Progress Report: DiaComp Pilot & Feasibility Grant 2014

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Project Title: Molecular Mechanisms of Kidney Disease in Diabetes and Obesity

BACKGROUND AND SIGNIFICANCE

The increasing prevalence of obesity and diabetes is a leading cause of chronic kidney disease (CKD) culminating in replacement therapy, thus representing a major health concern worldwide. Hyperfiltration and microalbuminuria are early signs of both obesity and diabetes (1, 2), which along with other elements of the metabolic syndrome, including hypertension and hyperlipidemia, contribute to the development and progression of obesity-related glomerulopathy (ORG) and diabetic nephropathy (DN).

There are several similarities in the histologic appearance of glomeruli from diabetic and obese individuals, and also overlap in some clinical features. The glomeruli of patients with diabetes are characterized by glomerular hypertrophy, widening of the glomerular basement membrane, mesangial expansion, and podocytopenia, leading to nodular glomerulosclerosis (Kimmelstiel-Wilson). Similarly, obesity-associated renal injury is characterized by glomerulomegaly, mesangial expansion, and podocytopenia, leading to focal glomerulosclerosis(3).

Both diabetes and obesity represent states of low-grade inflammation, oxidative stress, and pro-fibrosis, all of which may lead to progressive decline in GFR over time(4). However, this sequence of events may lead to obesity-related renal disease even in the absence of diabetes.

Pathways through which obesity might cause renal disease are not well understood, and early clinical biomarkers for ORG and incipient DN are currently lacking. Different pathogenic mechanisms, including abnormal glucose and lipid metabolism, profibrotic growth factors, proinflammatory cytokines, mitochondrial dysfunction, endoplasmic reticulum stress, and oxidative stress work in concert to result in progressive decline in renal function(5).

Recently we described ectopic lipid accumulation in kidney biopsies from patients with DN (fatty kidney) (6, 7). We have shown correlation of structural kidney alterations with RNA, and protein expression of lipid metabolism genes in diabetic kidneys. Furthermore, we have found correlation between these changes and the progressive decline in GFR (6).

The aim of this project was to study potential new treatment modalities that modulate the pathogenic pathways involved in lipid metabolism.

The intracellular nuclear receptor farnesoid X receptor (FXR) and the transmembrane G protein-coupled receptor (TGR5) respond to bile acids by activating transcriptional networks and/or signalling cascades. These cascades affect the expression of a great number of target genes relevant to bile acid, cholesterol, lipid and carbohydrate metabolism, as well as genes involved in inflammation and fibrosis.

Previous studies in rodent models of diabetes indicate that nuclear receptors, including the farnesoid X receptor (FXR) (8)and liver X receptor (LXR), transcriptional factors, including the sterol regulatory element binding proteins (SREBPs), and G protein coupled receptors, including TGR5,were effective in preventing or slowing down the progression of renal disease in obesity and diabetes(9, 10). However, it is not known whether these pathways are altered in human kidney disease in obesity or diabetes and whether the expression levels and activity of these pathways correlate with renal histopathology.

SPECIFIC AIMS

In **Specific Aim 1** we will extract mRNA, miRNA and protein from fixed and paraffin embedded human kidney biopsy samples from subjects with diabetic nephropathy (DN), obesity related glomerulopathy (ORG) and normal kidneys. The samples are obtained from the Department of Pathology, Columbia University, New York, NY, USA, and the Department of Pathology, Rabin Medical Center, Tel Aviv University, Israel. We will analyze pathways related to fibrosis, oxidative stress, inflammation and lipid metabolism, as well as nuclear receptors, transcription factors and G protein coupled receptors that are known to regulate these important pathogenic factors.

Progress toward stated aims:

We have extracted mRNA and proteins from 33 ORG and17 normal kidney FFPE kidney biopsy blocks from the Department of Pathology, Columbia University, New York, New York. We have already studied gene expression in kidney biopsy samples using RT-QPCR methods. Our focus is on genes involved in lipid and cholesterol metabolism and nuclear receptors.

Using laser-capture micro-dissection (LCM), we isolated RNA from glomeruli and tubulointerstitial tissues from these biopsies, which will enable us to further study gene-expression in ORG and DN by next generation sequencing (NGS) and RT-QPCRPCR(11, 12).

In **Specific Aim 2** we will correlate these molecular and metabolic pathways with estimated GFR, proteinuria and histopathology for each of the biopsy samples.

Progress toward stated aims:

FFPE kidney biopsies were identified by routine pathology analysis in pathology archive according to histopathology diagnosis. We revalidated percentage of glomerulosclerosis and histological subtypes of ORG (collapsing, tip, cellular, perihilar and not otherwise specified (NOS), re-analysed DN (pattern of nodular glomerulosclerosis) and also studied the degree of glomerulosclerosis and tubulointerstitial fibrosis.

We are currently collecting relevant patient clinical data to study their correlation with histopathology and gene expression metabolic pathway.

