UCD Diabetes Complications Research Centre

Research Symposium

Friday 19th January 2024

AGENDA

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Session I

Conway Lecture Theatre, UCD Conway Institute.

Chairs: Dr Jessica Davis and Dr Vanessa Cândido

09:15-09:30	**Introduction – Prof Catherine Godson**, UCD DCRC Director.
09:30-10:00	**Effective Science Communication** - **Mr Seán Duke**, Faculty of Science and Health, Dublin City University.
10:00-11:00	UCD DCRC Research Presentations **The type II diabetes medication metformin rescues attention deficits in a model of Huntington's Disease - Mr Cian Gavin** [Glennon lab] **Finely Modulating the Human Macrophage Phenotype: A Rationale for a Pro-resolving Approach Combined with Gold-standard Therapy in Atherosclerosis - Mr Braden Millar** [de Gaetano lab] **Induction of Let-7d-5p miRNA Modulates Aortic Smooth Muscle Inflammatory Signaling and Phenotypic Switching - Ms Tanwi Vartak** [Brennan lab] **Immune Cells Respond Differently to Immune Modulators Based on Metabolic Health - Ms Méabh Ní Chathail** [Roche lab] **Targeting BCAA Metabolism during Hepatic Stellate Cell Transactivation as a Potential Treatment for Liver fibrosis - Mr Rory Turner** [Wallace lab]

Kevin Barry Gallery, UCD Charles Institute of Dermatology.

11:00-11:30	Refreshments and Poster Session

Session II

Charles Seminar Room, UCD Charles Institute of Dermatology.

11:30-12:00	**Chairs:** Ms Delphi Mac Begg-White and Ms Tanwi Vartak **Injectable Stem Cell Hydrogels for Diabetic Wound Healing - Dr Jing Lyu**, Charles Institute for Dermatology & UCD School of Medicine.
12:00-12:15	**Rewiring Hepatic Mitochondrial Metabolism in Obesity - Asst Prof Christopher Shannon**, UCD Ad Astra Fellow, UCD DCRC.

12:15-13:30 UCD DCRC Research Presentations

PRRX1 is a Master Regulator of Renal Fibroblast Cell Fate and Regulates the Activation of Myofibroblasts in Response to TGF-β1 via Alteration of the Chromatin Landscape - Ms Ericka Bonifacio [Crean lab]

Metformin administration in TALLYHO/JngJ mouse models of Type 2 diabetes shows improvements in selective attention, complement signalling and mitochondrial respiration - Ms Mairéad Sullivan [Glennon lab]

A Novel Role for FERM Domain-containing Protein 3 (FRMD3) in Chronic Kidney Disease - Dr Ciarán Kennedy [Godson lab]

Development and Evaluation of a Novel Biomarker-based Risk Stratification Platform for Obesity-related Complications using Targeted Proteomics - Dr Rachel Byrne [McGillicuddy lab]

Non-caloric Sweeteners are Associated with Decreased Sperm Viability and Morphology in Male Mice - Ms Michelle Kearns [Reynolds lab]

Kevin Barry Gallery

13:30-14:30 Lunch and Poster Session

Session III

Charles Seminar Room

Chairs: Dr Dean Moore and Dr Darrell Andrews

14:30-15:00 **Emerging Medical Devices for the Treatment of Diabetes - Assoc Prof Eoin O'Cearbhaill**, UCD School of Mechanical and Materials Engineering.

15:00-15:45 **Understanding Obesity - Prof Donal O'Shea**, St Vincent's University Hospital & UCD School of Medicine.

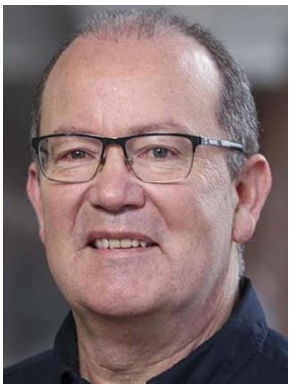
15:45-16:30 **Single Cell Analysis Strategies in Kidney Transplant Rejection - Clinical Assoc Prof Andrew Malone**, St Vincent's University Hospital & UCD School of Medicine.

Closing

Kevin Barry Gallery

16:30 Reception and Poster Session

GUEST SPEAKERS



Mr Seán Duke is a Communications Officer at the Faculty of Science and Health in Dublin City University. His role involves the implementation of the strategic communications plans for the faculty.

He has over 25 years of experience in science journalism and communication. He was a Science Journalist with *The Irish Times* and *The Irish Independent*, Broadcast Journalist with East Coast FM and a regular contributor to RTÉ Radio One and Newstalk. He was also a Podcast Host with Skillnet Ireland (*Ireland's Place in Space*) and UCD (*AgriFood Matters*).

Dr Jing Lyu is a Postdoctoral Researcher in Prof. Wenxin Wang's group at the Charles Institute, School of Medicine, UCD. Her research interests are in the areas of macromolecular design, synthesis, and structure control and their derived hydrogels for the treatment of skin wounds. She has published 28 peer-reviewed journal papers in journals such as Nature Reviews Chemistry, J. Am. Chem. Soc., Advanced Materials, etc. She has also secured three research fellowship/fundings.

In addition, Dr Lyu worked as a Product Development Scientist at Blafar Ltd during her PhD and Postdoc research (a biotechnology company, committed to producing functionalised biopolymer products for the cosmetic and medical devices industry around the world) and has built up good collaborations with worldwide leading researchers in both academia and industry.

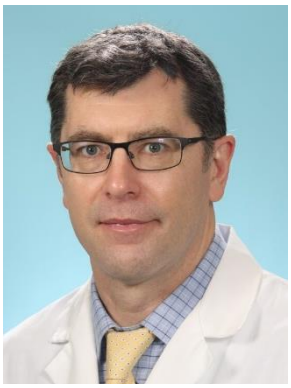
Assoc Prof Eoin O'Cearbhaill joined the School of Mechanical and Materials Engineering, UCD in 2013. He leads the UCD Medical Device Design Group where his interests include, 3D Printing of Medical Devices; Medical Device Innovation, Design and Commercialisation, including minimally invasive devices and delivery systems.

Prior to joining UCD, he was a Postdoctoral Fellow at Harvard Medical School, where his research focused on the conception and development of medical devices and the delivery of next generation therapeutics, in the laboratory of Prof. Jeffrey Karp.

