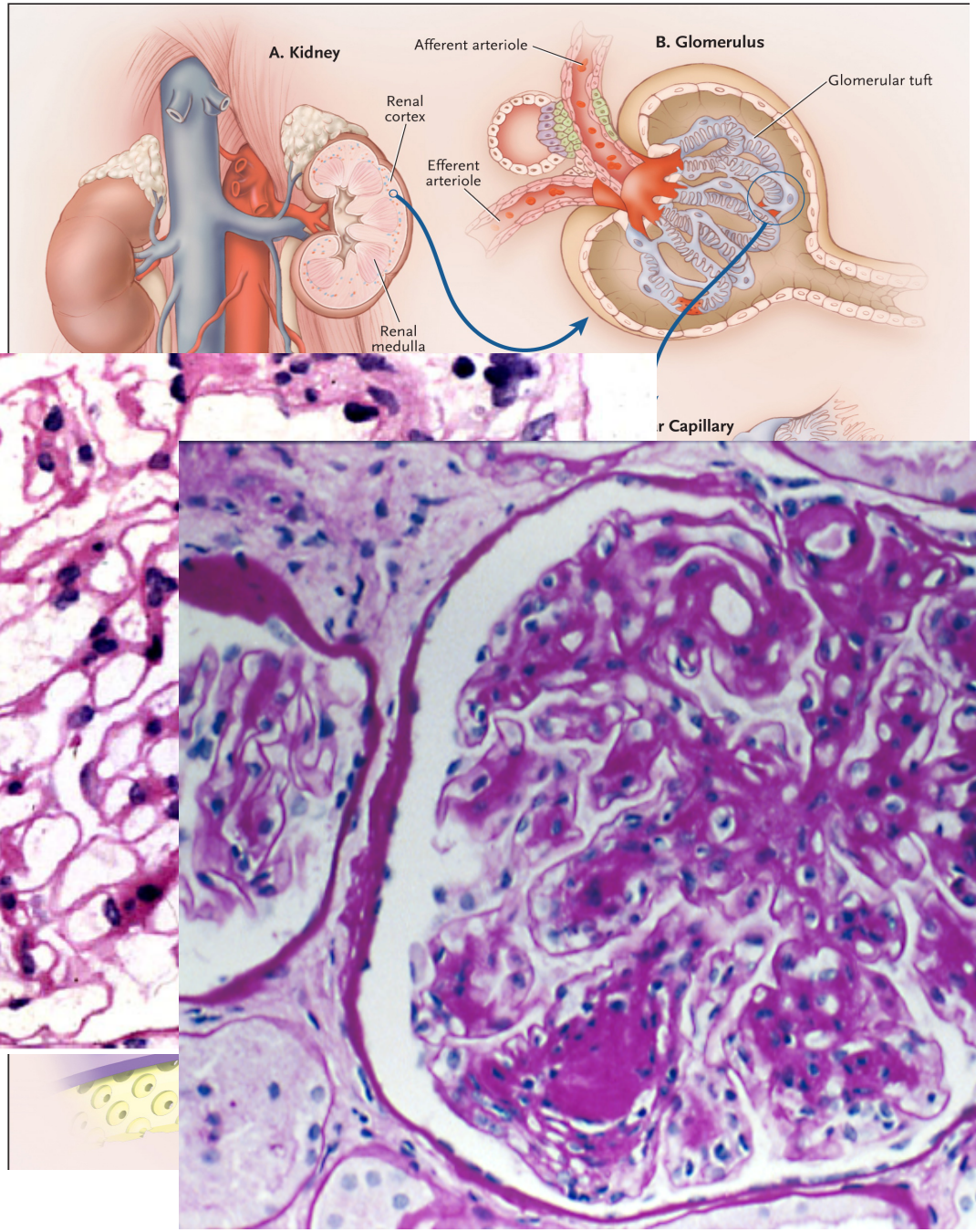
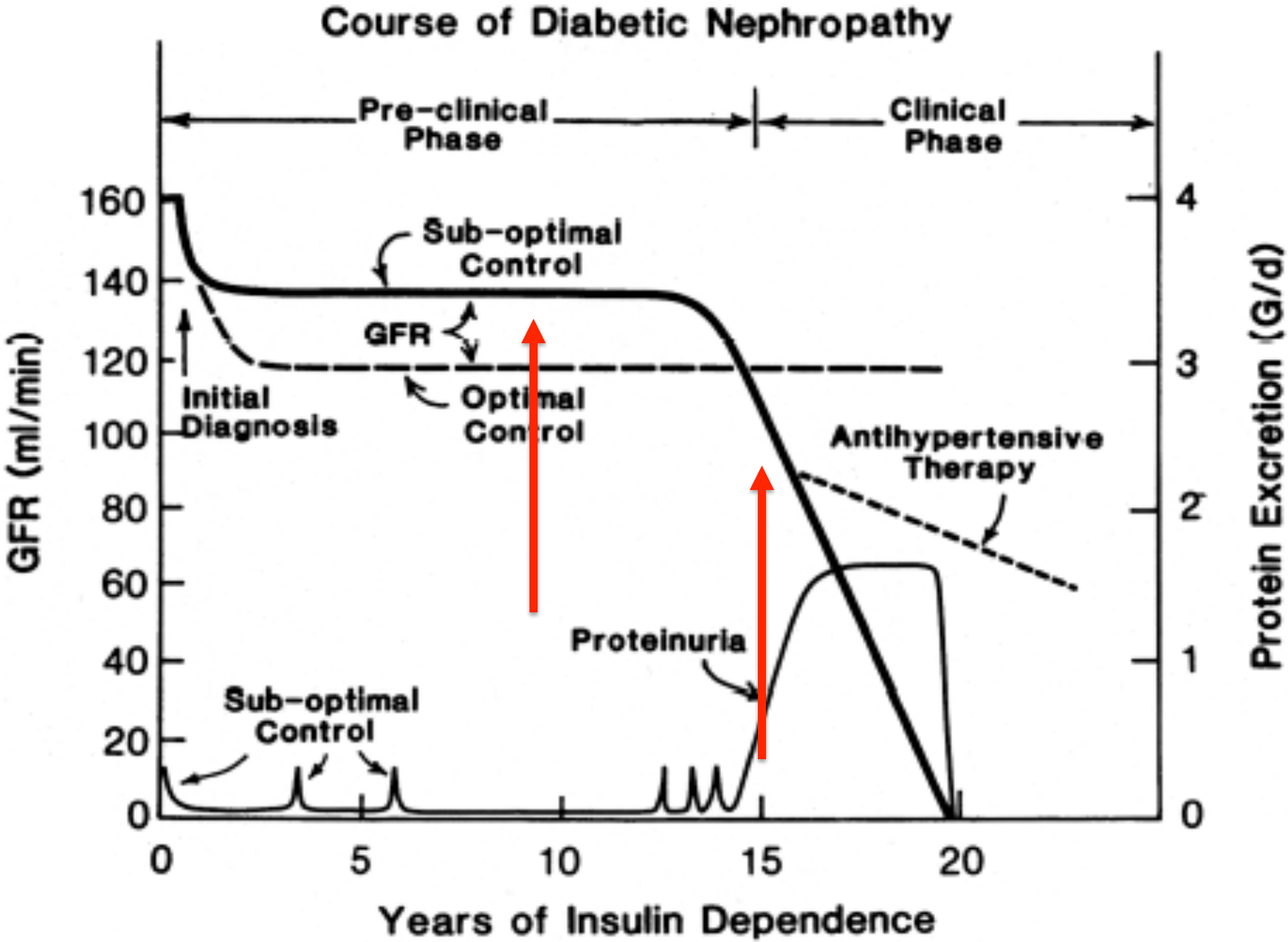
DYNAMO: Taking on the Challenge of Diabetic Nephropathy

Thomas Coffman, M.D.

