Precision Medicine: Tools to Define CKD Mechanisms

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Background: Precision medicine, identifying the right treatment for the right patient at the right time, must be applied in glomerular diseases to advance the field beyond the current “one size fits all” approach. Comprehensive genetic data from patient cohorts and animal model systems are presently generated for many disorders, including those of the glomerulus. The challenge of precision medicine, however, is designing an “informational commons” – a virtual space for researchers to share and explore large-scale datasets. To address this problem we have expanded a pair of software tools, tranSMART and NephroseqTM, to create a research platform that allows both user-specified exploration of cohort-study datasets and uniform meta-analysis across cohorts and datasets.

Methods/Results: Nephroseq is a web-based, systems biology data repository, search engine, and analysis toolkit, focused on renal gene expression datasets. It allows exploration of differentially-regulated transcripts using predefined cohorts and datasets with an extensive suite of systems-biology tools. Nephroseq’s intuitive interface allows scientists to interrogate data without requiring expertise in bioinformatics or statistics. Twenty-six datasets, representing nearly 2,000 samples and over 59 million gene expression values, were available as of March 2016. The tranSMART platform is an open-source translational medicine platform originally developed by Johnson and Johnson (5). The tranSMART allows user-specified exploration of cohort study datasets along the entire genotype-to-phenotype continuum. Users can explore the data by performing a variety of analyses, generating hypotheses and developing ancillary research questions to share with other fellow researchers.

Impact: Together, these tools facilitate access to human kidney disease clinical information and gene expression data for the renal research community, and will empower geographically-distributed research networks to jointly advance research and facilitate knowledge transfer from bench to bedside, thereby contributing to glomerular disease diagnosis, prognosis, intervention, and ultimately the improvement of patient care.
Molecular characterization of LN through serial biopsies

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Background: The response of lupus nephritis (LN) to treatment is assessed by clinical criteria, usually proteinuria and renal function, alone. The consequences of treatment for the kidney at the molecular level have not been explored in human LN, but could have important implications for modifying therapy to improve renal outcomes in LN. In this investigation changes in intra-renal transcript expression were measured and correlated with response in a LN cohort that underwent serial kidney biopsies.

Methods: SLE patients suspected of having LN had a kidney biopsy for diagnosis (Bx1) and patients with proliferative LN (n=19) were induced with high-dose corticosteroids plus either MMF or cyclophosphamide. After completing induction therapy, approximately 6 months, patients had a second kidney biopsy (Bx2) to determine histologic response to therapy. Intra-renal transcript expression was measured in Bx1 and Bx2 using Nanostring technology and a panel of over 500 immune response genes. Patients were segregated by clinical response at 6 months into group of complete responders (n=5, CR) and a group of non-responders (n=4, NR). Changes in transcript expression were compared between Bx1 and Bx2 in each responder group and between responder groups.

Results: Compared to healthy control kidneys (pre-implantation living donor transplant kidney biopsies, n=4), the CR group had 21 differentially-expressed transcripts at Bx1 and 28 at Bx2. In contrast the NR had 45 and 103 differentially-expressed transcripts at Bx1 and Bx2, respectively, compared to controls. The profiles of these differentially-expressed genes indicated that the type 1 interferon, the alternative complement and T cell signaling pathways discriminated CR from NR. At Bx1 transcripts regulated by type 1 interferon were over-expressed in CR and NR. During induction therapy the expression of type 1 interferon-inducible genes declined in CR but increased in NR, and additional type 1 genes were activated. Similarly, complement component transcript expression was increased at Bx1 in CR and NR and transcripts for regulators of the alternative pathway were suppressed in NR. At Bx2, these complement transcripts normalized in CR, but increased expression in NR. Transcripts related to T cell signaling became overexpressed at Bx2 in NR; this occurred to a lesser extent in CR. To determine whether changes in intra-renal transcript expression translated to changes in protein expression that could be measured non-invasively, complement component C5a was measured in the urine of an independent cohort of LN patients (n=34). Urine C5a concentration was significantly higher than normal in CR and NR at LN flare. After treatment urine C5a fell significantly in CR, but remained elevated in NR.