In **Specific Aim 3** we will study the similarities and differences of gene expression secondary to diabetes and obesity without T2DM to investigate the role of obesity in the mechanism of diabetic nephropathy.

Progress toward stated aims:

To look for novel genes and pathways that may play a role in the pathogenesis ORG, we chose to study wide gene expression with uunbiased approaches using NGS and proteomics. Transcriptome studies(13) support the need for using NGS in order to look for novel genes of potential involvement in the physiological mechanism and pathways or genes involved in diabetic nephropathy and obesity.Genes are expressed differentially in glomeruli and tubuli. However, since glomerular injury is of key importance in obesity, we decided to identify glomerular-specific transcripts by NGS (12-14). RNA was extracted using Laser Capture Microdissected (LCM) from human kidney biopsies of obesity-related glomerulopathy and normal tissue (glomeruli and tubulointerstitium). The kidney biopsies are provided by Columbia University and Rabin Medical Center pathology archive.

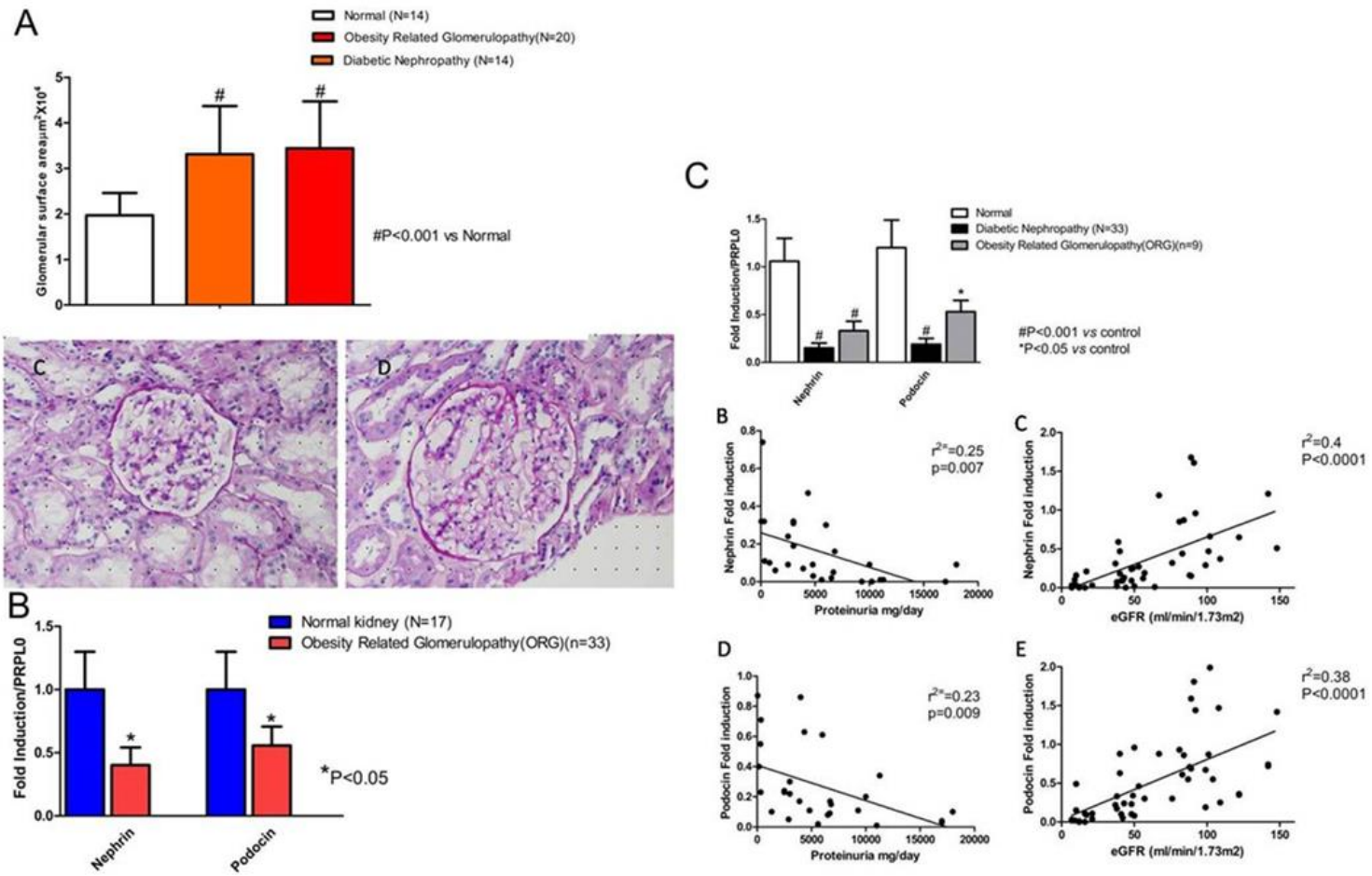
Molecular profiles and gene expression will be analyzed using illumine NGS. Our plans are to complete the above analyses and to try to link clinical and histological data using gene networks relevant to glomerular injury. We already collected the biological samples: Laser Capture Microdissected human kidney glomeruli (total of 30 samples; 10 ORG samples, 10 DN samples, 10 Healthy Control samples). We collected 44±30 glomeruli per sample, with a mean collected surface area of 1.3±0.9mm^2.We selected samples for sequencing according to RNA quantification and RNA integrity number (RIN).