He received his BE (Biomedical) and PhD from NUI Galway. Subsequently, he worked for Veryan Medical, before joining Creganna-Tactx, where he worked in both manufacturing and design service roles, helping to establish their Specialty Needles Division in Marlborough, MA.

Prof Donal O'Shea Consultant Endocrinologist at St Vincent's University Hospital (SVUH) and St Columcille's Hospital, Dublin and has been the Lead Clinician for a hospital-based multi-disciplinary obesity service in those hospitals since 1999. He is also the Head of the Obesity Research Group, Education and Research Centre, SVUH and Associate Professor of Medicine at UCD where he lectures in endocrinology.

Prof O'Shea has received many honours including the Patrick Meenan UCD MGA Inaugural Research Medal in 1996, the Norman Plummer Prize for Postgraduate Clinical Research in 1998, the Imperial College School of Medicine Undergraduate Teacher of the Year in 1999, the Irish Endocrine Society O'Donovan Medal for group's research activity in 2006 and the UCD Premier Award for Undergraduate Teaching Excellence in 2003-2008 & in 2010.



Clinical Assoc Prof Andrew Malone is a transplant nephrologist at St. Vincent's University Hospital, Dublin and UCD School of Medicine since 2024. His research interest lies in the field of transplantation genetics.

Following a fellowship in transplant nephrology Washington University (WU) St Louis he was appointed to faculty as Assistant Professor of Medicine in 2015. He has received several honours and awards including an American Society for Clinical Investigation Council Young Physician-Scientist Award (2020), an Early Career Development Award from the Central Society for Clinical and Translational Research (2021) and was chosen to join the JASN Editorial Fellows mentorship program, 2021-2023.

SHORT TALK ABSTRACTS

The type II diabetes medication metformin rescues attention deficits in a model of Huntington's disease.

Cian Gavin[1], Mairead Sullivan[1], Gabrielle Litovskitsch[1], Hannah Rapley[1], Eduordo Pisa[2], Simone Macri[2], Marian Tsanov[1] and Jeffrey Glennon[1].

[1]School of Medicine, Conway Institute of Biomedical and Biomolecular Research, University College Dublin, Belfield, Dublin 4, Ireland. [2]Centre for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Roma, Italy.

Huntington's Disease (HD) is a progressive and fatal neurodegenerative disorder caused by a polyglutamine expansion in the gene encoding the protein huntingtin (HTT). In HD, cognitive impairments precede the onset of the classical motor symptoms. Similar to the disease progression in humans, the yeast artificial chromosome (YAC) 128 HD mouse model expresses multiple copies of a full-length human huntingtin gene (HTT) modified in exon 1 to have a glutamine repeat expansion. YAC mice also exhibit cognitive dysfunction that precedes the onset of the neuropathological and motor impairments characteristic of HD. Recent work in our group has suggested a role for signalling elements downstream of insulin signalling in murine models of HD and highlighted neuro-inflammatory aspects. Here we evaluated (i) the anxiety and cognitive phenotypes of the YAC model, (ii) whether the anti-inflammatory aspects of the type II diabetes medication metformin modify the YAC phenotype and (iii) whether changes in Huntington's disease associated signalling is altered in a type II diabetes TALLYHO/JngJ mouse model and whether this is impacted by metformin.

YAC mice demonstrated increased anxiety in the elevated plus maze but did not show changes in spatial learning. However, YAC mice did show an increased number of errors in extra dimensional set shifting (EDS, a task assessing attentional switching). Ad libitum administration of metformin (2mg/ml) in the drinking water to YAC mice completely reversed the number of EDS errors made. Metformin had no effect on EDS in healthy C57/Bl6 control mice. Proteomic analyses in the nucleus accumbens (a region involved in EDS) in type II diabetic mice show decreased Huntington's Disease signalling which is attenuated by metformin administration in the TALLYHO/JngJ model. Metformin treated TALLYHO/JngJ mice show a rescue of HTT regulation of autophagy, perinuclear inclusions and AKT-mediated alterations in neurite outgrowth.

Together these findings confirm a deficit in attentional switching in the YAC mouse model of HD which can be rescued by metformin. It also highlights a role for mechanisms associated with HTT over-expression in the mechanism of action with metformin. Whether this involves HTT cross talk with AMPK activation will be the subject of forthcoming proteomic studies in the YAC mice.

Finely modulating the human macrophage phenotype: A rationale for a pro-resolving approach combined with gold-standard therapy in atherosclerosis.

Braden Millar[1], Ciarán Kennedy[2], and Monica de Gaetano[1].

[1]School of Biomolecular & Biomedical Science, University College Dublin. [2]School of Medicine, University College Dublin.

Atherosclerosis is a progressive, multi-factorial, inflammatory and dyslipidaemic disease characterised by the build-up of a plaque, *via* accumulation of lipid-laden foam cells and cell debris. An imbalanced lipid metabolism and failure to attenuate the inflammation attributes to disease progression. The lack of a therapeutic capable of tackling the associated residual inflammatory risk is an unaddressed need. A key aspect to the progression of this disease is the **monocyte-macrophage-**

foam cell axis, wherein monocytes are activated and differentiated into macrophages for the phagocytosis of infiltrated oxidised low-density lipoprotein (ox-LDL), generating foam cells which contribute to the development of an *atheroma* (atherosclerotic plaque). Chronic inflammation drives the continuing phagocytosis of ox-LDL and subsequently progresses the disease state.

We aim to generate, observe, and intervene on a novel *in vitro* cell model of athero-genesis, specifically monitoring the critical monocyte-macrophage-foam cell axis, in order to identify novel 'druggable targets' in the *Resolution of Atherosclerosis*.

In vitro model was set-up along the monocyte-macrophage-foam cell axis, characterizing a plethora of macrophage sub-phenotypes. In particular, genomic analysis revealed that M-CSF+IL-1β induced the optimal pro-inflammatory atherosclerotic macrophage; whilst Incucyte-generated data showed significant uptake of foam cells for subsequent foam cell formation in pro-inflammatory macrophage phenotype (TNF-stimulated) *vs* vehicle (N=3, $p<0.0001$). Subsequent genomic analysis of pro-inflammatory macrophages confirmed this showing down-regulation of efflux protein ABC-A1 in pro-inflammatory macrophages (N=3, $p<0.05$) and no significant difference in differential expression of influx protein CD36 (N=3, $p=0.391$), favouring lipid retention.

