevant pathways activated in the individual diabetic patient, integrating both environmental exposure and genetic background. In this review we summarize current understanding of how to link comprehensive gene expression data sets with biomedical knowledge and present strategies to build a transcriptional network of DN. Information about the individual disease processes of DN might allow the implementation of a personalized molecular medicine approach with mechanism-based patient management. Web based search engines like Nephromine are essential tools to facilitate access to molecular data of genomics, proteomics and metabolomics of DN.

Keywords

Diabetic nephropathy; personalized molecular medicine; patient tailored medicine; gene expression; transcription regulatory networks

Introduction

1. Background: diabetic nephropathy

Diabetic nephropathy (DN) is the most lethal diabetic microvascular complication and the leading single cause of end stage renal disease (ESRD) in the Western hemisphere. According to the United States Renal Data System Report of 2007, 43.8% of incipient patients with ESRD (46,851 patients) had diabetes mellitus as their primary diagnosis in 2005, resulting in 36.9% prevalence of DN in all ESRD (179,157) patients (1). DN has been reported in 25–40% of type 1 and type 2 diabetic patients (2). Moreover, even in patients with preserved kidney function, cardiovascular mortality in patients with DN is substantially increased compared to that in diabetic individuals without kidney disease (3). The human suffering as much as the economic burden of DN is immense. According to the American Diabetes Association the estimated total annual cost of diabetes in 2007 was \$174 billion. Medical expenditures totaled \$116 billion and were comprised of \$27 billion for diabetes care, \$58 billion to treat diabetes-related chronic complications, and \$31 billion for excess general medical costs (4).

2. The multi-faceted pathogenesis of DN

The pathogenesis of DN is multi-factorial and is comprised of genetic and environmental factors activating a multitude of intra- and extra-renal pathways. Genetic predisposition has been indicated as the decisive factor for the susceptibility and rate of progression to ESRD in diabetic patients by several observations:

1. Black Americans, Mexican Americans, and Native Americans are disproportionately affected by DN compared with White Americans (5).
2. Epidemiologic studies have shown that DN susceptibility and rate of progression is strongly clustered in families (5-7) and specific genetic factors for predisposition to DN were recently identified in several diabetic sibling studies (8-10).
3. The relationship between environmental factors like diabetes control/duration and the extent of glomerular injury shows a high degree of inter-individual variability pointing towards additional individual (genetic) risk factors (11). That these factors can be influenced was shown by multiple intervention studies targeting the known pathways activated in DN. Of those, the renin-angiotensin-aldosterone system (RAAS) plays a central role (12). This has been demonstrated by effectively slowing progression of DN via mechanisms additional to blood pressure control alone (8) by treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) (13) and as demonstrated by the AVOID study - the direct renin inhibitor Aliskiren (14). In addition, tight glycemic control (3,8) and aggressive treatment of hyperlipidemia (15) have been shown to contribute to a delay in DN progression.

3. From metabolic changes via structural alterations to a function-based disease definition

Specific metabolically driven glucose dependent pathways are activated within DN. These pathways induce oxidative stress, polyol pathway flux, hexosamine flux and accumulation of advanced glycosylated end-products (AGEs) in the kidney (16). Hemodynamic factors are further influencing the pathogenesis of DN and include elevations of systemic and intra-glomerular pressure and activation of vasoactive hormones including RAAS, endothelin and urotensin (17). These altered hemodynamics act independently, and in concert with metabolic pathways, to activate intracellular second messengers such as protein kinase C (PKC) and MAP kinase (MAPK), nuclear transcription factors such as nuclear factor- κ B (NF- κ B), and various growth factors such as the pro-sclerotic cytokines, transforming growth factor β (TGF β)-1, connective tissue growth factor (CTGF), and vascular endothelial growth factor (VEGF) (16). Ultimately these molecular mechanisms have been shown to lead to the specific histo-pathological pattern of DN not seen in other renal diseases. The first structural changes in DN are thickening of the basement membranes of glomerular capillaries, arterioles and tubules. Clinically these changes might appear together with microalbuminuria (18). Progression of DN is characterized by destruction of glomerular architecture and tubulointerstitial fibrosis resulting in a progressive decline of glomerular filtration rate (GFR) (19).

