sured using liquid chromatography-tandem mass spectrometry. Complex lipids and the effects of diabetes were largely tissue-specific, as lipid levels were primarily increased in the diabetic nerve, decreased in the diabetic retina, and glycerophospholipid remodeling was evident in the diabetic kidney. Only 15 lipids, primarily diacylglycerols and glycerophospholipids of the 36:4 composition, were shared across all 3 tissues. We identified co-regulated lipid sub-classes between plasma and each tissue, defining sub-classes of plasma lipids for use as surrogates of altered tissue lipid metabolism. While short chain diacylglycerols and long chain cardiolipins were similarly regulated between plasma and 2 of the tissues, most co-regulated sub-classes were between plasma and only 1 of the tissues examined. Furthermore, we tested the use of correlation analysis to integrate lipidomic and previously published transcriptomic data. We demonstrate this method can identify potentially pathogenic network-specific alterations in lipid metabolism, such as arachidonic acid metabolism in the diabetic kidney.

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18-LB

NLRC5 Deficiency Attenuates Diabetic Nephropathy by Alleviating Macrophage Infiltration and Inflammation

PEIPEI LUAN, JIANHUI ZHUANG, QING SU, WEIXIA JIAN, WENHUI PENG, *Shanghai, China*

Objective: Diabetic nephropathy (DN) is the main cause of end-stage kidney disease globally, and inflammation has a critical role in its pathogenesis. As the largest member of NOD-like receptors, NOD-like receptor family CARD domain containing 5 (NLRC5) has received extensive attention because of its important role in regulating immune and inflammatory responses. The current study tests the hypothesis that NLRC5 may play a critical role in the progression of DN.

Research Design and Methods: ODS Role of NLRC5 in DN was examined in genetic NLRC5 deficiency ($NLRC5^{-/-}$) and wild type (WT) mice those were streptozotocin-induced diabetic mice. Fasting blood glucose and 24-hour urinary albumin were measured. Kidney injury and molecular mechanism were observed and analyzed using transmission electron microscopy and molecular biological techniques including immunohistochemistry, qPCR and Western blot. Inflammation status and potential signaling pathways were tested in peritoneal macrophages treated with high glucose in vitro.

Results: NLRC5 expression was up-regulated in STZ induced diabetic mice, db/db mice and human diabetic kidney compared with controls. We found $NLRC5^{-/-}$ mice developed alleviated diabetic kidney injury relative to WT mice as evidenced by a significant decrease in albuminuria (105.5 ± 24.0 vs. 76.3 ± 20.5 μg/d, $P<0.05$), renal fibrosis (collagen IV and fibronectin), renal inflammation (interleukin-6 and tumor necrosis factor-α) and macrophage infiltration, and less reduced protein levels of nephrin and podocin in diabetic kidney. Our research also revealed alleviated inflammation in peritoneal macrophages from $NLRC5^{-/-}$ mice was associated with suppressive activation of nuclear factor-κB pathway.

Conclusions: NLRC5 plays a proinflammatory role in DN by regulating activation of NF-κB pathway in macrophages. Therefore, NLRC5 may represent a promising target for treatment of DN.

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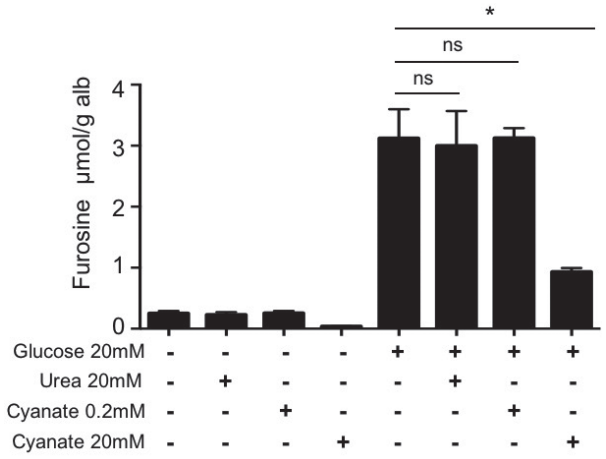
19-LB