Induction of Let-7d-5p miRNA modulates aortic smooth muscle inflammatory signaling and phenotypic switching.

Tanwi Vartak[1], Elena Giardini[1], Daniel Kelly[1], Bruce Moran[2], Ciarán Kennedy[1], Mary Barry[3], Catherine Godson[1], and Eoin Brennan[1].

[1]Diabetes Complications Research Centre, Conway Institute & UCD School of Medicine, University College Dublin, Dublin 4, Ireland. [2]St. Vincent's University Hospital, Dublin, Ireland. [3]Department of Vascular Surgery, St. Vincent's University Hospital, Dublin, Ireland.

Background and Aims: Activation of vascular smooth muscle cell inflammation is recognised as an important early driver of vascular disease. We have previously identified the let-7 miRNA family as important regulators of inflammation in in vitro and in vivo models of atherosclerosis. Here we investigated a dual statin/let-7d-5p miRNA combination therapy approach to target human aortic SMC (HAoSMC) activation and inflammation.

Methods: In vitro studies using primary HAoSMCs were performed to investigate the effects of let-7d-5p miRNA overexpression and inhibition. HAoSMCs were treated with combinations of the inflammatory cytokine tumor necrosis factor-α (TNF-α), and atorvastatin or lovastatin. HAoSMC Bulk RNA-seq transcriptomics of HAoSMCs revealed downstream regulatory networks modulated by let-7d-5p miRNA overexpression and statins. Proteome profiler cytokine array, western blotting and quantitative PCR analyses were performed on HAoSMCs to validate key findings.

Results: Let-7d-5p overexpression significantly attenuated TNF-α-induced upregulation of IL-6, ICAM-1, VCAM-1, CCL2, CD68, MYOCD gene expression in HAoSMCs ($p<0.05$). Statins (atorvastatin, lovastatin) significantly attenuated inflammatory gene expression and upregulated Let-7d levels in HAoSMCs ($p<0.05$). Bulk RNA-seq analysis of a dual Let-7d-5p overexpression/statin therapy in HAoSMCs revealed that let-7d-5p activation and statins converge on key inflammatory pathways (IL-6, IL-1β, TNF-α, IFN-γ). In silico analysis of smooth muscle cell phenotypic switching shows that overexpression of let-7d-5p in HAoSMCs maintains a contractile phenotype.

Conclusions: Targeting the Let-7 network alongside statins can modulate HAoSMC activation and attenuate key inflammatory pathway signals.

Immune Cells Respond Differently to Immune Modulators Based on Metabolic Health.

Méabh B. Ní Chathail[1], Christopher E. Shannon[1,2], Sinéad M. Mullin[1], Martina Wallace[3], Pamla Singh[4], Suzanne Norris[4], and Helen M. Roche[1,5]

[1]Nutrigenomics Research Group, UCD Conway Institute, and Institute of Food and Health, School of Public Health, Physiotherapy and Sports Science, University College Dublin. [2]School of Medicine, University College Dublin. [3]School of Agriculture and Food Science, Conway Institute, University College Dublin. [4]Hepatology Department, St James's Hospital. [5]Institute for Global Food Security, Queen's University Belfast.

Resolving chronic low-grade inflammation offers a therapeutic strategy to combat metabolic syndrome but requires an improved understanding of potential immunomodulatory mechanisms. We characterised the immunomodulatory effects of functional food ingredients in monocytes from patients with metabolic-associated fatty liver disease (MAFLD) (n=9), steatohepatitis (MASH) (n=5), and non-obese healthy controls (Con) (n=10) using an established innate immune training protocol. Monocytes stimulated with either yeast β-glucan (YBG), Compound-2, or vehicle control were re-stimulated with LPS, and cell supernatants were analysed for cytokine and metabolite concentrations.

Basal, LPS-stimulated, and YBG+LPS-stimulated TNF-α secretion were higher in MAFLD ($p<0.05$) compared to CON. There were no differences in TNF-α secretion in MASH upon stimulation with LPS/YBG+LPS/Compound-2+LPS ($p>0.05$). There were no differences in IL-6 secretion in MASH or MAFLD upon stimulation with LPS/YBG+LPS/Compound-2+LPS. LPS and YBG+LPS stimulated increased IL-6 in CON in comparison to baseline (no treatment) ($p<0.05$). Compound-2 suppressed LPS-stimulated TNF-α/IL-6 responses in MAFLD/CON with no differences between groups. There were no differences in IL-10 secretion between cohorts. In CON, YBG and YBG+LPS increased net lactate production, whereas Compound-2 had no effect in comparison to baseline ($p<0.01$). Citrate concentrations remained unchanged by any treatment.

This preliminary analysis reveals that the capacity of potential immune modulators to modulate immune cell function is variable depending on disease state. This indicates that the less progressed disease (MAFLD) is more malleable to adaptation than the more progressed disease (MASH). Further research will focus on the differences between MAFLD and MASH and how MAFLD can be further manipulated through dietary intervention.

Targeting BCAA Metabolism during Hepatic Stellate Cell Transactivation as a Potential Treatment for Liver fibrosis.

Rory Turner, Xiaofei Yin, Lorraine Brennan, and Martina Wallace.

School of Agriculture and Food Science, University College Dublin.

Non-alcoholic steatohepatitis, which is characterised by liver fibrosis, inflammation and steatosis, is one of the leading causes of chronic liver disease and cancer. Transactivation of quiescent hepatic stellate cells (HSC) to myofibroblasts is the primary driver of fibrosis in this context, yet the metabolic transformation required to drive this process is relatively uncharacterised. Here we carried out a meta-analysis of publicly available RNAseq data of primary HSCs treated with TGFβ and found that a decrease in BCAA catabolism was one of the primary metabolic signatures. Initial in vitro investigations have shown that LX-2 HSCs exhibit a significant decrease in expression of the rate-limiting BCAA catabolic gene, BCKDHB in response to TGFβ. This was associated with a simultaneous increase in the deaminating enzyme BCAT1 along with a build-up of both intracellular BCAAs and their deaminated counterparts, suggesting a dependency on this pool during HSC activation. Finally, treatment of LX-2 HSCs with the small compound BT2 to relieve inhibition of BCKDH resulted in both an increase in expression of HSC quiescence markers, a decrease in extracellular matrix deposition, alterations to the intracellular pool of BCAAs, an associated eight-fold increase in leucine flux to lipogenic acetyl-CoA, and a decrease in both mature collagen protein and extracellular matrix

deposition. Collectively this data indicates that BCAA metabolism may be a targetable feature of hepatic stellate cell transactivation that could be used to mitigate the development of liver fibrosis.