Gene expression is ongoing by Illumina NGS at the Weizmann Institute. (http://www.weizmann.ac.il/weizsites/incpm/our-units/genomics/).The data of interest (after bioinformatic study and obtaining a list of differential expressed genes) will be validated by RT-QPCR.

We also harvested protein from the biopsy for proteomics analysis. We plan to run a quantitative comparison profile of extracted proteins to compare tryptic peptide intensities across the different kidney samples. The analysis will be conducted on a quadruple-orbitrap mass spectrometer (Q Exactive, Thermo Fisher). We will use Progenesis LC-MS data analysis software for peak extraction and alignment. Sequence database searching will be conducted using Mascot Search Engine, v2.4 at the Israel National Centre for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel.

We hope to study correlation between protein/mRNA expressions.

New genes will be studied by immunohistochemistry or immunofluorescence staining to validate regulation of key metabolic pathways at the protein expression level.



Glomerular hypertrophy and decrease expression of podocyte markers in ORG.
Fig 1: (A) Glomerular surface area (SE) (B) Podocin and nephrin mRNA in ORG patients (C) Correlation to eGFR and proteinuria.

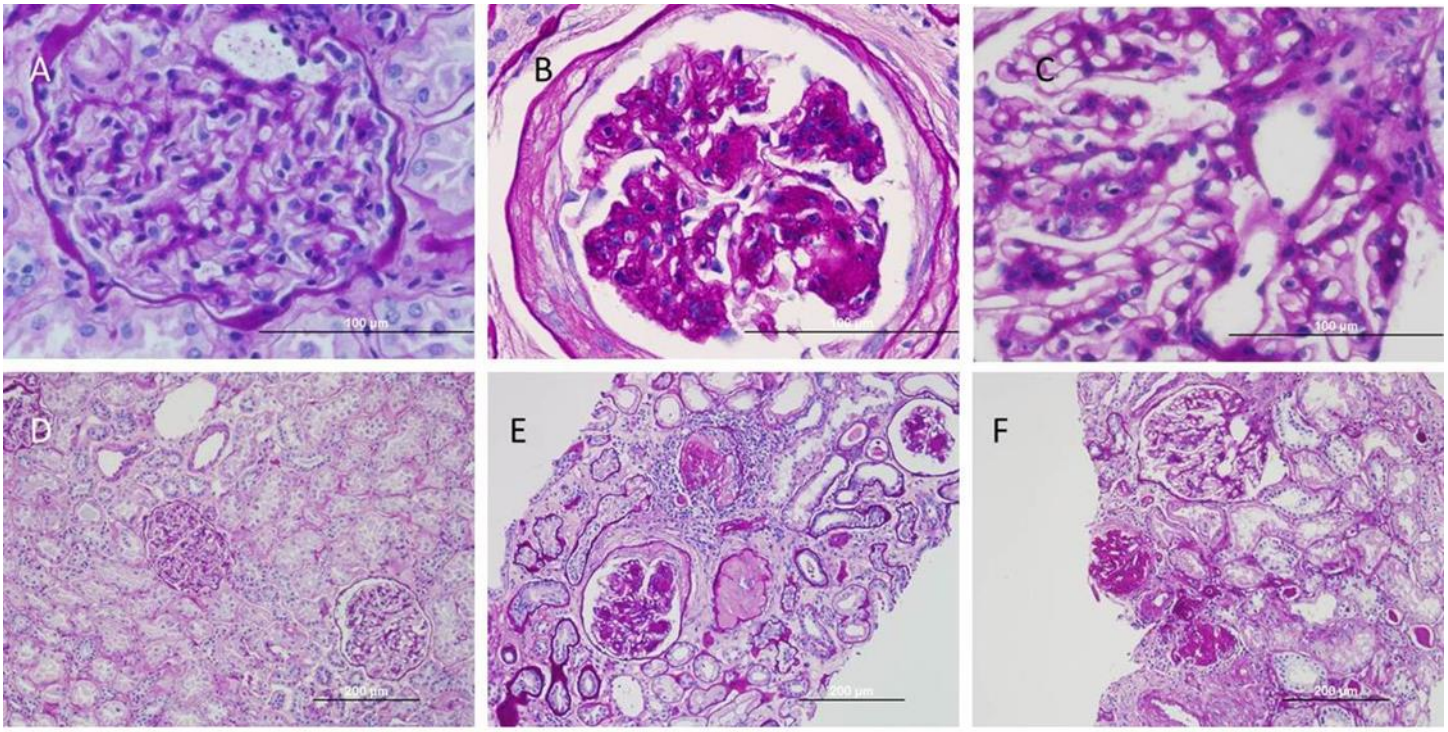


Fig 2 (A) PAS staining of (A,D)normal kidneys(A,D), DN(B,E) and ORG(C,F)