Glycation and Carbamylation Reciprocally Compete for Protein Modification

CAMILLE NICOLAS, STÉPHANE JAISSON, LAETITIA GORISSE, CHRISTINE PIETREMENT, PHILIPPE GILLERY, *Reims, France*

Diabetes and chronic kidney disease (CKD) accelerate protein molecular aging through glycation and carbamylation reactions, which are characterized respectively by the binding of sugars or urea-derived isocyanic acid on proteins. These reactions target the same protein amino groups, especially in diabetic patients with CKD. For example, they can compete for the major site of hemoglobin modification, the N-terminal Val residue of β-chains, modified in HbA_{1c}. This study aims to evaluate their competitive effect in vitro and in vivo. In vitro, albumin is incubated with glucose, urea or cyanate. In vivo, carbamylation is enhanced in normal and diabetic mice by sub-nephrectomy, or by cyanate consumption. Furosine, fructosamine and HbA_{1c} are measured by LC-MS/MS, colorimetric and immunological assays and homocitrulline and carbamylated hemoglobin (carbamylation-derived products) by LC-MS/MS. A reciprocal inhibition between carbamylation and glycation is observed in vitro. In vivo, 5 weeks after induction of CKD, plasma homocitrulline concentrations are similar in diabetic and nondiabetic mice. Fructosamine and HbA_{1c} decreased in CKD-diabetic mice compared to diabetic ones and also in cyanate-drinking compared to water-drinking diabetic mice. Carbamylation competes with glycation in vivo. Thus, classical markers of glycemic control, as the HbA_{1c}, should be interpreted with caution in diabetic patients with CKD.

Figure.



COMPLICATIONS—NEPHROPATHY—CLINICAL AND TRANSLATIONAL RESEARCH

20-LB

Novel Circulating Biomarkers Predict Rapidly Declining Renal Function in Type 2 Diabetes: The Fremantle Diabetes Study

KIRSTEN E. PETERS, WENDY A. DAVIS, JUN ITO, KAYE WINFIELD, THOMAS STOLL, SCOTT D. BRINGANS, RICHARD J. LIPSCOMBE, TIMOTHY M.E. DAVIS, *Fremantle, Australia, Perth, Australia*

The ability of baseline patient characteristics including albuminuria and estimated glomerular filtration rate (eGFR) to predict onset/progression of diabetic chronic kidney disease (CKD) is limited. We investigated the role of circulating diagnostic protein biomarkers (APOA4, APOC3, CD5L, C1QB, CFHR2, IBP3) in predicting renal function decline in type 2 diabetes (T2DM). A mass spectrometry platform was used to measure biomarkers at entry in 345 participants from the longitudinal observational Fremantle Diabetes Study (FDS) Phase II. Onset/progression of CKD was defined by i) ≥30% eGFR fall over 4 years, ii) incident CKD (eGFR <60mL/min/1.73m^2), iii) steepest eGFR trajectory, and iv) eGFR decline ≥5mL/min/1.73m^2/year. Multiple logistic regression identified clinical predictors of developing nephropathy. The incremental predictive value of biomarkers was then assessed. The 4 models were validated in an independent FDS cohort (n=447). Of the 345 initial participants, 30 had a ≥30% fall in eGFR over 4 years. After adjustment for the most parsimonious model, APOA4 and IBP3 independently predicted outcome (OR=4.85 [95% CI 2.04-11.50] and OR=0.32 [0.13-0.81], respectively) and improved model fit ($P<0.001$), discrimination (AUC from 0.84 to 0.88, $P=0.14$), and reclassification (NRI=0.81 [0.68-0.94] and IDI=8.1% [1.0%-15.1%]). For the other definitions of rapid decline, APOA4 and IBP3 improved model performance with CD5L and C1QB also independent predictors of steepest eGFR trajectory (OR=0.52 [0.29-0.93] and OR=2.41 [1.14-5.11], respectively). Applied to the validation cohort, the discrimination and accuracy of each model was good (AUC=0.65-0.83; mean square error=0.05-0.10), but calibration was poor ($P<0.05$) due to small numbers in lower deciles. The present study has identified novel plasma biomarkers (APOA4, IBP3, CD5L, C1QB) that improve prediction of indices of CKD in T2DM independently of conventional clinical variables.

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21-LB

miRNAs 1915-3p and 4532 as Novel Noninvasive Biomarkers to Detect Renal-Function Decline in Type 1 Diabetes Patients

MARIA BEATRIZ MONTEIRO, MARIANA CARDENAS-GONZALEZ, TATIANA PELAES, MIRA PAVKOVIC, VISHAL S. VAIDYA, MARIA LUCIA CORREA-GIANNELLA, *Boston, MA, São Paulo, Brazil*

Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease worldwide. Microalbuminuria (MA) is the earliest indicator of DKD in diabetes (DM). However, while MA is a sensitive marker, it is not spe-

For author disclosure information, see page LB107.