PRRX1 is a master regulator of renal fibroblast cell fate and regulates the activation of myofibroblasts in response to TGFβ1 via alteration of the chromatin landscape.

Ericka J. Bonifacio, Jessica L. Davis, Niall Carruthers, and John Crean.

School of Biomolecular & Biomedical Sciences, University College Dublin

Background: Emerging evidence suggests that changes in the chromatin landscape contributes to the pathogenesis of diabetic kidney disease (DKD). Previous studies have shown that TGFβ1 signalling to chromatin unleashes a programme of gene expression that initiates fibroblast to myofibroblast activation. We hypothesise that SMAD3 and the Polycomb Repressive Complex 2 core component, EZH2, co-occupy regulatory regions within chromatin to deposit histone post-translational modifications, thereby facilitating a landscape which drives fibroblast activation. Our data suggests that PRRX1 is a master regulator of fibroblast to myofibroblast differentiation through association with SMAD3/EZH2.

Methodology: CCD18Lu fibroblasts were treated with EZH2 inhibitor, GSK343, 1 hour prior to treatment with TGFβ1 for 48hrs. RNA was extracted from fibroblast cells, and subjected to TaqMan RT-PCR assay. Dermal BJ fibroblasts were subject to bulk-RNA sequencing and data was analysed through Galaxy pipeline.

Results: RT-PCR data suggests that PRRX1 expression is downregulated in response to TGFβ1 treatment. Interestingly, PRRX1 expression was sustained in fibroblasts pre-treated with GSK343. Western Blot analysis revealed upregulated expression of epigenetic mark H3K27me3 in tandem with downregulation of PRRX1 expression during TGFβ1 induced fibroblast to myofibroblast differentiation. Furthermore, Bulk RNA-seq analysis suggests a similar process during TGFβ1 induced myofibroblast activation in BJ fibroblasts.

Conclusion: TGFβ1 is a key driver of chromatin dynamics as evidenced by trimethylation on H3K27 and likely contributes to gene repression through interaction between SMAD3 and EZH2. PRRX1 regulates fibroblast cell identity, and loss of expression in response to TGFβ1 likely involves epigenetic silencing. This may render PRRX1 as a potential therapeutic target for ameliorating fibrosis in DKD.

Metformin administration in TALLYHO/JngJ mouse models of Type 2 diabetes shows improvements in selective attention, complement signalling and mitochondrial respiration.

Mairéad Sullivan[1], Simone Macri[2], Martina Presta[2], Angela Maria Ottomana[2], Adam Thompson[1], Jeffrey C. Glennon[1].

[1]School of Medicine, UCD Conway Institute, University College Dublin, Dublin, Ireland. [2]Instituto Superiore di Sanita, Rome, Italy, 0016.

Insulin signalling was previously identified as a key network involved in Obsessive-Compulsive Disorder symptomology, with impaired reversal learning (ability to switch behaviour depending on changed stimulus-reward associations), selective attention and spontaneous alternation observed in TALLYHO/JngJ mouse models of Type 2 diabetes/obesity. Here, we aimed to establish the causative mechanisms through which aberrant insulin signalling impacts behaviour through proteomic analysis of TALLYHO/JngJ blood and brain. Significant pathways and predicted causative networks were identified using Ingenuity Pathway Analysis (Qiagen). Proteomic results from blood highlight immune-related mechanisms at play, particularly immune-associated complement signalling. Complement signalling forms a major part of the innate immune system responsible for lysis of pathogens. Analysis

of brain areas, specifically the nucleus accumbens (addiction, motivation) and anterior cingulate cortex (decision making) in TALLYHO/JngJ highlight a role of altered synaptogenesis and mitochondrial respiration, particularly oxidative phosphorylation. The Type 2 diabetes drug metformin was administered to TALLYHO/JngJ mice to assess its potential to rescue aspects of their behavioural phenotype. This showed a significant improvement in selective attention in these TALLYHO/JngJ mice, with a corresponding rescue of complement signalling components in blood, particularly C8, forming part of the immune membrane attack complex. Additionally, oxidative phosphorylation was elevated in brain following metformin administration, suggesting a rescue of mitochondrial function. These results indicate a role of aberrant immune and respiratory signalling as a causative mechanism for behavioural inflexibility in insulin resistant mouse models. Future studies will aim to untangle immune-respiratory crosstalks.

A novel role for FERM domain-containing protein 3 (FRMD3) in chronic kidney disease.

CIARÁN KENNEDY[1,2], ROSS DOYLE[1], OISIN GOUGH[1], CAITRIONA MC EVOY[1], KATALIN SUSZTAK[3], MATTHIAS KRETZLER[4], JOSE FLOREZ[5], ANTHONY DORMAN[6], FINIAN MARTIN[1], PETER J. CONLON[7,8], DENISE M. SADLIER[2], GENIE CONSORTIUM, EOIN BRENNAN[1,2], and CATHERINE GODSON[1,2].

[1]UCD Diabetes Complications Research Centre, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland. [2]School of Medicine, University College Dublin, Belfield, Dublin 4, Ireland. [3]Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [4]Department of Internal Medicine, Division of Nephrology, University of Michigan, Ann Arbor, Michigan, USA. [5]Broad Institute of MIT and Harvard, Cambridge, MA, USA. [6]Department of Pathology, Beaumont Hospital, Dublin, Ireland. [7]National Kidney Transplant Service, Department of Nephrology and Kidney Transplantation, Beaumont Hospital, Dublin, Ireland. [8]Royal College of Surgeons in Ireland, Dublin, Ireland. [9]Mater Misericordiae University Hospital, Eccles St., Dublin 7, Ireland.