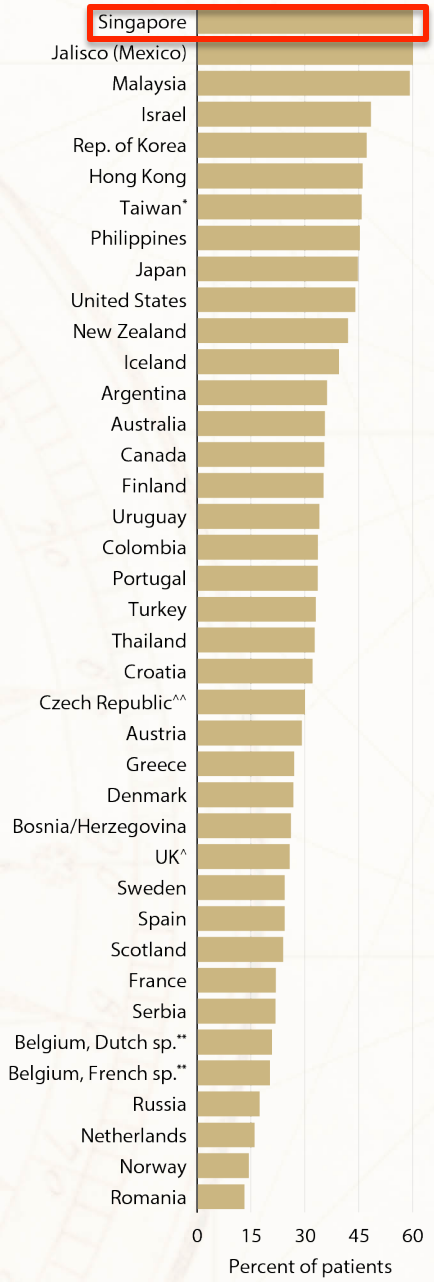
Dean, Duke-NUS Medical School


A. Kidney
Renal cortex
Renal medulla
Afferent arteriole
B. Glomerulus
Glomerular tuft
Efferent arteriole
r Capillary


Course of Diabetic Nephropathy
Pre-clinical Phase
Clinical Phase
Sub-optimal Control
GFR
Initial Diagnosis
Optimal Control
Antihypertensive Therapy
Proteinuria
Sub-optimal Control
GFR (ml/min)
Protein Excretion (G/d)
Years of Insulin Dependence
160
140
120
100
80
60
40
20
0
4
3
2
1
0
0
5
10
15
20

Percentage of incident patients with ESRD due to diabetes, 2011

Figure 12.4 (Volume 2)



Data presented only for countries from which relevant information was available. All rates unadjusted. ^UK: England, Wales, & Northern Ireland (Scotland data reported separately). *Latest data for Taiwan are from 2010. ^^ Czech Republic: Data on incident ESRD due to diabetes is an estimate. Data for France include 25 regions in 2011. .

Unmet Needs in Diabetic Nephropathy

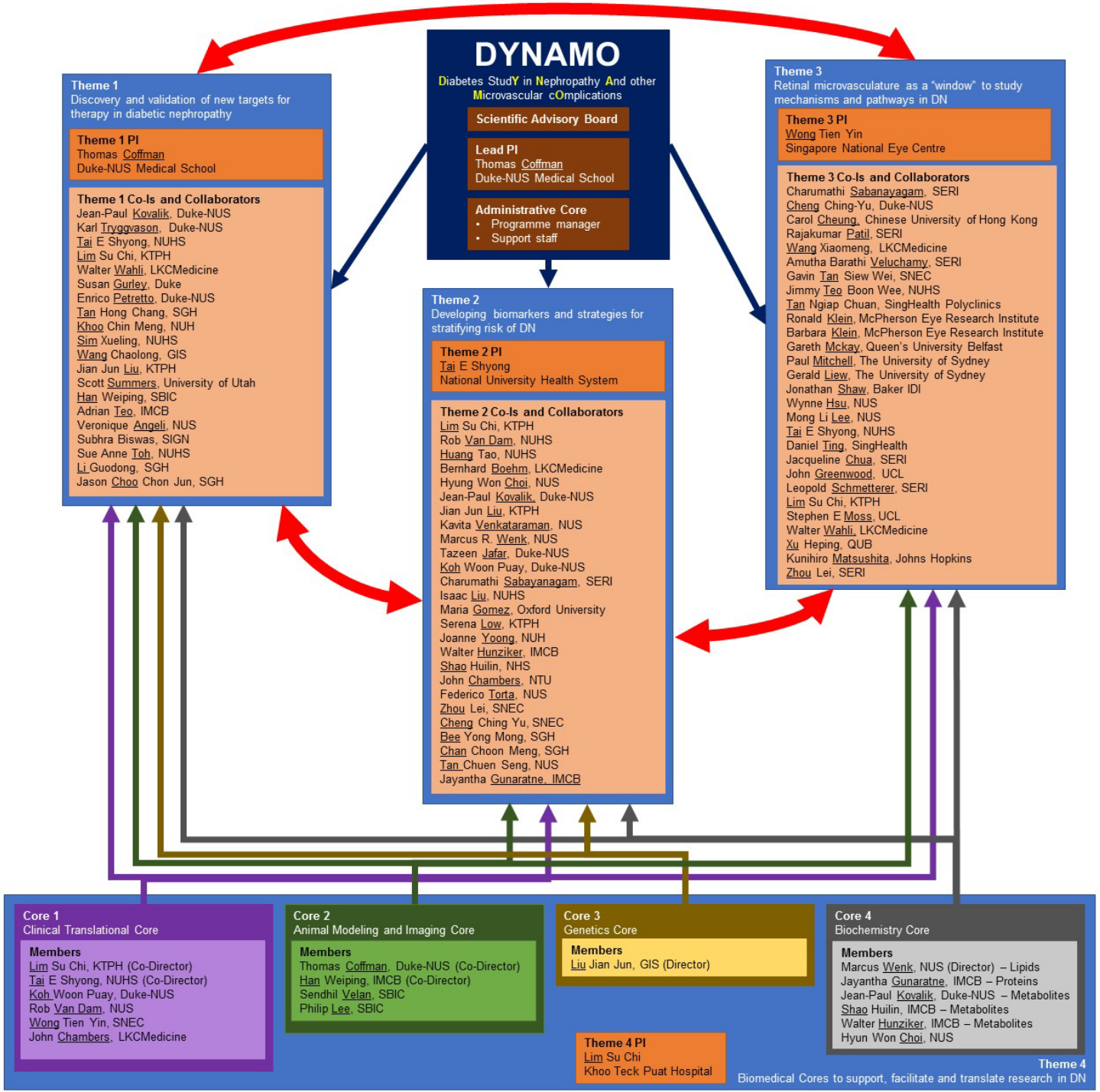
- Pathogenesis?
- Clinical strategies and/or biomarkers for early identification of susceptible patients
- More effective therapies to cure or regress kidney disease
- Sensitive and precise approaches for assessing progression and treatment efficacy
- Unexplained cardiovascular risk
- Poor understanding of genetic susceptibility mechanisms

DYNAMO

Diabetes stud**Y** in **N**ephropathy **A**nd other **M**icrovascular c**O**mplications

"The overall goal of this LCG proposal is to:

- ***address major unmet needs by identifying new mechanistic pathways in DN***
- ***discovering and validating potential new targets for treatment, and***
- ***defining novel biomarkers and strategies allowing early stratification of risk for DN within the larger population of people with type 2 diabetes"***