Integrating the specific regulatory pathways with the phenotypic heterogeneity in patients with DN will result in a function-based definition of distinct subgroups of patients. This function-based understanding of the disease processes will allow the development of a “personalized molecular medicine” for the management of DN and will identify specific progression factors active in defined subpopulations of the disease. “Personalized molecular medicine” implies in this context a medical treatment strategy that aims to interrupt disease mechanisms active in an individual patient. This treatment strategy is distinct from the current conventional therapy of DN, which tries to improve disease symptoms present in patients with similar clinical features, but potentially different underlying destructive mechanisms at the cellular and molecular levels. If applied, this strategy would have far reaching consequences for diagnostic

classification, disease prediction, and definition of patient cohorts for clinical trials and identification of patient tailored therapeutic regimen in type 1 and 2 diabetes mellitus in general and specifically DN (Figure 1).

4. Applying Genomics to define DN: lessons to be learned from oncology

Over the last decade we have witnessed an unprecedented expansion in our capability to define disease mechanism in a comprehensive genome wide manner. We are at the verge of being able to display the entire regulatory continuum covering DNA, mRNA, protein and metabolite profiles in a given individual. Analyzing these large data sets in isolation, linking them with clinical phenotype information and integrating them for a true systems understanding of the DN disease processes are daunting challenges (20). In this review we will focus on the study of transcriptomics as one of the most promising and advanced fields to identify biomarkers and monitor disease activity. Transcriptomics is defined as the analysis of mRNA expression in physiology and disease. Its advantages are a very comprehensive data capture and a high level of standardization in the field (21). The introduction of highly parallel mRNA profiling technologies has enabled the generation of genome-wide gene expression profiles of human disease. Combining microarrays with tissue banks allows for the display of virtually all mRNAs expressed at a given time point and provides a comprehensive, unbiased starting point for a systematic analysis of disease processes, resulting in mRNA fingerprints associated with defined clinical outcomes. These biomarkers may be applied towards early diagnosis, prognosis and prediction of response to therapy. Functional genomics seeks to decode gene-gene interactions and cellular pathways involved in disease biology that might be a suitable target of newer molecular therapeutics. Clear limitations concerning molecular target definition from transcriptomic data sets are the multiple layers of regulation interposed between mRNA level alterations in DN and downstream changes in protein function.

In oncology, gene expression fingerprints have been shown to predict disease outcome and treatment response in a series of studies (for review see (22-24)). Comprehensive molecular analysis of tumor tissue has allowed the definition of cancer-specific molecular signatures representing different disease mechanisms or states of neoplastic lesions. These expression signatures are currently evaluated as diagnostic parameters to predict disease course and response to therapy (25).

5. Gene expression profiling defines the transcriptional response to environmental factors and genetic predisposition in DN

Several challenges must be addressed prior to employing the high-throughput gene expression microarray techniques towards patients with DN:

1. As in oncology, tissue-based analyses will provide the most pertinent information on the intra-renal disease process active in the diabetic patient. However, routine clinical renal biopsy is only performed in a small proportion of patients with diabetes, necessitating large multicenter studies or protocol biopsies to procure a sufficiently large cohort of DN biopsies for study. Less invasive or even non-invasive markers such as peripheral blood or urine profiles provide an alternative source of biosamples from DN patients, but can be confounded by multiple non-renal effects.
2. A key difference between studies in oncology and renal disease is the considerable tissue heterogeneity of the nephron compared to the clonal expansion of a single cell seen in most cancers. This is further confounded by the small tissue volume procured by percutaneous renal biopsies. This can be addressed to some extent by microdissection of the biopsied specimen, either in the tissue section by laser capture or in the tissue core by manual microdissection. These strategies allow the isolation of glomeruli and tubuli for molecular analysis. For many renal diseases including DN,

glomeruli are of special interest since they are the first compartment to show ultrastructural damage (26,27).