Currently there are limited mechanisms to link current disease severity and risk of disease progression in Chronic Kidney Disease (CKD). To better understand this link, we interrogated the renal transcriptomic profile of CKD patients with measures of CKD severity and identified FERM-domain containing protein 3 (FRMD3) as a candidate gene for follow-up study.

RNA-seq was used to profile the transcriptome of CKD biopsies from two independent cohorts within the North Dublin Renal BioBank (NDRBB) (n=24 and N=17), the results of which were correlated against various clinical parameters. To explore the function of FRMD3, one of the candidate genes most strongly correlated with disease state, lentiviral transduction was used to both knock-down and overexpress FRMD3 in human renal proximal tubule epithelial cells (HK-2). Mass spectrometry was used to interrogate the interactome of FRMD3 in HK-2 cells.

We identified a subset of 93 genes which are significantly correlated with eGFR and %TIF at time of biopsy and with disease progression 60 months post-biopsy. One of the top-ranking genes from this subset, FRMD3, has previously been associated with the risk of developing DKD. Interrogating the interactome of FRMD3 in HK-2 cells revealed interactions with cytoskeletal components cell-cell junctions. Knockdown of FRMD3 in HK-2 cells resulted in an increase in pro-apoptotic activity within the cells as well as dysregulation of E-Cadherin.

To summarize, We have identified a panel of kidney-specific transcripts correlated with severity and progression of kidney disease, and from this have identified FRMD3 as a key player in tubule cell structure and health.

Development and evaluation of a novel biomarker-based risk stratification platform for obesity-related complications using targeted proteomics.

Rachel Byrne[1], Aleksandra Dudzik[1], Stephen Pennington[2], and Fiona McGillicuddy[1].

[1]Diabetes Complications Research Centre, School of Medicine, UCD Conway Institute, University College Dublin, Ireland. [2]School of Medicine, UCD Conway Institute, University College Dublin, Ireland.

Targeted mass spectrometry (T-MS) can be used for sensitive quantification of specific proteins in complex biological samples. By selectively monitoring predefined peptide ions, T-MS enables accurate measurement of protein abundance, aiding biomarker discovery.

MetHealth aims to utilize T-MS to measure proteins associated with high-density lipoprotein particles (HDL-P), to develop and validate a biomarker-based risk stratification platform for assessing metabolic health in individuals with obesity.

A high-throughput method for HDL isolation has been developed. Prototypic peptides assays for proteins of interest, related to metabolic health in people with/without obesity, were developed, selected based on literature searches and discovery proteomics experiments. The development and optimization of multiple reaction monitoring (MRM) peptide assays were performed using Skyline software (Version23.1; MacCoss Lab). MRM peptide analysis was performed using an Agilent 6490 triple-quadrupole MS with Jet-Stream electrospray source coupled to a 1290 Quaternary Pump HPLC system.

MRM assays encompassing 82 proteins, and 196 peptides have been developed. Preliminary reproducibility of HDL-P across multiple sample injections of the same HDL-P sample obtains a coefficient of variation (CV) <10%, indicating high reproducibility. The peptides associated with ApoA-1, the most abundant HDL-associated protein, have a CV <20%.

Our findings demonstrate that HDL-associated proteins can be measured on MRM assays with high reproducibility. Upon completion of preliminary validation experiments (within-day variability; stability over days; and intra/inter operator), patient's samples with/without obesity will be run to delineate high-risk people with obesity, who are likely to develop life-limiting complications, to low-risk people with obesity, who can maintain their metabolic health despite excessive adiposity.

Non-caloric sweeteners are associated with decreased sperm viability and morphology in male mice.

Michelle Kearns[1], Reynolds Clare[1], Sabine Koelle[2], and Caoimhe Neville[2].

[1]Conway Institute, [2]School of Medicine, University College Dublin, Dublin, Ireland.

Background: Infertility is global issue affecting approximately 15% of couples worldwide, with male infertility contributing up to 50% of cases. There is growing evidence that obesity can alter sperm parameters and negatively affect sperm function, cause sub-fertility in males, and induce epigenetic changes to spermatozoa, increasing the risk of metabolic dysfunction in offspring non-caloric sweeteners (NCS) are widely consumed owing to their intense sweetness while providing minimal or no energy, however their detectability in different body tissues, including the reproductive system is unclear. Limited rodent studies have shown high NCS intakes can reduce sperm function, however most studies to date have looked at excessive NCS consumption.

Objectives: The present study was carried out to evaluate the impact of modest NCS intakes, in combination with High fat diet (HFD) on sperm parameters and glucose tolerance in male mice.

Methods: 32 adult male mice were randomly assigned to a control diet (CD) (10% kcal fat) or HFD (45% kcal fat) and assigned to 8 groups of 4 mice each and received either water (control), fructose (20% solution in water) or NCS (Ace-k at a concentration of 12.5mM solution in water or rebaudioside a at a concentration of 1mM solution in water). Mice were weighed weekly, and food and solution

intakes were monitored for the duration of the study. At week 5, an oral glucose tolerance test (OGTT) was performed on mice fasted for 6 hours. Following 8 weeks, mice were humanely killed. Testes were weighed and sperm was isolated from the cauda epididymis and parameters, including sperm viability and morphology were evaluated. Sperm abnormal morphology and live viability were evaluated for at least 200 spermatozoa of each animal. The percentage of morphologically abnormal spermatozoa and viable sperm were assessed by Eosin and Eosin-Nigrosin staining respectively and imaged using light microscopy.

Results: Following 8 weeks on the diet, HFD mice gained significantly higher body weight than CD mice ($p<0.001$) with a mean body weight of 30.1g ±1.0 for CD and 33.7±1.4 for HFD mice. Following NCS consumption, mice had increased abnormal sperm morphology (Ace-k 68.0± 9.2, Reb-a 78.3±10.3) compared to CD fed mice (CD control 59.6±6.8, HF control 64.2±9.0). Sperm viability decreased in CD NCS groups (Ace-k 27.9±1.1, Reb-a 39.9±11.2) compared to the CD water group (43.2±8.5), however sperm viability increased in both HFD NCS mice (Ace-k 38.1±0.6, Reb-a 46.6±4.0) compared to CD NCS fed mice. There was no significant difference in testes weight between the groups.

HFD-control fed mice had a significantly higher glucose AUC compared to CD-control fed mice (CD Control 1393±102, HF Control 1937±177).