DYNAMO
Diabetes StudY in Nephropathy And other Microvascular cOmplications
Scientific Advisory Board
Lead PI
Thomas Coffman
Duke-NUS Medical School
Administrative Core
• Programme manager
• Support staff
Theme 1
Discovery and validation of new targets for therapy in diabetic nephropathy
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Duke-NUS Medical School
Theme 1 Co-Is and Collaborators
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Karl Tryggvason, Duke-NUS
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Developing biomarkers and strategies for stratifying risk of DN
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Cheng Ching Yu, SNEC
Bee Yong Mong, SGH
Chan Choon Meng, SGH
Tan Chuen Seng, NUS
Jayantha Gunaratne, IMCB
Theme 3
Retinal microvasculature as a "window" to study mechanisms and pathways in DN
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Paul Mitchell, The University of Sydney
Gerald Liew, The University of Sydney
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Mong Li Lee, NUS
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Jacqueline Chua, SERI
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Kunihiro Matsushita, Johns Hopkins
Zhou Lei, SERI
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Sendhil Velan, SBIC
Philip Lee, SBIC
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Genetics Core
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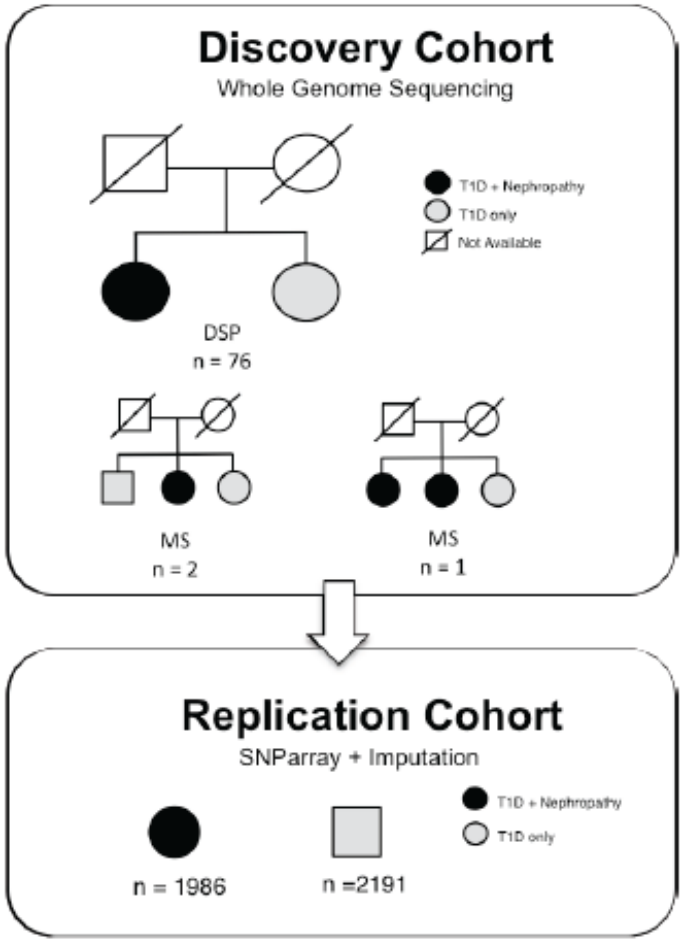
Liu Jian Jun, GIS (Director)
Core 4
Biochemistry Core
Members
Marcus Wenk, NUS (Director) – Lipids
Jayantha Gunaratne, IMCB – Proteins
Jean-Paul Kovalik, Duke-NUS – Metabolites
Shao Huilin, IMCB – Metabolites
Walter Hunziker, IMCB – Metabolites
Hyun Won Choi, NUS
Theme 4 PI
Lim Su Chi
Khoo Teck Puat Hospital
Theme 4
Biomedical Cores to support, facilitate and translate research in DN

PROJECT 1.2. Genetic causes of DN in Singaporeans and identification of drug targets.

Karl Tryggvason
Enrico Petretto
Jing Guo



Discovery cohort

criteria for cases:

(i) patients with overt persistent proteinuria (AER≥300 mg/24 hours or ACR >30 mg/mmol)

(ii) were on dialysis

(iii) have been kidney transplanted or

(iv) had died from kidney disease

Cases also had retinopathy, but no clinical or laboratory evidence of non-diabetic renal or urinary tract disease

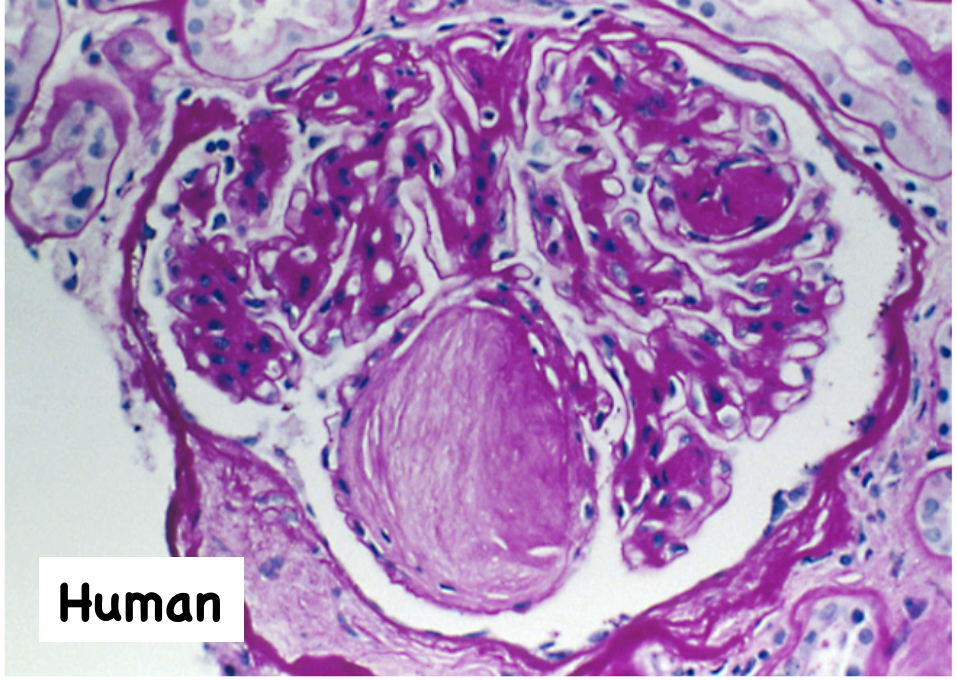
criteria for (family) controls:

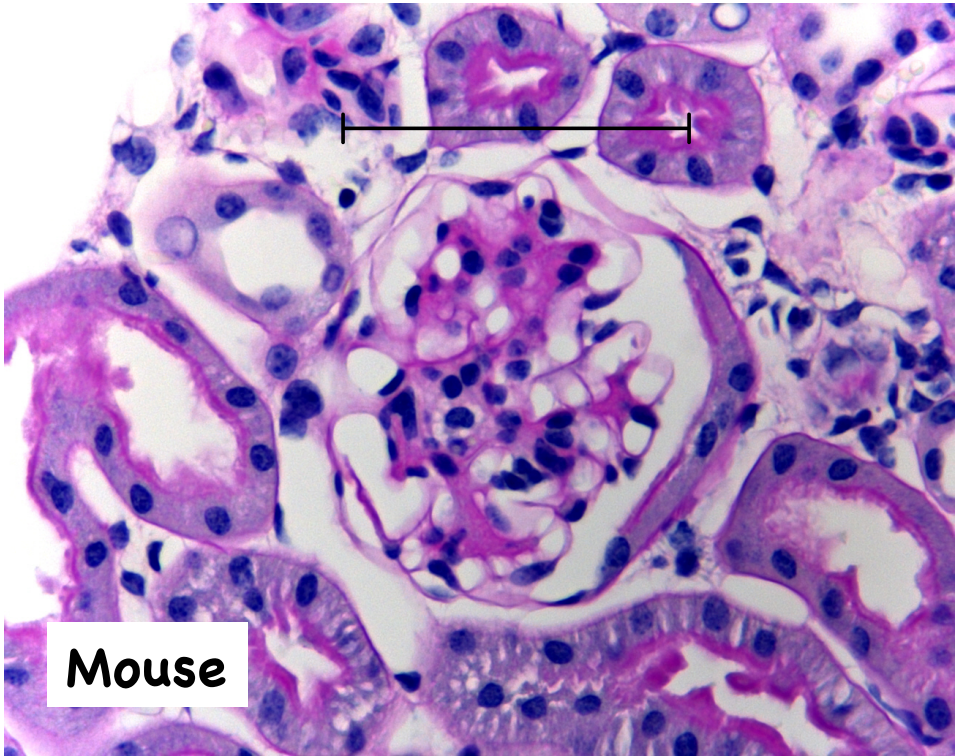
T1D for over 15 years at the initiation of the study in the late 1990s, so they have presently had diabetes for over 30 years without developing nephropathy and they have never been treated with ACE inhibitors or angiotensin receptor blockers (ARBs)