3. The integration of gene expression data into their functional context is one of the greatest challenges to employ this information towards “personalized molecular medicine” of DN. The strategies used to date are based on our current knowledge of transcriptional regulation and biological processes in general. Genes influenced by a common environmental challenge or genetic predisposition are assumed to show co-regulation in the examined tissue resulting in similarities of their patterns of expression. These expression similarities are detected by classification systems (cluster algorithms) and form the starting points for further analyses (28-30). Co-regulated genes can be integrated into established or de-novo generated functional pathways derived from our current biological knowledge to define their overall functional context (31). Additional strategies can integrate the mRNA alterations of available genomic information into underlying transcriptional regulatory mechanisms (32).

6. Integrating comprehensive genome wide data sets into current biomedical knowledge

6.1 Generation of comprehensive expression profiles by gene expression arrays

—For comprehensive gene expression studies multiple platforms are in use employing highly parallel solid phase or liquid phase approaches. The most widely used platform, solid phase gene expression arrays consist of thousands of single DNA sequences spotted onto a glass slide that are the reverse complement to the target RNA sequence in the biological sample. These can be detected by fluorescence-based signal detection. Oligonucleotide microarrays use single-stranded DNA of 25-70 bp, cDNA microarrays use double-stranded DNA of 200-500 bp. The advantages of gene expression arrays include the possibility of robust and standardized genome-wide analyses, the integration of the information with other genome wide sources of information, and the possibilities for inter-organ, inter-cell type, inter-species comparison. As mentioned earlier the analysis of gene expression arrays with its required complex statistical analysis is the key challenge of this method. Schena et al. published the first paper describing gene expression arrays in 1995 (33). Since then the use of this technique has expanded and multiple mRNAs involved in the pathogenesis of renal diseases were identified.

Baelde et al. conducted a gene expression profiling study in a small group of human DN patients (34). Comparing gene expression in four deceased kidney donors with and without DN 615 mRNAs were found to be differentially regulated and associated with a disturbed cytoskeleton formation and tissue repair mechanisms, especially in the endothelium of renal vessels in patients with DN.

Gene expression arrays of human proximal tubular cells exposed in vitro to high glucose levels were conducted by Qi et al. High glucose increased gene expression of Thioredoxin-interacting protein (Txnip) and its promoter activity. TGF β -1 silencing experiments in the same setting were highly suggestive that increased Txnip expression is TGF β -1-independent (35). These findings are in line with others showing that high glucose can induce many chemokines such as interleukin-8, monocyte chemoattractant protein (MCP)-1 and nuclear factor-kappaB (NF- κ B) and activator protein (AP)-1 (36,37).

As systemic inflammation is considered to be a key driving force of DN (38), several studies have analyzed inflammatory mRNA signatures in non-renal tissue of DN patients. Gene expression profiles of peripheral blood mononuclear cells (PBMCs) from diabetic patients with and without DN (with proteinuria or with normoalbuminuria - both with at least 20 years of diabetes duration) focused on 198 candidate genes for diabetic nephropathy (Pubmed search

based on gene variants or gene expression analyses) and revealed only thrombospondin 1 (THBS1) and cyclooxygenase 1 (COX1) to be overexpressed in DN PBMCs and matrix metalloproteinase 9 (MMP9) and cyclooxygenase 2 (COX2 genes) repressed in DN PBMCs (39). These differentially regulated molecules have been associated with DN in various model systems, but might also be a consequence of renal disease or proteinuria in general.

Starting from the hypothesis that the beneficial effect of angiotensin inhibition is at least in part mediated by a direct anti-inflammatory effect, Tone et al. investigated the gene expression profiles in angiotensin II stimulated cultured macrophages. Among the 19 high-glucose upregulated genes the induction of chemokine MCP-2 was prevented by angiotensin II inhibition (40) potentially interrupting a vicious cycle of micro-inflammation in diabetes.