Conclusion: These data demonstrate that activity of intra-renal inflammatory genes induced at LN flare begins to fall in patients who respond clinically to induction therapy, but increases in patients who do not respond. The functional profiles of the protein products of these transcripts suggest that non-responders may benefit from interventions targeted at the type 1 interferon, alternative complement and T cell signaling pathways.
AT2R agonist protects high salt-induced kidney injury

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Background: Salt-sensitivity has been implicated in chronic kidney damage (CKD) and impairing renal function, particularly in obesity. Earlier we have reported blood-pressure and oxidative stress reducing effects of angiotensin AT2 receptor (AT2R) agonist C21 in kidney of high sodium diet (HSD)-fed obese Zucker rat (OZR).

Methods: Present study tested the effects of C21 on indices of renal function in HSD-fed OZR. Obese rats were fed normal sodium diet (NSD) or 4% HSD for 14 days, with/without C21 (1 mg/kg/day) delivered subcutaneously via osmotic pump.

Results: Compared to NSD controls, HSD rats exhibited severe glomerulosclerosis, interstitial fibrosis, decline in estimated glomerular filtration rate (GFR), and an increase in urinary leak and activity of N-acetyl-ß-D-glucosaminidase, a lysosomal enzyme and a marker of tubular damage, which were all improved by C21. C21 treatment reduced protein-to-creatinine (uPcr), albumin-to-creatinine (uAcr) as well as fractional excretion of protein (FEpro) and albumin (FEalb) in HSD-fed OZR, which is independent of changes in protein recycling receptors – megalin and cubilin. HSD intake has also altered renal excretory and reabsorptive capacity as evident by elevated plasma urea nitrogen-to-creatinine (UN-to-cr) and fractional excretion of urea nitrogen (FEUN), and reduced urine-to-plasma creatinine (UPcr), which were modestly, but insignificantly, improved by C21.

Conclusion: AT2R agonist C21 reduced glomerular protein leak and preserved lysosomal function and thus reduced exposure of nephrotic proteins to tubular lumen. These studies suggest a potential role of AT2R in protecting glomerulus and renal tubules during HSD intake in obesity. (Supported by NIH R01 grant DK61578)
Tubular subsegmental omics and 3D imaging of human biopsies

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The extent and rate of tubulointerstitial fibrosis are the primary determinants of outcome in all renal pathologies, including primary glomerulopathies. Remarkably, patients with apparently identical initial biopsy readings exhibit highly variable rates of progression to terminal tubulointerstitial fibrosis. To date, extensive investigation of the glomerular lesions and tubulointerstitium has failed to account for this unpredictable rate of progression. In particular, the tubulointerstitium has been approached as “one entity” without careful regard to its various components. Herein, we propose that the overall rate and extent of tubulointerstitial fibrosis, regardless of the initiating glomerular lesion, are determined by molecular signatures specific to individual tubular subsegments and the immune make-up of the interstitium. To this end, we developed novel techniques to examine the molecular signature of tubular subsegments as well as the immune cell make-up of the renal interstitium. These techniques include broad “omics” readouts conducted on tubular subsegments obtained by laser microdissection of human biopsy specimens. We also developed a novel microscopy-based quantitative approach to examine the immune cell make-up of the interstitium conducted on 3-D samples of human biopsies. Our studies aim to support the hypothesis that at a subsegmental level a unique molecular signature exists which primarily determines the progression of most kidney diseases. Identifying this unique molecular and cellular signature will guide our ability to determine best therapy and prognosis for individual patients with kidney disease. It will also help validate some of the many animal models used to study human kidney disease and identify novel targets for therapeutic intervention.
Identification of biomarkers and therapeutic targets for CKD

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Background: The goal of "precision medicine" is to characterize diseases based on the underlying molecular biology, in order to identify specific biomarkers and therapeutic targets that will ultimately improve clinical outcomes (1). Chronic kidney disease (CKD) affects 8-16% people worldwide, with an increasing incidence and prevalence of end-stage kidney disease (ESKD). Identifying the molecular mechanism responsible for progression of CKD is a critical step to contain the worldwide CKD epidemic. Our hypothesis is that both clinical phenotypes (e.g. GFR, proteinuria) and renal tissue alterations seen in CKD are associated with, and a consequence of, the dynamic molecular mechanism reflected in the transcriptional programs of the diseased renal tissue. Transcriptomic analysis of kidney biopsy samples of CKD patients therefore provides a strong foundation for precision medicine and the feasibility to identify potential biomarkers and therapeutic targets in CKD.