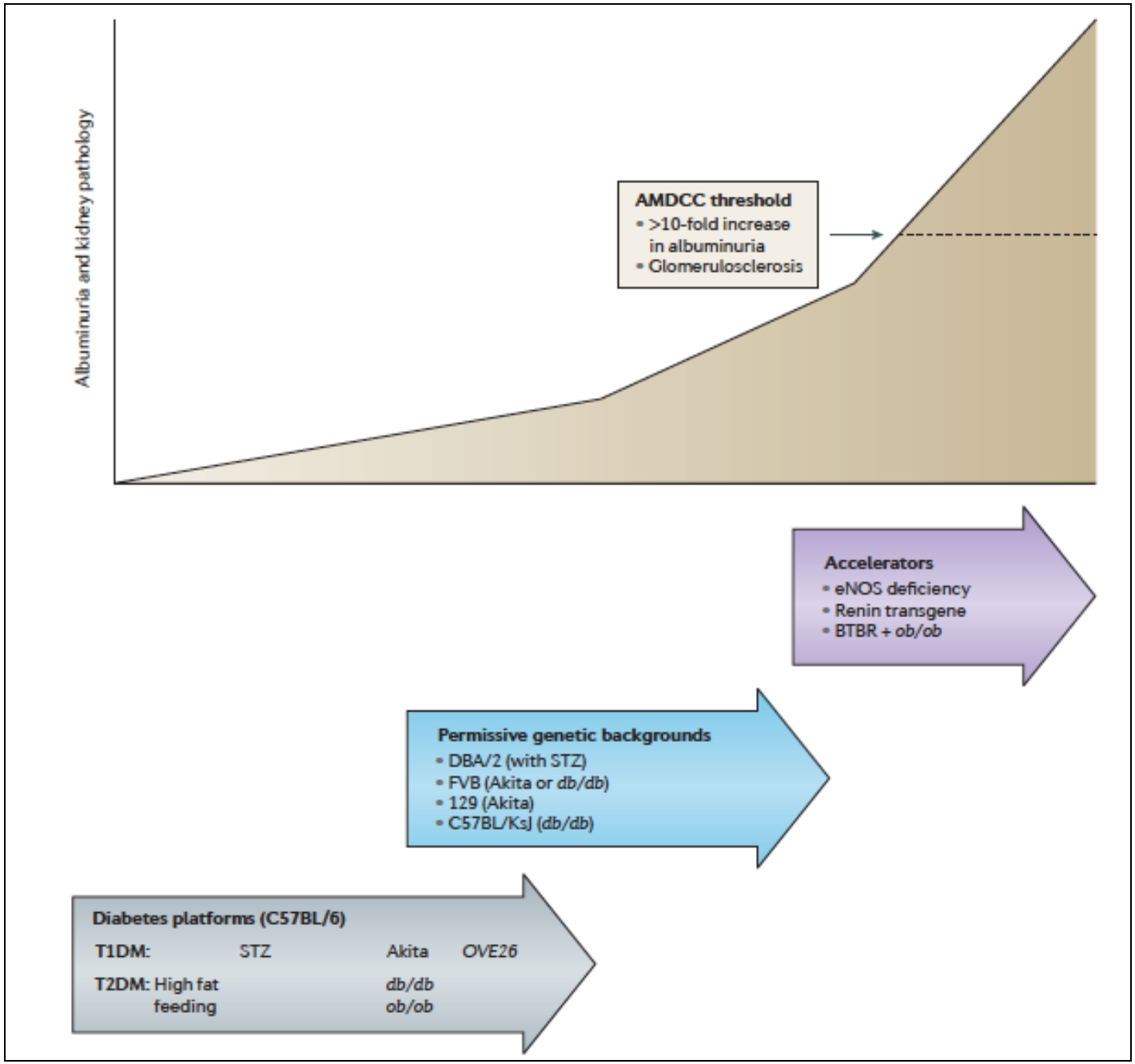
Guo et al.

PROJECT 1.3. Mechanisms of Genetic Susceptibility to DN

Thomas Coffman
Susan Gurley
Kengo Azushima


Human


Mouse

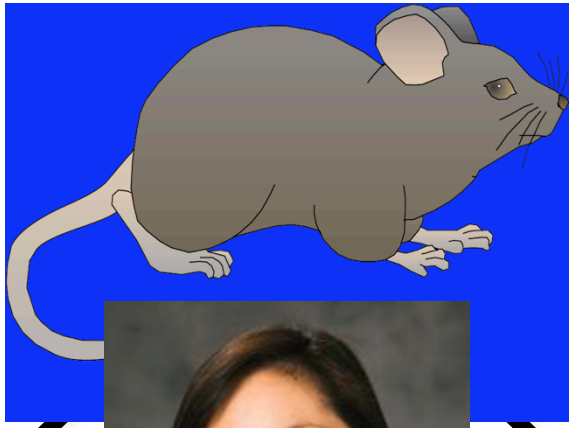


Azushima et al. *Nature Rev Nephrology* 14:48-56 , 2018

"Accelerated" Model of DN

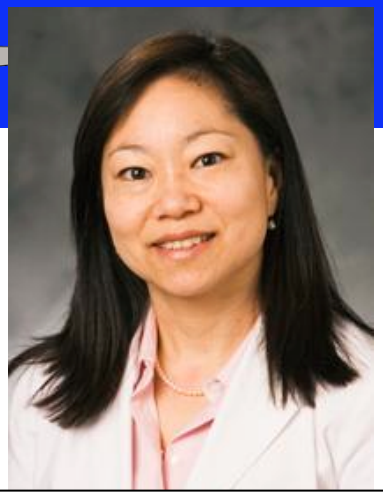
- **Akita ($Ins2^{+/C96Y}$) T1DM platform**
- **Single-copy mouse renin transgene targeted into the *Apoa1/Apoc3* locus (Caron K, et al. *PNAS* 99:8288)**
- **Constitutive expression of renin under control of the albumin promoter**