For the evaluation of systemic inflammation in DN skin fibroblasts gene expression profiles from patients with type 1 diabetes were compared between either fast or slow progression of DN towards ESRD (41). In this study genes involved in pathways of oxidative phosphorylation and their related upstream pathways were significantly differentially regulated indicative for an involvement in the pathogenesis of DN.

6.2 Beyond the identification of mRNA differential regulation - building the transcriptional network of DN—A large body of studies have described significant regulation of specific mRNAs in DN including: osteopontin, myeloperoxidase (MPO), Notch, TNF-related apoptosis inducing ligand (TRAIL), fibronectin (FN)-1, hepatocyte nuclear factor (HNF)-4 α , transient receptor potential channel (TRPC)-1, TGF β -1, CTGF, Gremlin, sterol regulatory element-binding protein (SREBP)-1c, c-Jun, collagen type VIII, PVT1, MCP1, NF κ B, Rantes and bone morphogenetic protein (BMP)-7 (for review (42)). These studies have also related these observations to the specific functional consequences seen in model systems of DN.

Beyond single mRNA alterations, a comprehensive display of mRNA regulation in DN allows the identification of transcriptional networks activated in DN. A recently developed approach examines transcriptional elements and higher order promoter structures in promoter modules linked to regulatory pathways involved in DN (32). This approach appears to be justified since differential gene expression is regulated at the level of transcription initiation by regulatory transcription factors that bind directly to their transcription factor binding sites within the promoter sequence. Their binding results in an activation or repression of target gene expression. Transcription factors physically and functionally interact with each other and with regulatory sequences within the DNA. These transcription regulatory networks play a pivotal role in pathophysiological states and also in developmental processes and physiological responses (for review (43)).

Using the above rationale the transcription regulatory networks involved in the pathogenesis of diabetic nephropathy were studied in a cohort of patients with progressive DN (44). Gene expression based unsupervised hierarchical clustering of the study cohort displayed two discrete branches within the DN group. The molecular stratification associated with clinical characteristics, segregating a group of patients with mild renal impairment and less severe histopathology scores for tubulo-interstitial damage from a group of patients with progressed DN. Evaluating the genes responsible for the gene expression based DN sub-stratification revealed the enrichment of inflammation-stress response genes and among the stress response genes a significant enrichment of known NF- κ B target genes. Transcription factors such as NF- κ B work in concert with other transcription factors in order to communicate selectivity and specificity of transcriptional responses. Cooperative binding of a transcription factor in a specific orientation and spacing is one core feature of an effective transcription initiation complex. The systematic promoter module analysis of the upregulated NF- κ B-dependent genes

identified a NF- κ B-IRFF transcription factor binding module in the progressive DN regulated gene set. Based on these studies, the DN dependent regulation of nine mRNA was predicted and verified in an independent cohort via qRT-PCR (44).

Identification of NF- κ B dependent transcriptional networks is particularly intriguing, as the activation of NF- κ B-linked regulatory pathways has been shown to be associated with the inflammatory processes in DN (45,46). Recent studies by Yang et al. showed an association between NF- κ B and diabetic nephropathy. In patients with DN, but not in a cohort of diabetic patients with comparable diabetes duration without DN, NF- κ B induced aldose reductase (AKR1B1) expression. NF- κ B dependent AKR1B1 activation is considered to be one mechanism contributing to the pathogenesis of DN (47).

The sequential strategy of genome wide expression profiles, definition of molecularly defined diseased subtypes, extraction of subtype defining mRNAs, and analysis of underlying transcriptional regulation revealed with NF- κ B a pathway with ample independent evidence for relevance in DN and added with the IRFF response element a new candidate for tissue and disease specificity of the NF- κ B response in DN.

Currently, NF- κ B inhibition is investigated as a therapeutic target in diabetes and its complications, for example in retinal neovascularization (48,49), DN itself (50) and beta cell dysfunction (51).