Method: Our work started from the identification and cross-validation of pathways and candidate intrarenal biomarkers for CKD progression in 261 kidney biopsies. We then used a sequential prioritization strategy to prioritize candidates for non-invasive biomarkers to allow broad clinical applicability. Next, intra-renal transcript levels of the top candidate biomarkers were correlated with the urinary levels of the encoded proteins. Urinary protein levels were assessed for their correlation with CKD progression. Finally, the biomarker was tested for its ability to increase predictive power of established clinical marker panels for CKD progression prediction in three cohorts. Cox proportional hazards models was used to evaluate the predictive value of marker on CKD outcome, and likelihood ratio tests, C-statistics, and Akaike information criterion (AIC) were used to assess the goodness of fit and improved prediction ability.

Results: Using Epidermal Growth Factor (EGF) as a proof of principle, we could demonstrate that prediction of renal survival by eGFR and albuminuria was significantly improved by addition of uEGF to the model in diverse CKD populations with a wide spectrum of causes and stages. uEGF may contribute to the improved risk prediction as it can capture the degree of tubular differentiation and regeneration potential, mechanisms essential to retain renal function with the acute and chronic insults seen in CKD, but not be well reflected by the conventional predictors (proteinuria or baseline GFR).

Conclusions/Impact: uEGF shows promise as an independent risk predictor of CKD progression. Inclusion of uEGF significantly improved prediction of composite end points by eGFR and proteinuria in diverse populations worldwide with a wide range of CKD. An immediate benefit of our work can be an improved stratification of CKD patients for the selections of high-risk patients into clinical trials, addressing a critical hurdle for novel molecular target validation in CKD.
Gene expression based dissection of CKD traits

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Background: Chronic kidney disease (CKD) affects more than 10% of the US population. The two key manifestations of CKD are functional (GFR decline) and structural changes (glomerulosclerosis and tubulointerstitial fibrosis). We used gene expression profiles in microdissected human kidney tubule samples as quantitative traits to describe the changes associated with these traits in CKD, establish their relatedness and characterize pathways that likely underlie them.

Methods: Transcriptome data from ninety-five human kidney samples with a range of functional and structural changes were used for the primary analysis and data obtained from 41 samples were available for validation. We used linear correlations, pathway analysis, weighted gene co-expression network analysis (WGCNA) and prediction models, to analyze the data.

Results: Functional and structural changes strongly correlated in our dataset. We first used linear correlation at transcript level and at system level (using WGCNA) to identify transcripts or transcript clusters whose expression showed correlation with eGFR, and with fibrosis. Similar to the strong correlation between eGFR and fibrosis, we found a 78% identity between correlated transcripts, representing the molecular commonalities between these processes. 490 genes, whose expression strongly correlated with fibrosis but not with eGFR were enriched for immune system and programmed cell death processes. Transcripts whose expression exclusively correlated with eGFR were enriched for metabolic processes. Further functional trait dissection was directed to identify stage-specific expression changes. Genes enriched for TGFß, Wnt signaling and ECM receptor interactions correlated with eGFR only in earlier CKD stages. Finally, we have identified a signature gene set, whose combined expression profile accurately classifies samples according to CKD severity.

Conclusion and impact: Our transcriptome-based CKD trait dissection method highlights potential differences in the underlying mechanisms of eGFR deterioration and fibrosis, and in different CKD stages, that may guide future diagnostics and therapeutics development.
Detecting Genotype-Driven Expression Change in Human Kidney