Conclusion: This study has shown that NCS consumption reduced sperm parameters in male mice**.** Furthermore, consumption of a HFD had a protective effect on sperm viability in mice consuming artificial sweeteners compared with control and fructose groups. HF Control fed mice had significantly increased AUC for glucose compared to CD Control mice. Our results indicate that modest consumption of artificial sweeteners may induce negative effects on sperm parameters in mice, however these negative effects on sperm viability may be reduced with the addition of a high fat diet.

POSTER ABSTRACTS

Poster 1

Generating induced pluripotent stem cells from urine-derived epithelial cells.

Jessica Lauren Davis[1], Sara Knezevic[1,2], Ciara McMahon[1], and John Crean[1].

[1]School of Biomolecular and Biomedical Science, University College Dublin. [2]School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons.

Induced pluripotent stem cells (iPSCs) are derived from somatic cells that have been reprogrammed back into a pluripotent state using the ectopic expression of defined embryonic transcription factors. The most important advantage of iPSCs is the possibility to use mature somatic cells from patients who suffer from genetically defined diseases, opening up the possibility to characterize specific phenotypes in patient derived cells. To date fibroblasts are still the most commonly used primary cell source and are established from a skin punch biopsy, however this method is very painful and the cells are often slow to reprogram. Urine epithelial cells (URECs) are an attractive alternative as they have fewer genetic alterations then iPSCs derived from skin and have higher levels of reprogramming efficiency. We collected urine from several donors and isolated and characterised the subsequent URECs. Next, we reprogrammed the URECs using episomal plasmids for OCT4, SOX2, L-MYC, and KLF4. After 15 days, newly reprogrammed iPSC colonies were observed and clones were selected and

amplified. Characterisation of iPSCs by immunocytochemistry revealed clones expressed Nanog, SOX2, SSEA4 and TRA-1-60. UiPSCs were successfully differentiated into kidney organoids.

Poster 2

Modelling therapeutic responses to statins using iPSC-derived human blood vessel organoids.

Tanwi Vartak[1,2], Jessica Davis[1,3], Niall Treacy[1,3], Dermot Brougham[4], John Crean[1,3], and Eoin Brennan[1,2].

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Aim: Recently, efforts have been made to establish induced pluripotent stem cell (iPSC) derived self-organizing human capillary blood vessel organoids (BVOs) as robust models that enumerate key processes of vasculogenesis and angiogenesis. This study investigated if we could model tumor necrosis factor-alpha (TNF-α) activation seen in human vascular disease in BVOs and assessed whether BVOs can be exploited as a tool to determine therapeutic responses to atorvastatin.

Methods: HPSI1213i-babk_2 iPSCs were subjected to BVO culture protocols using the STEMdiff™ BVO Kit for 14-22 days. BVOs were stimulated with TNF-α (1-100ng/ml; 24h) and Atorvastatin (1-5uM; 24h). BVOs were investigated by qPCR and immunostaining [smooth muscle cell marker ACTA2; pericytes (PDGFR-β); endothelial tubes (PECAM1, VEGF); vascular basement membrane (Col IV)].

Results: Confocal imaging showed the presence of endothelial tubes (PECAM1/CD31+), smooth muscle cells (ACTA2+), and basement membrane (Collagen IV+) within the BVOs. BVO dose-response experiments of TNF-α (1-100ng/ml) and atorvastatin (1-5μM) were performed to determine optimal concentrations. TNF-α (100ng/ml; 24h) evoked a significant upregulation of inflammatory genes ICAM1, VCAM1, IL-6, TNFRSF1A as well as ACTA2, PDGFR-β, PECAM1 and VEGF (*$p<0.05$). BVO treatment with atorvastatin (5μM; 24h) significantly attenuated TNF-α-mediated effects on ICAM1, VCAM1, ACTA2, PDGFR-β and VEGFA genes (*$p<0.05$), suggesting that BVOs are amenable to investigating drug responses. RNA-seq transcriptomics studies are ongoing to characterize BVO global responses to statins.

Conclusions: BVOs express markers of endothelial and vascular smooth muscle cells and are amenable to investigating therapeutic responses to drugs. Further studies are required to clarify how closely BVOs recapitulate human blood vessels.

Poster 3

The HDL proteome represents a novel, sensitive biomarker of metabolic inflammation and metabolic health in obesity that can track both disease progression and regression.

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Not available.

Poster 4

Investigation of the role of Teneurin Transmembrane Protein 2 (TENM2) in podocyte dysfunction in diabetic kidney disease.

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Background and Aims: It is known that familial aggregation of diabetic kidney disease (DKD) occurs independent of known risk factors. This highlights the influence of undiscovered heritable risk factors for DKD. To address this, the GENIE consortium (**Ge**netics of **N**ephropathy - an **I**nternational **E**ffort) have performed genome-wide association studies (GWAS) of type 1 diabetes individuals of European ancestry with and without DKD and discovered several promising genetic loci reaching genome-wide significance, resulting in the identification of a novel intronic variant (rs72831309) in *tenm2* (Teneurin Transmembrane Protein 2). Integration of renal biopsy omics data confirmed that *tenm2* expression correlates positively with eGFR and negatively with tubulointerstitial fibrosis. **Building on these findings, this project aims to characterise the role of TENM2 in DKD.**

Methods: Conditionally immortalised human podocytes (hPODs) were maintained at 33°C and transferred to 37°C to induce differentiation for 14 days. hPODs were treated with PDGF and TNF-α ± (10ng/ml; 24h). hPODs were transfected using RNAiMAX and Qiagen siRNA pools targeting *tenm2* (20nM; 24h). Markers of podocyte function and inflammatory pathway activation were assessed by qPCR.

Results: Using publicly available renal biopsy scRNA-seq data (Humphreys Lab) we determined that podocyte cells are the primary expression source of *tenm2*. hPOds were cultured and characterized to confirm the expression of established podocyte markers (NPHS1, PODXL, CD2AP). To induce hPOD injury, cells were treated with PDGF and TNF-α ± (24h), and markers of podocyte function and inflammatory pathway activation were determined. To model the observations of reduced TENM2 expression in DKD, we performed siRNA knockdown experiments of TENM2 in hPODs (20nM; 24h). Studies are ongoing to determine the efficiency of knockdown and whether loss of TENM2 expression in hPODs leads to cellular dysfunction.