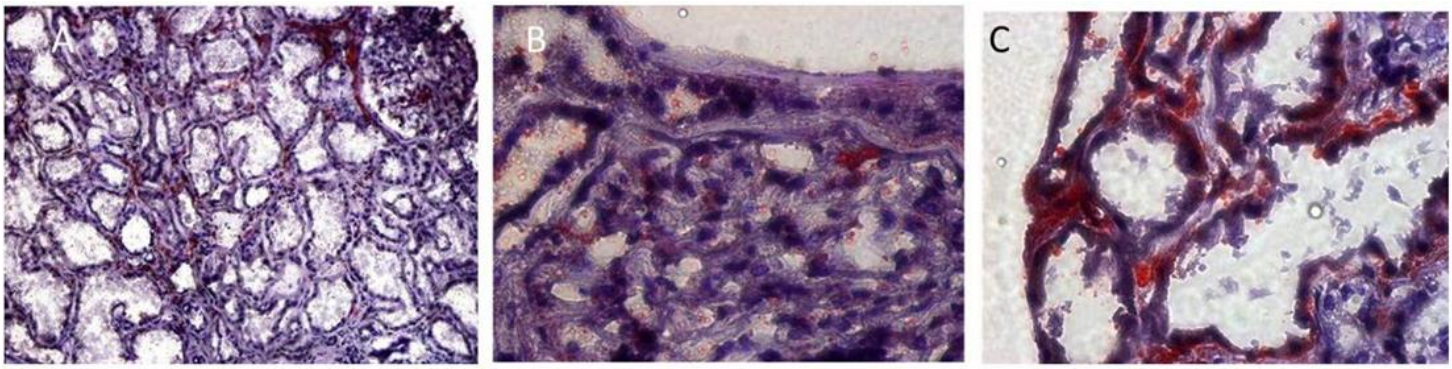


Fig 3: Lipid droplet in human ORG by Oil red O positive lipid deposits in human ORG (A). in the glomeruli X40 (B) and in the tubuli of ORG(C)

Clustered lipid droplets appeared, with different electron densities and are commonly seen in the podocyte and other cells both in DN and in ORG.