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There are more than 5 million genomic sequence variants in humans. Some variants influence transcript levels and therefore have the ability to interfere with cell and organ function. These variants are called expression quantitative trait loci (eQTL). The goal of our study was identify functionally important variants in the human kidney at the genome-wide level. Our analysis required genotype and expression data for a large number of human tissue samples. Here we used 99 human kidney samples of Central European descent. RNAseq and genotype data was normalized and genotype data imputed using 1,000 Genome reference data. The association between genotype and transcript levels was performed using Matrix eQTL, controlled for association within 1 megabase. After multiple testing correction, we identified 1,155 statistically significant target genes (eGenes) and 63,284 significant SNPs (eSNPs). We compared kidney eSNPs to those published for other organs in the database Genotype-Tissue Expression project (GTEx). Of the 1,155 eGenes, 720 were common between the kidney and other organs, indicating cell-type-specific and independent eGenes. Using kidney-specific epigenome maps, we found that eSNPs were enriched in kidney-specific regulatory elements, including promoters and enhancers. We also found greater overlap between eSNPs and SNPs discovered in chronic kidney disease genome-wide association studies, compared to other traits (digestive, nervous, immune system diseases, hematological measurement, cardiovascular, and metabolic disease). We identified transcript level changes associated with genotypic variants. These results highlight kidney-specific regulatory elements and potentially identify target genes for polymorphisms associated with traits related to kidney function.
Melamine induces Ca2+-sensing receptor activation and elicits apoptosis in proximal tubular cells

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Ingestion of melamine (2,4,6-triamino-1,3,5-triazine)-contaminated foods have been shown to cause kidney stone development and acute kidney injury (AKI). While the mechanism of such kidney diseases contributed by melamine is not clear, some predisposing factors such as proapoptotic pathways, have been identified, induced by an upregulation of intracellular calcium ([Ca2+]i). Melamine, which used to mimic the protein enrichment in infant formula, has some structural similarities with L-amino acid, such as L-phenylalanine (L-Phe). We have demonstrated the role of L-Phe as an endogenous agonist for calcium sensing receptors (CSR) in salivary gland cells. Here we show that melamine activates CSR, and results in a sustained calcium (Ca2+) entry and elevated [Ca2+]i in the renal epithelial cell line, LLC-PK1. Moreover, we found that melamine-induced CSR activation, results in [Ca2+]i rise, leading to apoptosis and necrosis. Additionally, cells exposed melamine displayed a rise in phospho-ERK (pERK) activation and lactate dehydrogenase (LDH) release, due to cytotoxicity. Finally, melamine failed to exert its deleterious effects (cell cytotoxicity, necrosis, and apoptosis) when exposed to NPS-2143 (a CSR blocker). These results, thus, demonstrate that melamine activation of CSR cause a sustained elevation of [Ca2+]i, leading to apoptosis and cell cytotoxicity, which may subsequently result in AKI and kidney stone. Keywords: melamine, kidney cells, calcium sensing receptor, intracellular calcium, apoptosis, necrosis, acute kidney injury and kidney stone.
Adenosine Restored Na+ transport in Acute Kidney Injury

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Background: Acute kidney injury, due to ischemia/reperfusion injury (IRI), associates with high morbidity and mortality and lacks effective therapeutic procedures. Adenosine via adenosine receptor activation protects the kidney against IRI. However, adenosine mode of reno-protection has not been established in its complexity. Na+ re-absorption is severely compromised in IRI and both surface and total protein of the principal proximal tubule Na+ transporter, the Na+/H+ exchanger 3 (NHE3), are markedly decreased following IRI. This study aims to determine the effect of adenosine receptor on NHE3 in renal IRI.

Methods: IRI was induced in Sprague-Dawley rats through bilateral cross-clamping of renal artery followed by reperfusion.

Results: Adenosine A2A receptor (A2AR) protected against IRI; compared to vehicle-treated ischemic rats, A2AR agonist, given after ischemia, decreased the peak of plasma creatinine (Cr) and blood urea nitrogen (BUN) 24 hours after IRI and accelerated their reversal thereafter. A2AR activation affected protein expression of NHE3 in IRI. A2AR agonist, given after ischemia, reversed the decrease in NHE3 surface but did not affect the loss of NHE3 total protein due to renal IRI. Interestingly, a combined activation of A2AR, after ischemia, and A1R, before ischemia, acted in conjunction. Dual A2AR/A1R activation was beneficial for kidney function; it synergistically reduced the increase in both Cr and BUN when compared to the effect of A2AR activation alone. A2AR and A1R activation preserved both cell surface and total protein of NHE3 following IRI.