Conclusions: Integration of genetic and transcriptomics data have identified TENM2 as a novel candidate gene associated with DKD. Studies are ongoing to clarify its role in the progression of this complication of diabetes.

Poster 5

Glucose-lowering Drugs for the Treatment and Prevention of Diabetes-associated Atherosclerosis.

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Introduction: In recent decades, type-2 diabetes has become increasingly common, particularly in younger individuals. Diabetes leads to micro- and macro-vascular complications, including atherosclerosis.

Atherosclerosis is a cardiovascular disease characterised by lipid-rich plaques within the vasculature. These plaques develop overtime, occluding blood flow; and can give rise to major adverse cardiovascular events, including myocardial infarction and stroke.

Overarching Aim: We aim to investigate the effect of using GLP-1 receptor agonists (e.g., Liraglutide and Semaglutide) on THP-1-derived macrophages to assess the anti-atherosclerotic effect of these medications on an in-vitro cell model of diabetes-associated atherosclerosis.

Specific Aim: Initial experiments involved optimising conditions for the THP-1-derived cell model of diabetes-associated atherosclerosis. Firstly, cells exposed to low (5mM), and high (25mM) glucose environment were cultured in serum-free (0%), serum-starved (0.1%) and high-serum media (10%) to explore the effect of serum on glucose uptake over 48-hours.

Results: Transcriptomic analysis was conducted to evaluate the expression of glucose transporters (GLUT-1 and GLUT-4) under the different conditions. Serum-starvation and serum-restriction decreased GLUT-1 and GLUT-4 expression under high glucose conditions.

Conclusion: Glucose-transporter expression, which is a key modulator of the monocyte-macrophage inflammatory response is regulated by serum and glucose concentrations. In serum-free/ -starved and high glucose conditions THP-1 cells are less metabolically active and hence downregulate glucose-transporters. Conversely, low glucose and low serum conditions leads to enhanced glucose-transporter expression. Therefore, conventional 10% serum may influence the expression of key genes and proteins, and hence serum-restricted media may be superior to model the in-vivo effects of hyperglycaemia in diabetes-associated atherosclerosis.

Poster 6

Characterisation of iPSC-derived retinal organoids by single cell transcriptomic analysis reveals their potential to model disease.

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"i) The failure of many therapies to translate effectively from pre-clinical animal models to human patients may be attributed, in large part, to the fundamental species differences that exist between humans and other animals (Huch et al., 2017). This is particularly apparent in the field of retinal research and has highlighted the need to develop an ex vivo human model.

ii) Retinal organoids (ROs) were differentiated from human induced pluripotent stem cells (hiPSCs) using an optimised protocol adapted from Chichagova et al. (2019) over a period of 150 days. ROs were stimulated with TGFβ2 for a period of 7 days before undergoing dissociation and being subjected to single cell RNA sequencing (scRNAseq). ROs were also characterised by qPCR at various timepoints during differentiation, as well as by immunofluorescent imaging.

iii) Analysis of scRNAseq results is currently still ongoing, but preliminary results indicate that that iPSC-derived ROs develop into all of the major retinal cell types, organised in the same manner as in the native retina. This has been demonstrated with positive staining for retinal markers such as CRX, HUC/D, NRL and RXRγ by confocal microscopy and by the expression of key developmental genes indicating the presence of retinal ganglion cells (ATOH7), amacrine cells (GAD1), photoreceptor precursor cells (CRX).

These results indicate that iPSC-derived ROs produced using this protocol display many similarities to the native human retina, pointing to their suitability for use in modelling retinal disease. Further analysis of TGFβ2-treated ROs will provide more insight into their ability to model a disease state.

Poster 7

The FPR-2 agonist AT-01-KG attenuates polymicrobial sepsis and reduces cardiac and renal inflammation.

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The failure to resolve an inflammatory response underpins numerous prevalent pathologies and may lead to fibrosis, scarring and organ failure. We have identified endogenous mediators that drive the resolution of inflammation and developed synthetic mimetics including the small molecule AT-01-KG. Polymicrobial sepsis is a life threatening condition reflecting exuberant, uncontrolled inflammation. We have investigated the potential of AT-01-KG to modulate pathologic responses in the murine caecal ligation and puncture [CLP] model. Treatment with 2 ug/kg 1 hour post CLP injury was associated with significant attenuation of systemic and end organ [cardiac and renal] inflammation and injury. AT-01-KG significantly reduced phospho-ERK1/2 in kidney and phospho-AKT in the heart. The molecular target of AT-01-KG is FPR2, a GPCR, interestingly we did not find protective responses to AT-01-KG in CLP-induced polymicrobial sepsis in FPR2 -/- mice. The immunomodulatory role of AT-01-KG was supported by a reduction in blood and peritoneal bacterial load. Importantly, addition of AT-01-KG [1x10-10 M] to human whole blood significantly enhanced bacterial killing suggesting the therapeutic potential of the compound. These data from murine and human models support the resolution of inflammation as a tractable target in complex pathologies which may support antibiotic and steroid-sparing regimens.

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Poster 8

Utilising mechanically tuneable gelatin methacryloyl (GelMA) hydrogels to investigate the effects of stiffness on the maturation of hiPSC-derived kidney organoids.

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Human induced pluripotent stem cell (hiPSC) derived kidney organoids show remarkable similarity to the human kidney in terms of histology and gene expression. In recent years, our group has utilised kidney organoids to model diabetic nephropathy using kidney organoids and integrated single-cell multi-omic sequencing. However, kidney organoids produced using current state of the art methods resemble the first or second trimester fetal kidney. Our work with mechanically tuneable gelatin methacryloyl (GelMA) hydrogels as support matrices for kidney organoids aims to improve the generation of kidney organoids from hiPSCs through controlled modifications of biophysical parameters such as stiffness.

We use GelMA hydrogels to study the effects of stiffness on kidney organoid maturation by encapsulating developing organoids in GelMA hydrogels of differing mechanical strength (defined by the storage modulus G'). We have shown through immunofluorescence and electron microscopy that kidney organoids grown within GelMA hydrogels contain all the major components seen in the human kidney and form segmented nephron structures with podocytes containing foot processes and proximal tubules with open lumen and extensive brush borders.