Generating Inbred *Ins2*$^{+/C96Y}$-*Renin Tg*$^{+}$ Lines

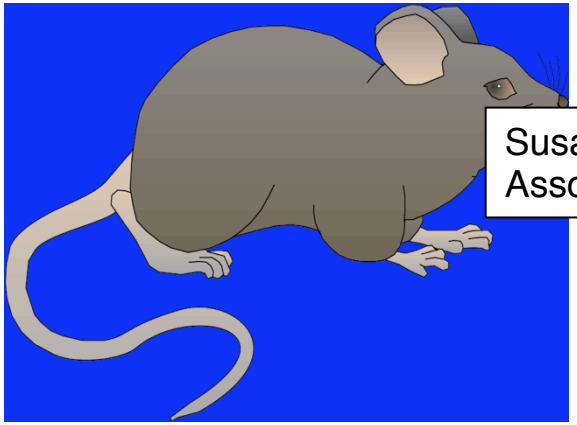


Back Cross With C57BL/6

Back Cross With 129/SvEv



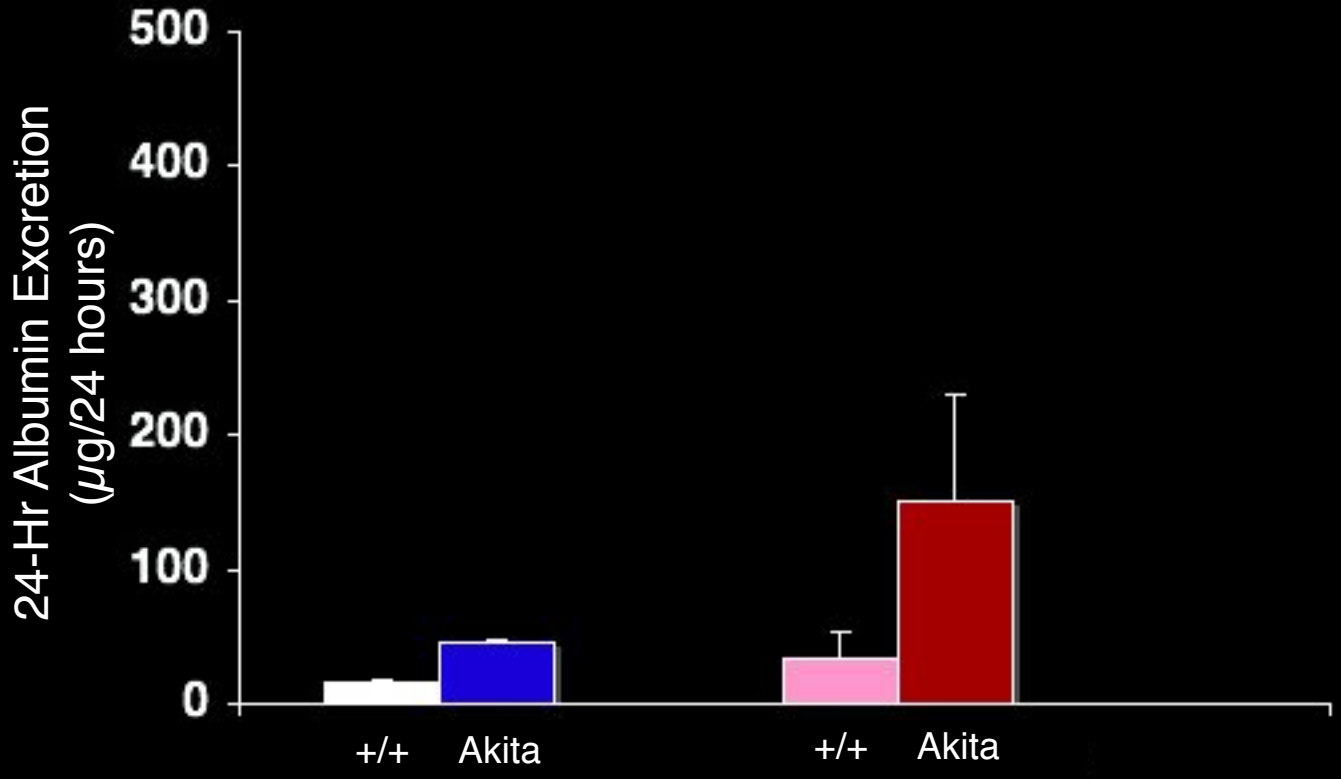
Susan Gurley, MD PhD
Associate Professor of Medicine



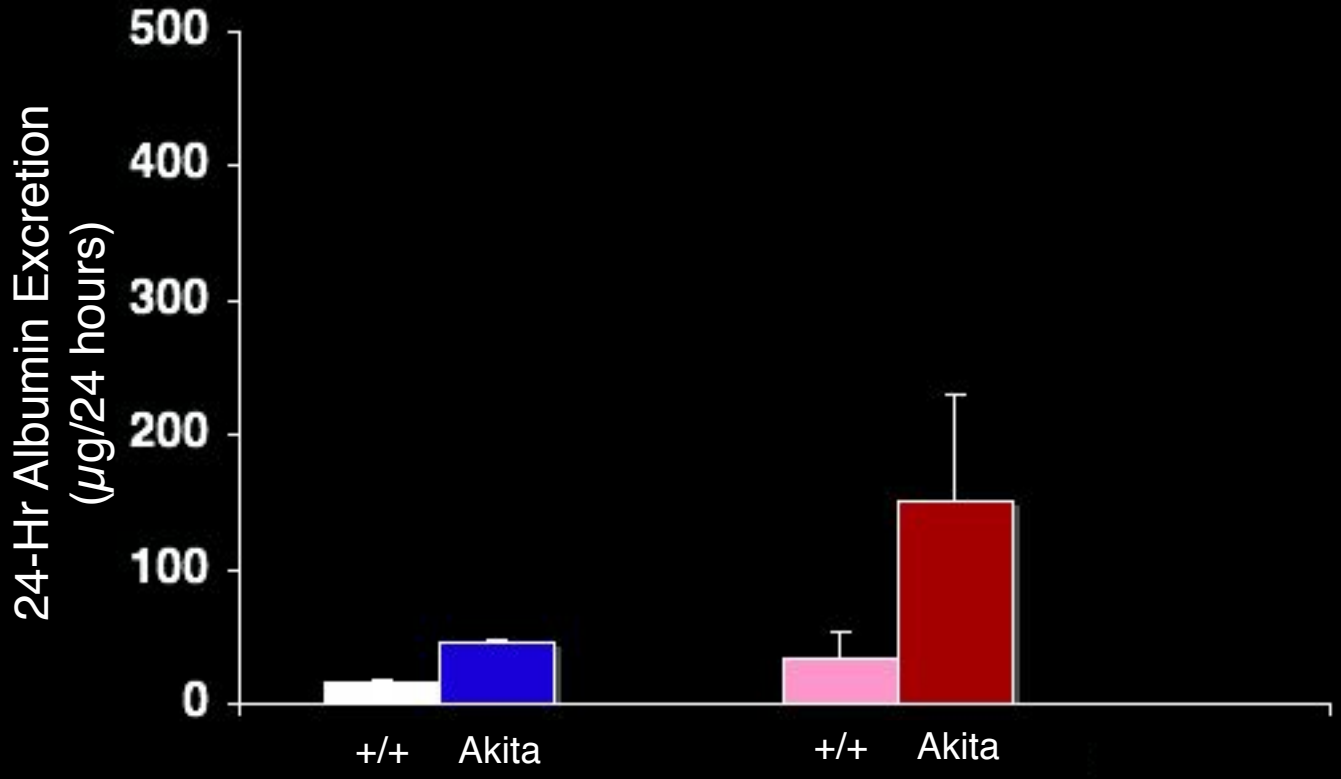
C57BL/6*Ins2*$^{+/C96Y}$-*ReninTg*$^{+}$