Conclusion and Impact: Control of sodium homeostasis via modulation of NHE3 protein expression may be a key mechanism by which A2AR in conjunction with A1R activation mediates reno-protection against IRI.
Kidney fibrosis is the histological manifestation of chronic kidney disease (CKD). Fibrosis is associated with changes in expression of a large number of genes. Using gene expression datasets it is often difficult to identify causal pathways for kidney fibrosis. We generated gene expression microarray data from a large cohort (n=95) of human microdissected normal and CKD tubule samples. Using an adjusted linear regression model we identified genes whose expressions significantly associated with phenotypic changes. In addition, we performed RNA sequencing for four mouse fibrosis models; folate induced fibrosis (FA), unilateral ureteral obstruction (UUO), tubule specific Notch transgenic and podocyte specific APOL1 transgenic mice. The expression of large number (GFR: 2,603, tubulointerstitial fibrosis: 3,101 and glomerular sclerosis: 3,403) of genes correlated with fibrosis severity in human samples. To narrow the list of potential targets we identified the mouse orthologs of human genes and examined their correlation with fibrosis development. Our analysis identified 2,117 differentially expressed genes from mouse models and 761 conserved expression changes between mouse and human. Genes identified from mouse models allowed proper clustering of control and diseased human kidney samples. Ontology analysis indicated that genes with immune response show positive correlation with fibrosis development both in mouse models and in patient samples. On the other hand, genes involved in metabolism and oxidative reduction showed negative correlation. Comparative analysis of human and mouse kidney fibrosis have identified conserved genes and pathways in kidney fibrosis. These genes can serve as potential new biomarkers or targets for kidney disease development.
The Million Veteran Program (MVP) is a major initiative launched by the VA to advance precision medicine. The MVP has enrolled 50,000 veterans so far and has become the largest US database linking genetic, clinical and lifestyle information. In the MVP 200,000 subjects have been genotyped. The VA funded four MVP-consortiums to study chronic diseases that are high priority for the VA. One of these grants/consortiums title “Pharmacogenomics of Risk Factors and Therapies Outcomes for Kidney Disease” focuses on advancing precision medicine for the care of people at risk of or with progressive kidney disease. This consortium (PI is Adriana M Hung, MD MPH from Nashville VA/ Vanderbilt University) centers around the two main risk factors for CKD: type 2 diabetes and hypertension and also renal transplantation. Aim 1 is devoted to diabetes management. This aim will address the genetic determinants of the glycemic response to metformin. Although the work by this consortium is just starting, the MVP already provides the largest dataset of genotyped metformin users (n=42,711) with prescription and HbA1c data. The diabetic aim will also explore metformin nephro-protective effects and genetic risk for diabetic kidney disease, as well as for hypoglycemia. Aim 2 will explore genetic risk for hypertension, resistant hypertension and associated kidney disease. Aim 3 will evaluate the pharmacogenomics of immunosuppressive drugs used in kidney transplantation and replicate genetic findings in tacrolimus, whose clinical utility is currently being tested at Vanderbilt. Most phenotypes have been developed, tested and published in studies using the entire VA population.
An integrative genomics approach to predict patients with TNF activation in progressive nephrotic syndrome

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Background: The histopathology-based classification of FSGS and MCD does not accurately represent the molecular basis of these diseases or predict response to therapy. Targeted treatment approaches (anti-TNF, FONT trial, Joy et al., Am J Kidney Dis. 2010) led to remission in only 25% of unselected FSGS patients. We describe methods using transcriptomic and proteomic data to predict network activation and potentially treatment response to targeted intervention in patients.

Methods: Transcriptomic profiles from micro-dissected clinically indicated renal biopsies were generated from NEPTUNE study participants with FSGS or MCD. Gene expression levels were analyzed by clustering and functional networks analysis and validated in the ERCB cohort. Functional networks were assessed for relationships with serum and urinary biomarker profiles generated on the Luminex platform.