Comprehensive expression profiles also offer the opportunity to employ hypothesis driven focused approaches. The above gene expression data set of progressive DN was mined for apoptosis associated transcriptional networks (52). A high proportion of known cell death related genes were found to be significantly differentially regulated (112 of 455) with maximal changes observed among death receptors and the death ligand TRAIL. Follow-up studies confirmed the TRAIL protein induction in DN glomeruli and proximal tubuli by immunohistochemistry. In vitro studies demonstrated that apoptosis was only observed in the presence of proinflammatory cytokines *and* high glucose levels. TRAIL was found to activate NF- κ B, a further indicator for the vicious cycle of apoptosis and inflammation in DN (52).

These results confirm on one hand the pivotal role of inflammation in the pathogenesis of DN and offer on the other hand tools for identification of currently unknown target genes using a strategy outlined in Figure 2.

6.3 Nephromine - an online search tool of renal genome-wide gene expression data for the renal community—With the rapidly expanding amount of molecular data and their interactions generated by the different approaches in the fields of genomics, proteomics and metabolomics there is an increasing need for tools to make this knowledge easily accessible and usable for molecular biology researchers as well as clinician-scientists.

To address this need an online search tool of renal genome-wide gene expression data sets was developed in a collaboration of bioinformatics and renal research teams using a highly successful tool in oncology as a model (53). The search engine, Nephromine, has been developed as a resource for the scientific community allowing integration of available deep expert knowledge with the modern tools of molecular biology and clinical patient phenotypes. Nephromine (freely accessible for academic users at <http://www.nephromine.org>) combines transcriptome maps and data sets of human studies generated by the renal research community with automated searches of gene ontology terms: molecular function, cellular components and biological processes, KEGG pathways, therapeutic target databases, predicted microRNA targets, HPRD Interaction Sets, literature-defined concepts and the Interactome database among others.

Nephromine can search for either genes or more general terms (i.e. diseases, studies). The profile search for the search term “diabetic nephropathy” reveals an overview of the search results including, for example, links to the therapeutic target database for the selected profile. It allows the application of various filters, shows enrichment within the chosen dataset and displays Interactome results. Interactome results show gene-gene interactions according to the Human Protein Reference Database (HPRD contains genes and their corresponding proteins) and lists for DN i.e. 640 interactions. Among the first hits the gene tubulin β (Gene ID: 203068) is interacting with a variety of genes on a highly significant level. Although tubulin β itself is not co-cited with DN, its main Gene Ontology function is GTP binding and GTPase activity. Activation of GTPases was recently reported for the various pathways in the pathogenesis of DN (54). Nephromine enables users to search for the interacting partners of tubulin β either to confirm known molecular interactions, look for therapeutic targets or to generate hypotheses for pathway interactions.

The aim of Nephromine and similar data mining tools is to empower the non-bioinformatic scientist with the ability to search on the web large datasets of stage-specific, conserved gene expression signatures in an explicit, focused research context, as has been described above for the definition of apoptosis pathways in DN.

7. Conclusion

A more comprehensive understanding of individual pathomechanisms involved in DN will enable physicians to provide an early and precise diagnosis for a specific patient. Furthermore, this will be the prerequisite allowing researchers to identify therapeutic molecular targets and test them in patients with activation of this target. An important aspect of molecular biology and medicine is that the amount of molecular data generated by the different approaches in the fields of genomics, transcriptomics, proteomics and metabolomics is growing exponentially. Therefore, as introduced in this review, tools like Nephromine are crucial to make this knowledge easily accessible and usable to a broad spectrum of molecular biology researchers as well as clinician-scientists.

Acknowledgements

We thank Anna Henger and Celine Berthier for helpful discussion and Chrysta Lienczewski for carefully proofreading the manuscript. This study was supported in part by NIHU54 DA021519 ‘National Center for Integrative Biomedical Informatics’ and by a MICHM Multidisciplinary Research Grant to MK.