129/SvEv*Ins2*$^{+/C96Y}$-*ReninTg*$^{+}$

Effect of Renin Transgene + 129/SvEv Background on Albumin Excretion



Effect of Renin Transgene + 129/SvEv Background on Albumin Excretion



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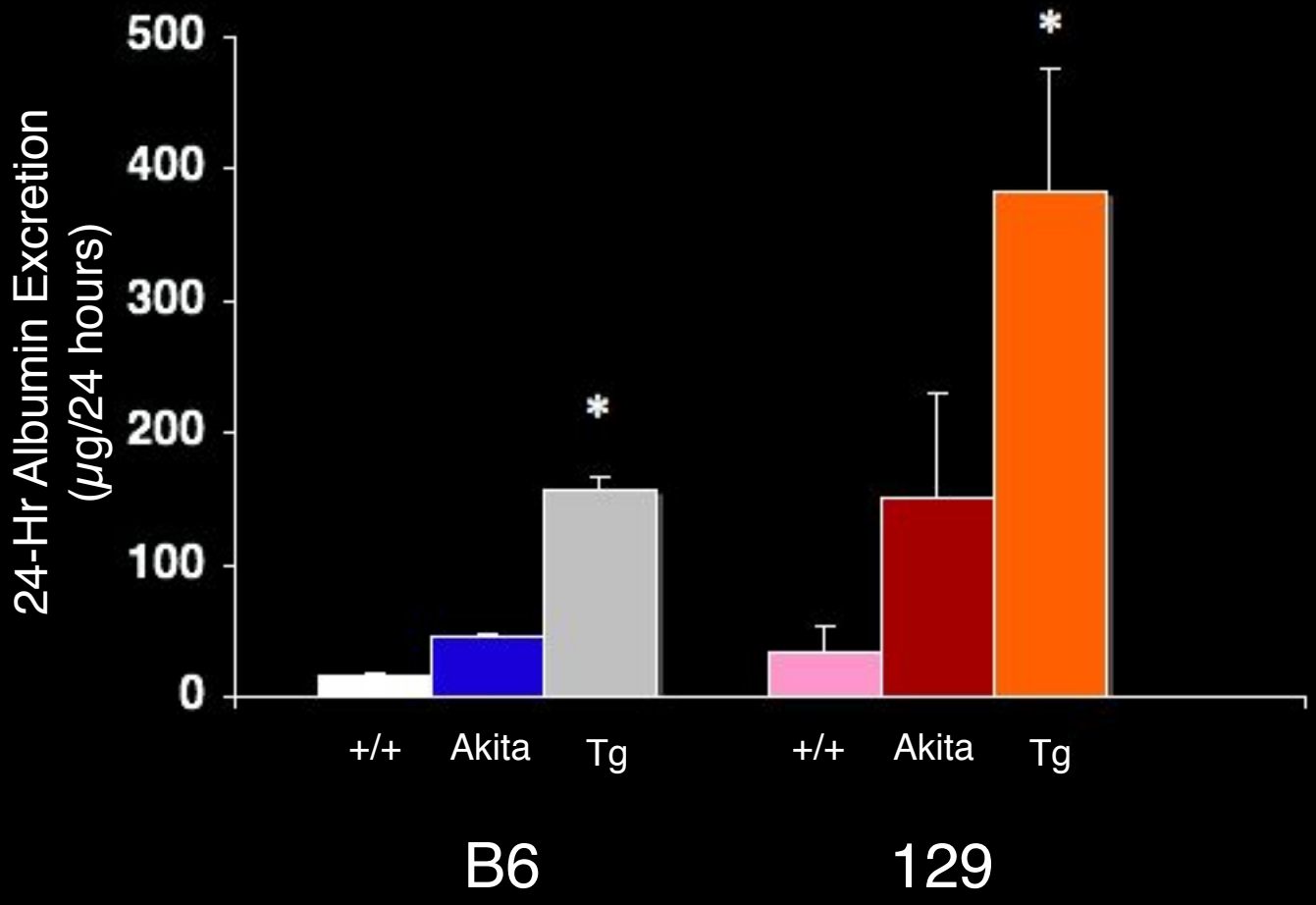
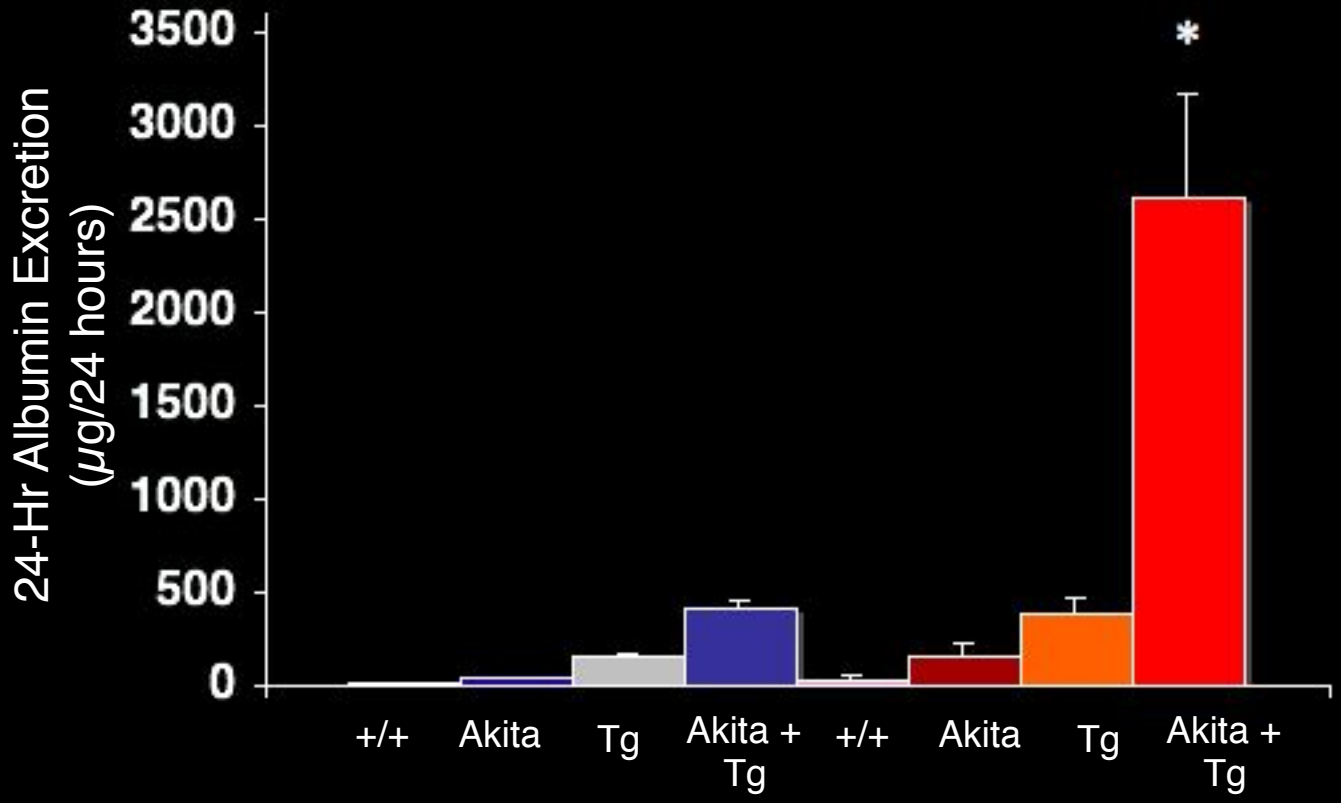
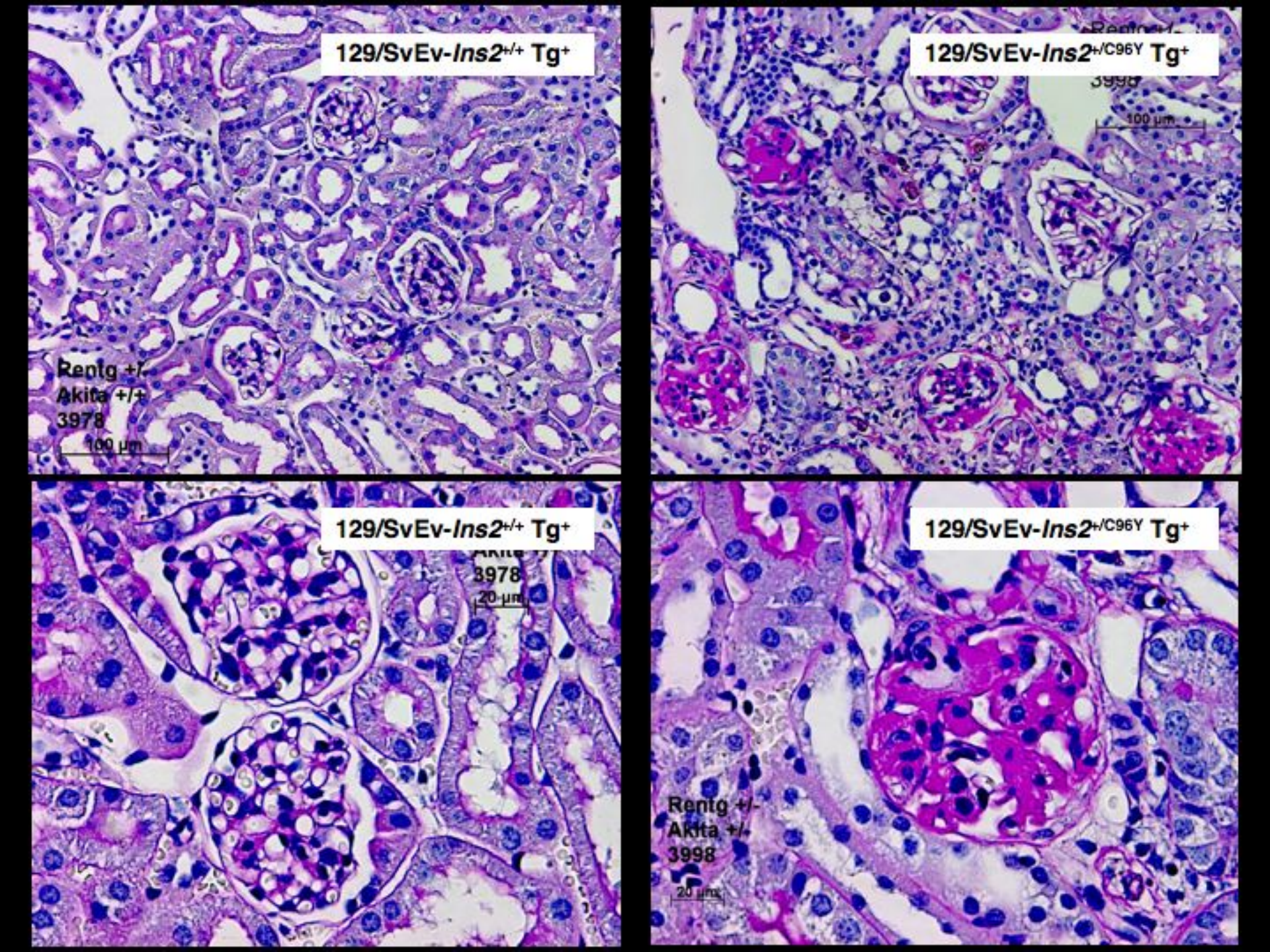
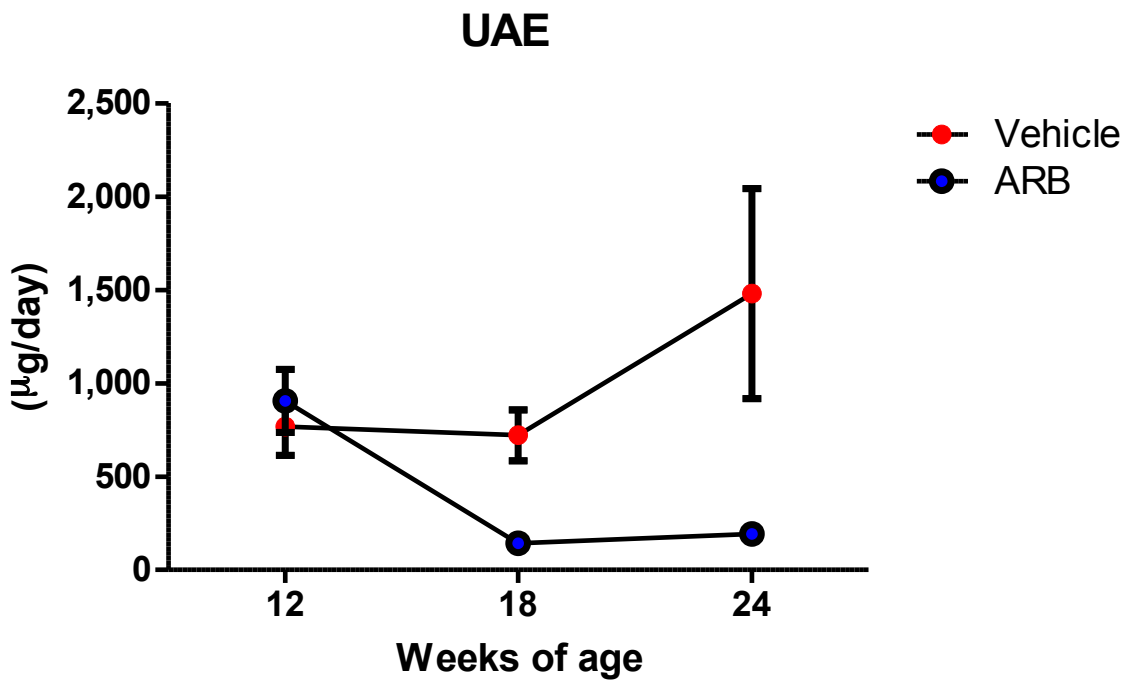