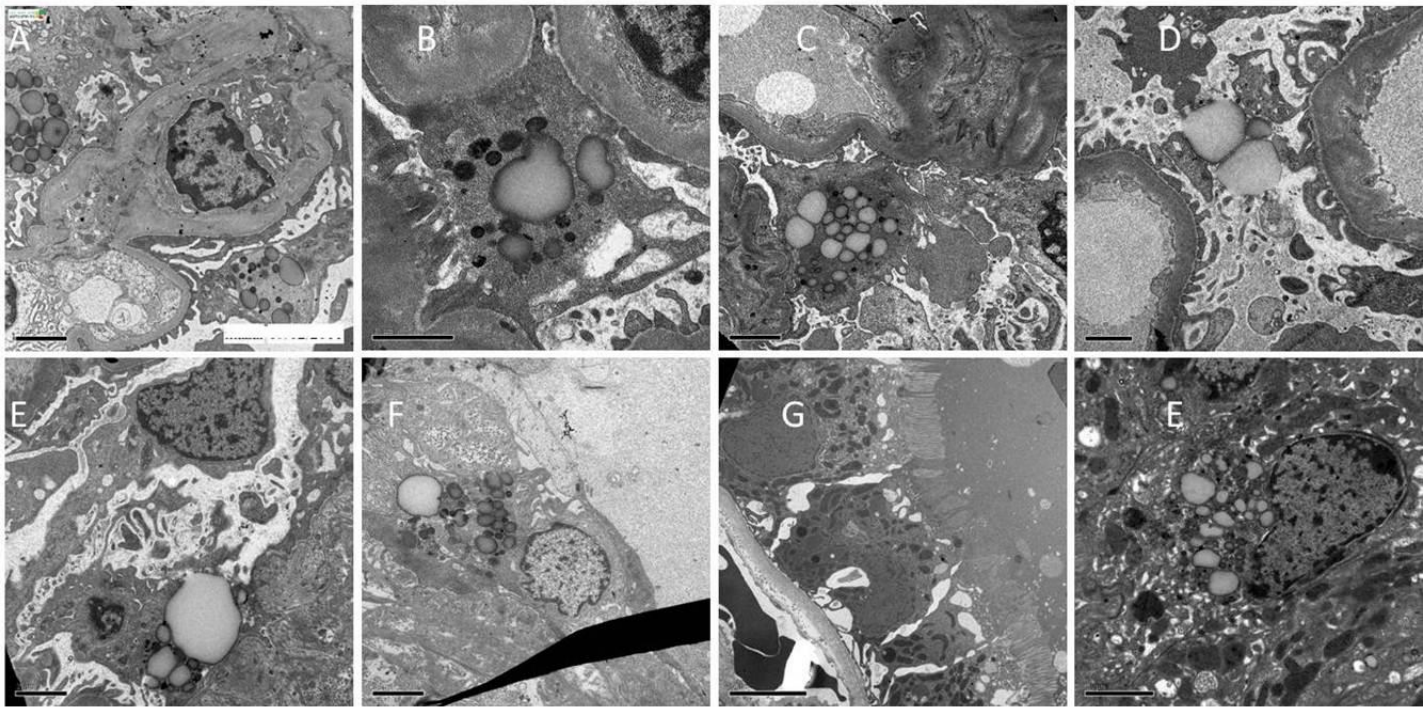


Fig 4: Lipid droplets in human kidney by EM. (A) DN (B-D) in podocyte in ORG (F)in parietal cell and(G-H) in proximal tubules.

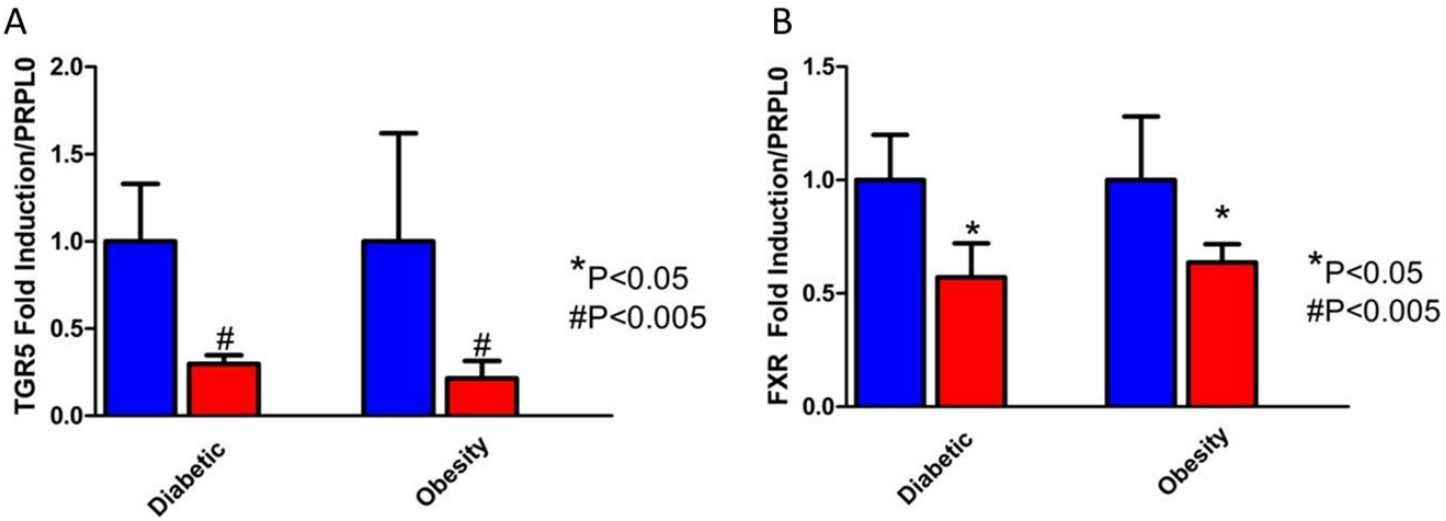
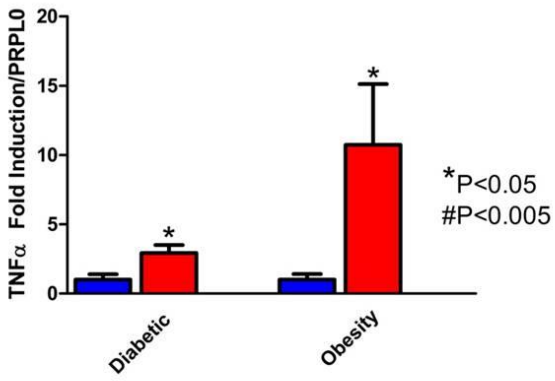


Fig 5a: Expression of bile acids in human kidney : The intracellular nuclear receptor farnesoid X receptor (FXR) and the transmembrane G protein-coupled receptor (TGR5) respond to bile acids by activating transcriptional networks and/or signaling cascades. These cascades affect the expression of a great number of target genes relevant to bile acid, cholesterol, lipid and carbohydrate metabolism, as well as genes involved in inflammation and fibrosis. (A) Expression of TGR5 mRNA in DN and ORG is reduced. (B) Expression of FXR mRNA in DN and ORG is reduced.