References

1. USRDS. 2007 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: 2007.
2. Ritz E, Orth SR. Nephropathy in patients with type 2 diabetes mellitus. *N Engl J Med* 1999;341:1127–1133. [PubMed: 10511612]
3. Patel A, MacMahon S, Chalmers J, Neal B, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008;358:2560–2572. [PubMed: 18539916]
4. Economic costs of diabetes in the U.S. In 2007. *Diabetes Care* 2008;31:596–615. [PubMed: 18308683]
5. Iyengar SK, Schelling JR, Sedor JR. Approaches to understanding susceptibility to nephropathy: from genetics to genomics. *Kidney Int* 2002;61:S61–67. [PubMed: 11841615]
6. Ng DP, Krolewski AS. Molecular genetic approaches for studying the etiology of diabetic nephropathy. *Curr Mol Med* 2005;5:509–525. [PubMed: 16101480]
7. Rogus JJ, Poznik GD, Pezolesi MG, Smiles AM, et al. High-density SNP genome wide linkage scan for susceptibility genes for diabetic nephropathy in type 1 diabetes: Discordant sib-pair approach. *Diabetes*. 2008

8. Bleyer AJ, Sedor JR, Freedman BI, O'Brien A, et al. Risk factors for development and progression of diabetic kidney disease and treatment patterns among diabetic siblings of patients with diabetic kidney disease. *Am J Kidney Dis* 2008;51:29–37. [PubMed: 18155530]
9. Schelling JR, Abboud HE, Nicholas SB, Pahl MV, et al. Genome-wide scan for estimated glomerular filtration rate in multi-ethnic diabetic populations: the Family Investigation of Nephropathy and Diabetes (FIND). *Diabetes* 2008;57:235–243. [PubMed: 18003762]
10. Tanaka N, Babazono T. Assessing genetic susceptibility to diabetic nephropathy. *Nephrology (Carlton)* 2005;10(Suppl):S17–21. [PubMed: 16174281]
11. Iyengar SK, Adler SG. The application of the HapMap to diabetic nephropathy and other causes of chronic renal failure. *Semin Nephrol* 2007;27:223–236. [PubMed: 17418690]
12. Luetscher JA, Kraemer FB, Wilson DM, Schwartz HC, et al. Increased plasma inactive renin in diabetes mellitus. A marker of microvascular complications. *N Engl J Med* 1985;312:1412–1417. [PubMed: 3887168]
13. Sarafidis PA, Stafylas PC, Kanaki AI, Lasaridis AN. Effects of Renin-Angiotensin System Blockers on Renal Outcomes and All-cause Mortality in Patients With Diabetic Nephropathy: An Updated Meta-analysis. *Am J Hypertens*. 2008
14. Parving HH, Persson F, Lewis JB, Lewis EJ, et al. Aliskiren combined with losartan in type 2 diabetes and nephropathy. *N Engl J Med* 2008;358:2433–2446. [PubMed: 18525041]
15. Rosario RF, Prabhakar S. Lipids and diabetic nephropathy. *Curr Diab Rep* 2006;6:455–462. [PubMed: 17118229]