Effect of Renin Transgene + 129/SvEv Background on Albumin Excretion




129/SvEv-*Ins2*$^{+/+}$ Tg^{+}
Rentg +/-
Akita +/+
3978
100 µm
129/SvEv-*Ins2*$^{+/C96Y}$ Tg^{+}
100 µm
129/SvEv-*Ins2*$^{+/+}$ Tg^{+}
3978
20 µm
129/SvEv-*Ins2*$^{+/C96Y}$ Tg^{+}
Rentg +/-
Akita +/-
3998
20 µm

Losartan administration causes sustained reduction of albuminuria in 129 Akita ReninTg mice



Azushima et al

DukeNUS Medical School

Phenotyping Protocol

Organ Harvest

-DNA
-Kidney
-Heart
-Liver
-Skel Muscle
-Adipose
-Serum
-Urine

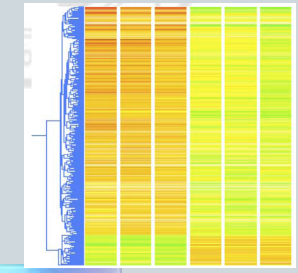
3
weaned

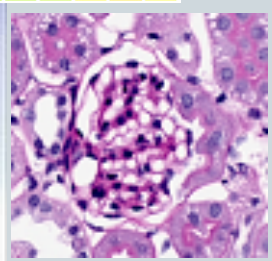
8
BG/BW
Urine

12
BG/BW
Urine

13

Age (weeks)