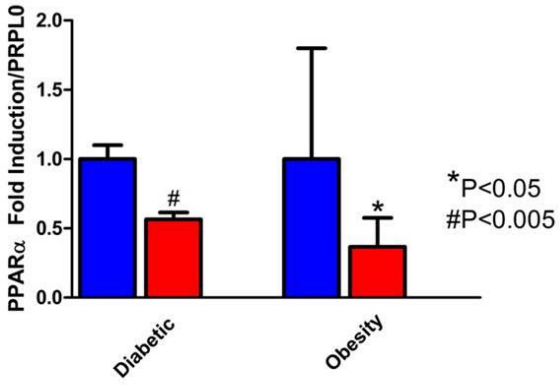
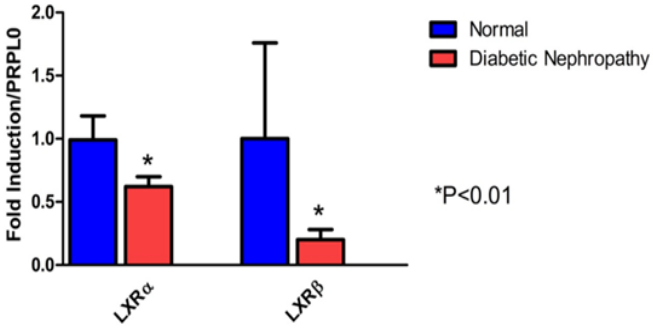


FIG 5b: TNFα mRNA in DN and ORG patients and PPARα mRNA in ORG and DN.



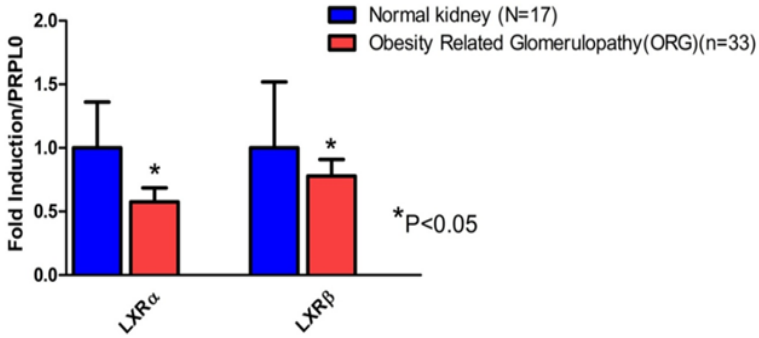
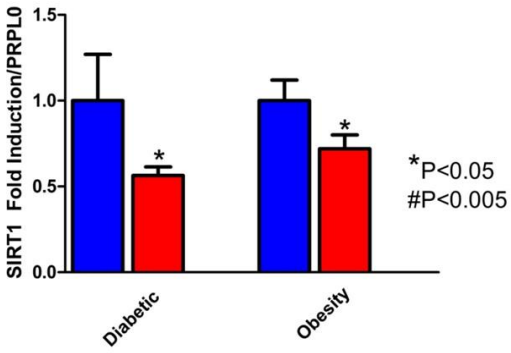


Fig5c : Dysregulation of cholesterol metabolism is central in the pathogenesis of diabetes and obesity renal complications. LXRs receptors have essential roles, in the regulation of cholesterol , fatty acid and glucose metabolism in the kidney. We have shown that expression of LXR receptors and cholesterol metabolism target genes are altered in diabetic kidney biopsies and correlate with eGFR . LXRs mRNA expression is decreased also in ORG.



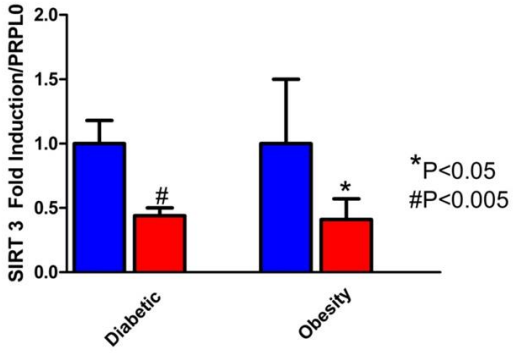


FIG 5d: Sirtuin 1 (SIRT1) plays important roles in maintaining metabolic functions and immune responses. Both SIRT1 and SIRT3 are metabolic sensors. We found down regulation of SIRT1and SIRT3 expression both in diabetic and obesity kidneys.