16. Forbes JM, Fukami K, Cooper ME. Diabetic nephropathy: where hemodynamics meets metabolism. *Exp Clin Endocrinol Diabetes* 2007;115:69–84. [PubMed: 17318765]
17. Maric C. Vasoactive hormones and the diabetic kidney. *ScientificWorldJournal* 2008;8:470–485. [PubMed: 18454258]
18. Wolf G, Ziyadeh FN. Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. *Nephron Physiol* 2007;106:26–31.
19. Niehof M, Borlak J. HNF4 alpha and the Ca-channel TRPC1 are novel disease candidate genes in diabetic nephropathy. *Diabetes* 2008;57:1069–1077. [PubMed: 18184923]
20. Sieberts SK, Schadt EE. Moving toward a system genetics view of disease. *Mamm Genome* 2007;18:389–401. [PubMed: 17653589]
21. Morrison N, Cochrane G, Faruque N, Tatusova T, et al. Concept of sample in OMICS technology. *OMICS* 2006;10:127–137. [PubMed: 16901217]
22. Malinowski DP. Multiple biomarkers in molecular oncology. II. Molecular diagnostics applications in breast cancer management. *Expert Rev Mol Diagn* 2007;7:269–280. [PubMed: 17489734]
23. Martinelli G, Iacobucci I, Soverini S, Cilloni D, et al. Monitoring minimal residual disease and controlling drug resistance in chronic myeloid leukaemia patients in treatment with imatinib as a guide to clinical management. *Hematol Oncol* 2006;24:196–204. [PubMed: 16988930]
24. Sanga S, Frieboes HB, Zheng X, Gatenby R, et al. Predictive oncology: a review of multidisciplinary, multiscale in silico modeling linking phenotype, morphology and growth. *Neuroimage* 2007;37 (Suppl 1):S120–134. [PubMed: 17629503]
25. Wulfschuhle JD, Speer R, Pierobon M, Laird J, et al. Multiplexed cell signaling analysis of human breast cancer applications for personalized therapy. *J Proteome Res* 2008;7:1508–1517. [PubMed: 18257519]
26. Kretzler M, Cohen CD, Doran P, Henger A, et al. Repuncturing the renal biopsy: strategies for molecular diagnosis in nephrology. *J Am Soc Nephrol* 2002;13:1961–1972. [PubMed: 12089394]
27. Chabardes-Garonne D, Mejean A, Aude JC, Cheval L, et al. A panoramic view of gene expression in the human kidney. *Proc Natl Acad Sci U S A* 2003;100:13710–13715. [PubMed: 14595018]
28. Chan YH. Biostatistics 302. Principal component and factor analysis. *Singapore Med J* 2004;45:558–565. [PubMed: 15568116]
29. Gasch AP, Eisen MB. Exploring the conditional coregulation of yeast gene expression through fuzzy k-means clustering. *Genome Biol* 2002;3:RESEARCH0059
30. Qin ZS. Clustering microarray gene expression data using weighted Chinese restaurant process. *Bioinformatics* 2006;22:1988–1997. [PubMed: 16766561]