Results from previous single cell transcriptomic studies and qPCR suggest an improvement in the maturation of podocytes as well as other glomerular components in kidney organoids grown within hydrogels where the storage modulus is closer to that of the adult kidney (G'=5-8kPa) compared to softer hydrogels that more closely mimic the mechanical microenvironment of the gastrulation stage embryo (G'=0.4-1kPa). Recent work in this project has looked at how light sheet fluorescence microscopy (LSFM) can be used to study the effects that a variable G' (stiffness) has on the derivation of the different renal cell types present within kidney organoids. These investigations have uncovered differing morphologies of developing CD31+ endothelial structures in kidney organoids matured in soft (G'~0.4kPa) or stiff (G'~6kPa) GelMA hydrogels.

Overall, our work points towards the idea that modification of simple biophysical such as matrix stiffness can be used as an instructive cue to develop more physiologically authentic kidney organoids which can then serve as 3D multicellular models of disease or drug development.

Poster 9

Biomarkers of Wasting and low Skeletal muscle index in a gastrointestinal cancer cohort.

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Not available.

Poster 10

Determining the extent to which innate immune training can be induced in monocytes from older individuals.

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Not available.

Poster 11

The KCNQ1 channel controls behavioural flexibility via regulation of mitochondrial respiration.

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The voltage-gated potassium channel KCNQ1 is expressed in tissues including brain, heart and gut, and governs processes including neuroexcitability and insulin secretion. Recent analyses of Obsessive-Compulsive Disorder GWAS studies have identified insulin signalling as a candidate mechanism and KCNQ1 as a highly significant candidate gene. Here, we aimed to further examine the precise behaviours impacted by KCNQ1 action, and the mechanisms through which it acts. We first conducted behavioural tasks in a mouse model of a full-body KCNQ1 knockout. Additionally, mass spectrometry proteomic analysis was conducted in blood and brain to identify signalling mechanisms at play. Electrophysiological analysis of the hippocampal field postsynaptic potential (fEPSP) following hypoxia and long-term potentiation (LTP) was also carried out. KCNQ1 knockout mice showed significantly increased compulsive circling behaviour and tics ($p<0.001$), in addition to reduced spontaneous alternation, a reflection of behavioural flexibility, in a Y-maze ($p<0.01$). KCNQ1 knockout mice also showed significantly impaired spatial learning and memory in a Barnes maze relative to controls ($p<0.01$). Interestingly, KCNQ1 knockout mice spend significantly more time in the centre of an open field apparatus, indicating impaired environmental sensitivity. Mass spectrometry analysis of brain regions shows a consistent signature of mitochondrial dysfunction, particularly with disrupted oxidative phosphorylation. Following hypoxia, KCNQ1 knockout hippocampal slices were slower to recover fEPSP than wildtype, with overall reduced LTP. To conclude, KCNQ1 is a strong regulator of compulsive behavioural patterns, potentially via regulation of mitochondrial bioenergetics. Additionally, this channel is a mediator of neuronal resistance to hypoxic stress and appropriate LTP.

Poster 12

PRRX1 as a master regulator of fibroblast identity and of myofibroblast activation in kidney disease.

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Not available.

Poster 13

Investigating the mechanisms of efferocytosis-mediated inflammation resolution.

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Not available.

Poster 15

Screening synthetic FRP2 agonists for pro-resolving activity using a novel real-time imaging-based efferocytosis assay.

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Efferocytosis is the process through which apoptotic cells are cleared via phagocytic engulfment, primarily by professional phagocytes such as macrophages. This process is vital for maintaining tissue homeostasis and occurs in every major organ. The failure to adequately clear apoptotic cells results in the release of intercellular contents leading to tissue damage and persistent inflammation. FPR2 agonists such as lipoxin (LX) have been previously shown to enhance macrophage efferocytosis. Our group has previously demonstrated that synthetic LX mimetics phenocopy many of the anti-inflammatory and pro-resolving actions of LX, however whether these mimetics can also enhance macrophage efferocytosis remains unexplored. To investigate efferocytotic uptake by macrophages in response to stimulation with synthetic FPR2 agonists, human neutrophil line (HL60) cells were rendered apoptotic with 1uM staurosporine for 6 hours, stained with pHrodo red (pH sensitive dye) and added to monocyte derived macrophages (THP-1 cells treated with PMA) which had been pre-treated for 30 mins with FPR2 agonists (LX and synthetic LX mimetics). Efferocytotic uptake of apoptotic neutrophils by macrophages was monitored using the Incucyte S3 live-cell analysis system by imaging the cells every 30 mins for 6 hours. Efferocytosis was quantified using the Incuyte's in-built Cell by Cell analysis software. The synthetic FPR2 agonist AT-KG-01, but not AT-02-CT, enhanced macrophage efferocytosis. These findings suggest that synthetic FPR2 agonists have pro-resolving activity and reveal new mechanistic insights into the effect of the agonists on macrophages.

Poster 16

Multiomic integration of ScRNA_seq and ScATAC_seq to characterize transcriptional and enhancer drivers of Chronic Kidney Disease.

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Dysregulation of TGFβ1 signaling is implicated in metabolic diseases such as Chronic Kidney Disease (CKD). TGFβ1 plays a central role as a pro-fibrotic factor and is key in the development and progression of CKD. Multiomic integration of ScRNA-seq and ScATAC-seq of induced pluripotent stem cells (IPSC) provides insights into cell heterogeneity and chromatin regulators involved in the progression of CKD. Using iPSC-derived kidney organoids, the Crean lab has shown that this differentiation is a TGFβ1/Smad3-driven phenomenon and that Smad3 and PRC2 co-occupy the genome, with specific co-enrichment at putative enhancers and Super-enhancers (SE). Enhancers are cis-regulatory (cREs) regions in the DNA that augment the transcription of associated genes and play a key role in cell-type-specific gene expression. This project aims to link fibrotic genes uncovered using ScRNA-seq to super-enhancers that regulate the underlying gene expression networks underpinning kidney fibrosis. Transcription factors (TF) typically regulate gene expression by binding cREs known as enhancers by recruiting coactivators and RNA polymerase II (RNA Pol II) to target genes. CRISPR cas9 knockdown of SE-associated fibrotic gene drivers in CKD will disrupt the expression of fibrotic genes. This will provide more insights into future drug development for kidney fibrosis.

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