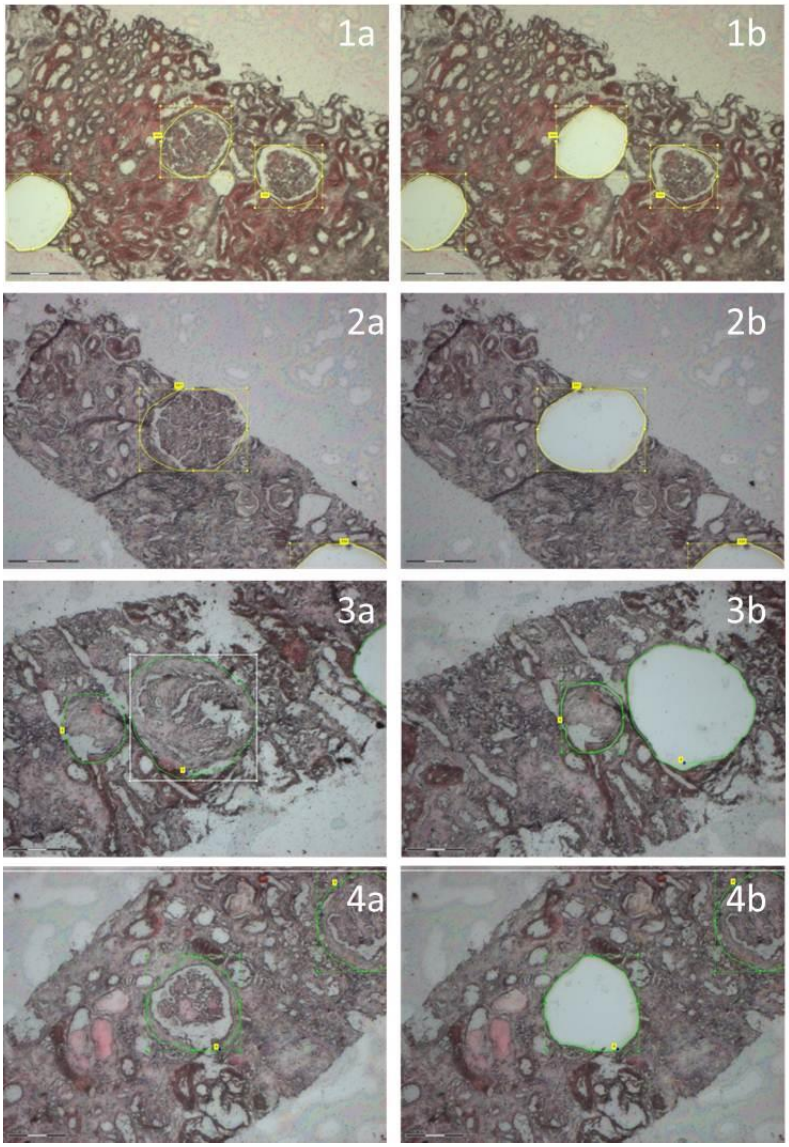
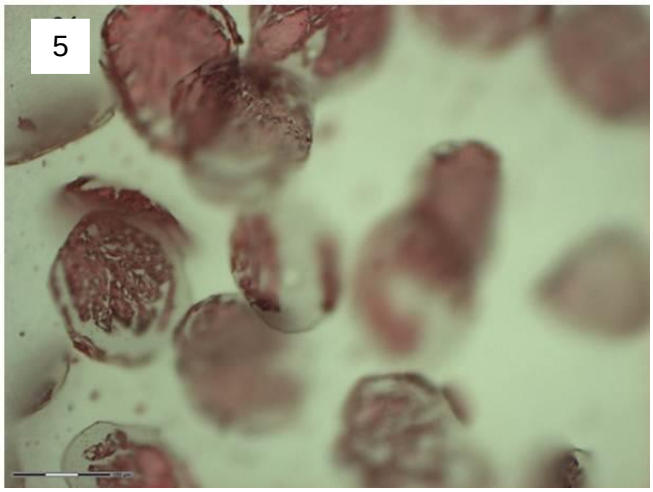


Fig 6 Kidney biopsies:
(a) before captured glomeruli, and (b) after LCM showing the remains surrounding intact tubulointerstitium. (1) Normal (2) ORG (3) ORG global sclerosis (4) DN.
(5) Capttured glomeruli collcted in LCM cap-tube.



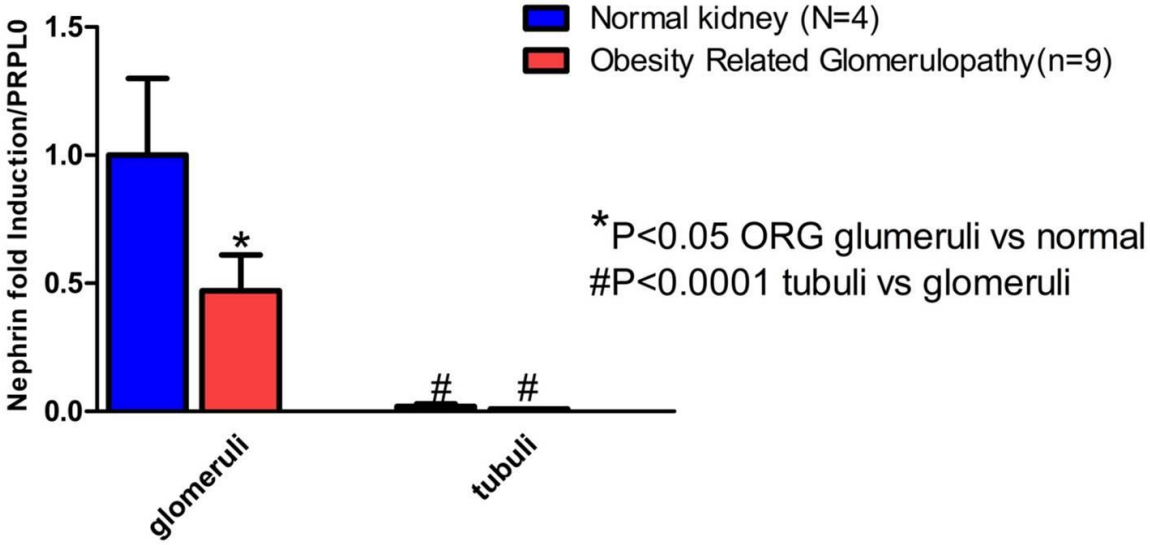


Fig6: Nephrin mRNA expression in LCM glomeruli, *vs* expression in surrounding tubulointerstitium.