Results: Hierarchical clustering grouped subjects into 3 distinct molecular subgroups with significant differences in clinical outcomes: proteinuria remission and eGFR at baseline. Cluster identification was replicated in the ERCB cohort. Functional analysis identified a transcriptional network consistent with activated TNF in cluster 3. A TNF activation score was generated from TNF-regulated transcripts; the TNF activation score correlated with serum TNF levels, and differentiated cluster 3 subjects in NEPTUNE and ERCB cohorts. TIMP1 and MCP1 correlated with the TNF activation score and logistic regression was able to predict intra-renal TNF activation with high accuracy.

Conclusion and Impact: A molecular classification of primary proteinuric kidney identifies TNF activation in a subset of patients that is linked to urinary biomarkers. Patient stratification approaches aid in rational clinical trial design, identifying patients most likely to benefit from targeted therapies.
Comprehensive RNA sequencing analysis of human kidney disease

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Comprehensive RNA sequencing analysis of human diabetic and hypertensive kidney disease Chengxiang Qiu, Matthew Palmer, Mendy Liang, Melanie Sweeney, Julie Hawkins, Jon Hill, Paolo Guarnieri, Gregory Warnes, Carine Boustany, Steven Pullen and Katalin Susztak Background One in ten people in the United States suffers from chronic kidney disease (CKD). Progress in CKD research and drug development has been limited, and no drugs have been registered for the last 15 years. One key limitation has been that animal models do not recapitulate common forms of CKD (diabetic and hypertensive kidney disease). The aim of the current study was to collect and analyze a large number of human kidney tissue samples and identify transcript level changes in microdissected human glomeruli and tubuli. Methods Human kidney tissue samples were collected in RNAlater from non-neoplastic regions of tumor nephrectomies. Detailed clinical information was collected using an “honest broker” system. Histologic sections were scored for 19 independent pathological parameters reflecting glomerular, tubulointerstitial, and vascular compartments. RNA sequencing of 256 human kidney tissue samples (154 control and 102 CKD) was performed on glomeruli and tubuli separately using Illumina Trueseq v3 library kits and Hiseq2000 instruments. Reads were aligned using STAR aligners and annotated using Gencode human genome (GRCh37). Results Glomerular filtration rate was best associated with tubulointersitial fibrosis. Using statistical modeling, a large number of genes showed correlation with fibrosis and glomerulosclerosis. In addition, we applied weighted gene coexpression network analysis (WGCNA) to identify key transcriptional nodules that correlate with structural and functional changes. Both analyses highlighted pathways associated with immune system activation and dysmetabolism (eg. oxidoreductase) in CKD.
Linking renal structure to molecular function for outcome prediction in Diabetic Kidney Disease

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Background: Genome wide transcriptional profiling identifies active regulatory and transcriptional networks in kidney disease. Integrating structural changes with molecular profiles may identify functional correlates of early structural damage that predict subsequent disease progression.

Methods: Gene expression profiling and quantitative morphometric analysis was performed on protocol kidney biopsies from 49 Pima Indians with type 2 diabetes. Transcriptional co-expression modules were generated and associated with morphometric and long term clinical traits using Weighted Gene Coexpression Network Analysis. Urinary protein Epidermal Growth Factor (uEGF) levels were assessed for their correlation with the VvInt and GFR

Results: Several structural parameters were associated with molecular profiles. The degree of tubulointerstitial damage, assessed by measurement of cortical interstitial fractional volume (VvInt), showed the strongest association and was linked to long term clinical outcomes. Enrichment for migratory, inflammatory and cell-cell/cell-matrix interaction pathways was found in the transcripts that correlated positively with VvInt and enrichment for metabolic pathways, turnover of amino acids, sugars and lipids in those that correlated negatively. A subset of VvInt associated transcripts correlated with GFR and ACR measured ~8 years after biopsy, including EGF. uEGF showed strong positive correlation with intrarenal EGF transcript and negative correlation with with VvInt. uEGF was strongly associated with GFR measured at baseline and 8 years after biopsy.

Conclusion & Impact: VvInt-associated gene expression in preclinical to early DN was associated with ACR/GFR progression. 81% of these transcripts were also regulated in a diabetic European cohort. Molecular-morphometric approaches may capture molecular mechanism activated at a preclinical disease stage permitting early intervention in those affected.