31. Kanehisa M. The KEGG database. Novartis Found Symp 2002;247:91–101. [PubMed: 12539951] discussion 101–103, 119–128, 244–152
32. Werner T. Bioinformatics applications for pathway analysis of microarray data. Curr Opin Biotechnol 2008;19:50–54. [PubMed: 18207385]
33. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995;270:467–470. [PubMed: 7569999]
34. Baelde HJ, Eikmans M, Doran PP, Lappin DW, et al. Gene expression profiling in glomeruli from human kidneys with diabetic nephropathy. Am J Kidney Dis 2004;43:636–650. [PubMed: 15042541]
35. Qi W, Chen X, Gilbert RE, Zhang Y, et al. High glucose-induced thioredoxin-interacting protein in renal proximal tubule cells is independent of transforming growth factor-beta1. Am J Pathol 2007;171:744–754. [PubMed: 17675577]
36. Panchapakesan U, Sumual S, Pollock CA, Chen X. PPARgamma agonists exert antifibrotic effects in renal tubular cells exposed to high glucose. Am J Physiol Renal Physiol 2005;289:F1153–1158. [PubMed: 15886275]
37. Srinivasan S, Bolick DT, Hatley ME, Natarajan R, et al. Glucose regulates interleukin-8 production in aortic endothelial cells through activation of the p38 mitogen-activated protein kinase pathway in diabetes. J Biol Chem 2004;279:31930–31936. [PubMed: 15145956]
38. Mora C, Navarro JF. Inflammation and diabetic nephropathy. Curr Diab Rep 2006;6:463–468. [PubMed: 17118230]
39. Moculski DK, Fojcik H, Wielgorecki A, Trautsolt W, et al. Expression pattern of genes in peripheral blood mononuclear cells in diabetic nephropathy. Diabet Med 2007;24:266–271. [PubMed: 17263765]
40. Tone A, Shikata K, Ogawa D, Sasaki S, et al. Changes of gene expression profiles in macrophages stimulated by angiotensin II--angiotensin II induces MCP-2 through AT1-receptor. J Renin Angiotensin Aldosterone Syst 2007;8:45–50. [PubMed: 17487826]
41. Huang C, Kim Y, Caramori ML, Moore JH, et al. Diabetic nephropathy is associated with gene expression levels of oxidative phosphorylation and related pathways. Diabetes 2006;55:1826–1831. [PubMed: 16731849]
42. de Borst MH, Benigni A, Remuzzi G. Primer: strategies for identifying genes involved in renal disease. Nat Clin Pract Nephrol 2008;4:265–276. [PubMed: 18364721]
43. Werner T, Fessele S, Maier H, Nelson PJ. Computer modeling of promoter organization as a tool to study transcriptional coregulation. FASEB J 2003;17:1228–1237. [PubMed: 12832287]
44. Schmid H, Boucherot A, Yasuda Y, Henger A, et al. Modular activation of nuclear factor-kappaB transcriptional programs in human diabetic nephropathy. Diabetes 2006;55:2993–3003. [PubMed: 17065335]
45. Mezzano S, Aros C, Droguett A, Burgos ME, et al. NF-kappaB activation and overexpression of regulated genes in human diabetic nephropathy. Nephrol Dial Transplant 2004;19:2505–2512. [PubMed: 15280531]
46. Sakai N, Wada T, Furuichi K, Iwata Y, et al. Involvement of extracellular signal-regulated kinase and p38 in human diabetic nephropathy. Am J Kidney Dis 2005;45:54–65. [PubMed: 15696444]
47. Yang B, Hodgkinson A, Oates PJ, Millward BA, et al. High glucose induction of DNA-binding activity of the transcription factor NFkappaB in patients with diabetic nephropathy. Biochim Biophys Acta 2008;1782:295–302. [PubMed: 18325349]
48. Nagai N, Izumi-Nagai K, Oike Y, Koto T, et al. Suppression of diabetes-induced retinal inflammation by blocking the angiotensin II type 1 receptor or its downstream nuclear factor-kappaB pathway. Invest Ophthalmol Vis Sci 2007;48:4342–4350. [PubMed: 17724226]
49. Yoshida A, Yoshida S, Ishibashi T, Kuwano M, et al. Suppression of retinal neovascularization by the NF-kappaB inhibitor pyrrolidine dithiocarbamate in mice. Invest Ophthalmol Vis Sci 1999;40:1624–1629. [PubMed: 10359349]
50. Chen L, Zhang J, Zhang Y, Wang Y, et al. Improvement of inflammatory responses associated with NF-kappaB pathway in kidneys from diabetic rats. Inflamm Res. 2008
51. Saisho Y, Hirose H, Horimai C, Miyashita K, et al. Effects of DHMEQ, a novel nuclear factor-kappaB inhibitor, on beta cell dysfunction in INS-1 cells. Endocr J 2008;55:433–438. [PubMed: 18385532]

52. Lorz C, Benito-Martin A, Boucherot A, Ucerro AC, et al. The death ligand TRAIL in diabetic nephropathy. *J Am Soc Nephrol* 2008;19:904–914. [PubMed: 18287563]
53. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* 2007;9:166–180. [PubMed: 17356713]
54. Peng F, Wu D, Gao B, Ingram AJ, et al. RhoA/Rho-kinase contribute to the pathogenesis of diabetic renal disease. *Diabetes* 2008;57:1683–1692. [PubMed: 18356410]

Figure 1.
Progression of renal diseases over time indicating time points for detection of the disease and treatment initiation.

Figure 2.

Strategy used to define human transcriptional networks and their confirmation by functional analysis: bioinformatic approaches for gene expression data are able to define common regulatory mechanisms in DN in a stepwise approach. First, differentially regulated genes are assigned, which are then mapped to known and de-novo generated pathways. Their transcriptional control can be confirmed by a qRT-PCR of identified downstream targets and their function can be studied in (cell-type specific) transgenic mouse models.