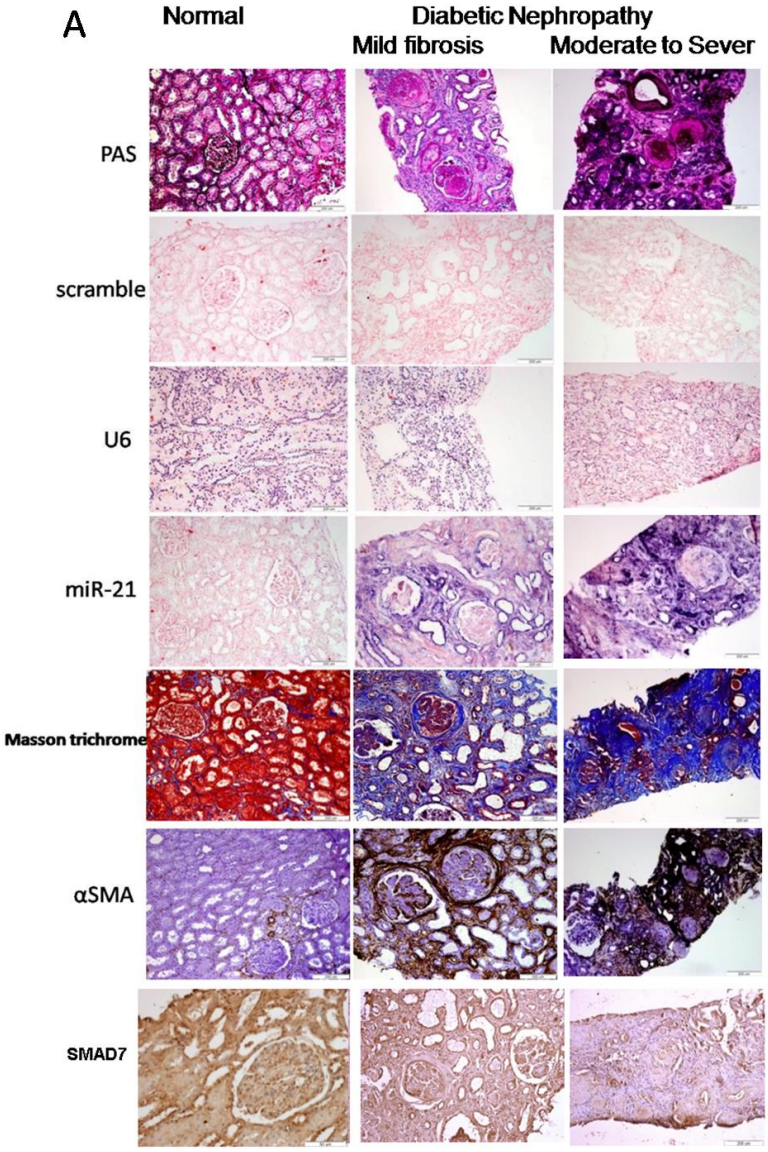
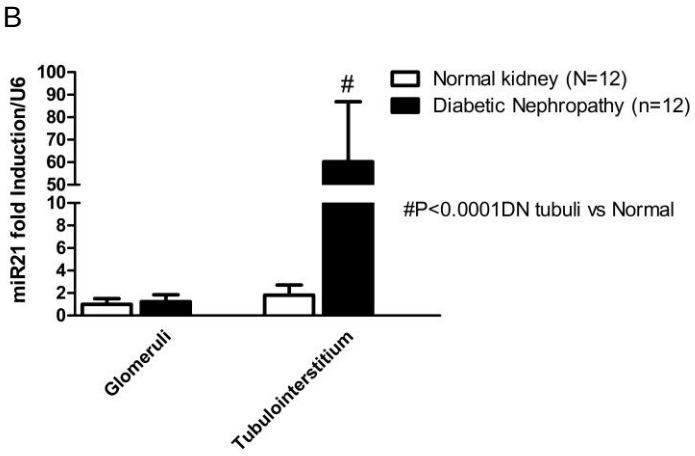


Fig 7: RNA samples were already used for preliminary study of differential miR expression in glomeruli *vs* tubulointerstitium in diabetic kidney. We found correlation between miR21 expression seen by in situ hybridisation (ISH) (A) and by using RT-QPCR in isolated LCM fractions (B).



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