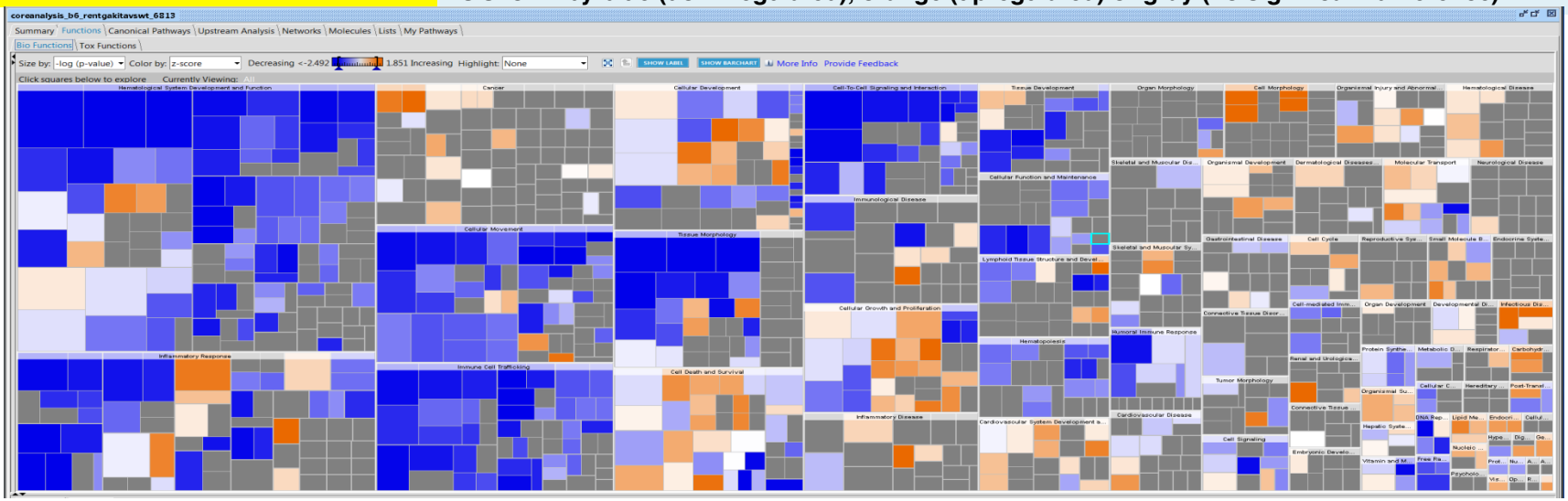


DukeMedicine

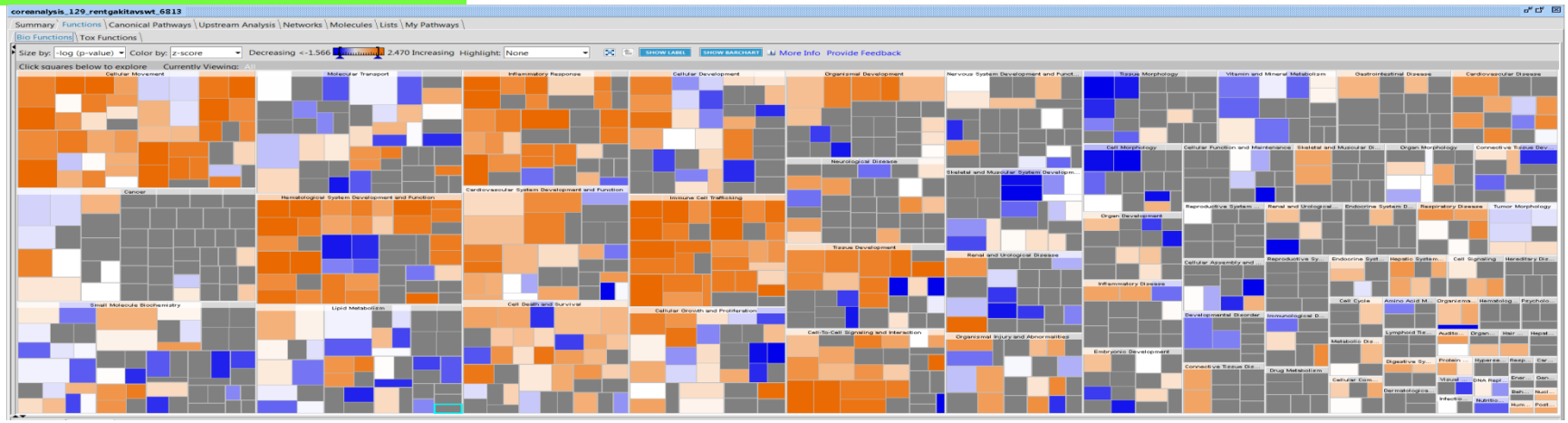
Comparative changes in B6 and 129 (RenTg/Akita vs WT)

B6 RenTg/Akita vs WT

Genes are categorized into 'biological functions'. Overall effect of gene expression on a 'function' is shown by blue (downregulated), orange (upregulated) or gray (no significant difference).

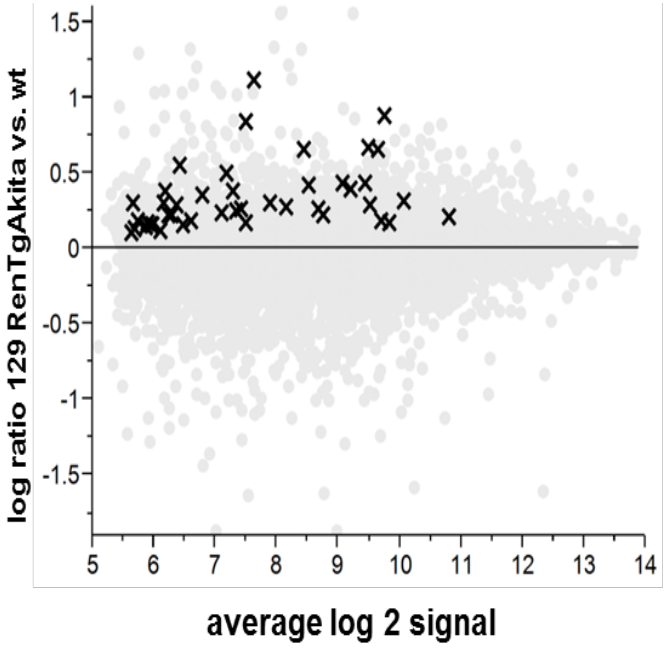


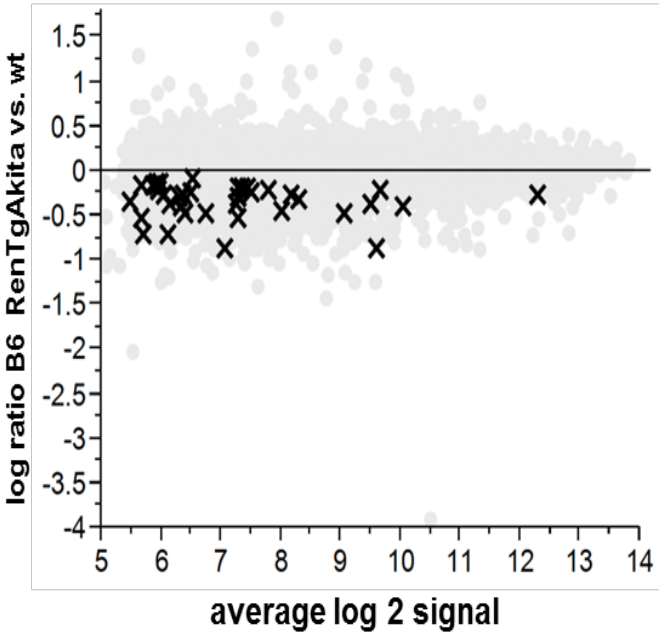
129 RenTg/Akita vs WT



Gurley et al. ***Submitted***

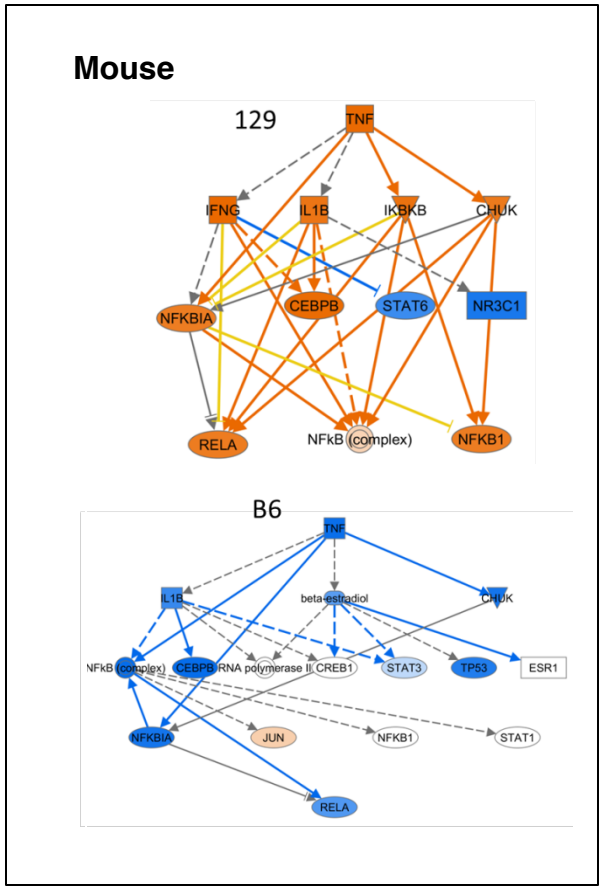
Fold-Change in Gene Expression in Cytokine Cytokine-Receptor Pathways

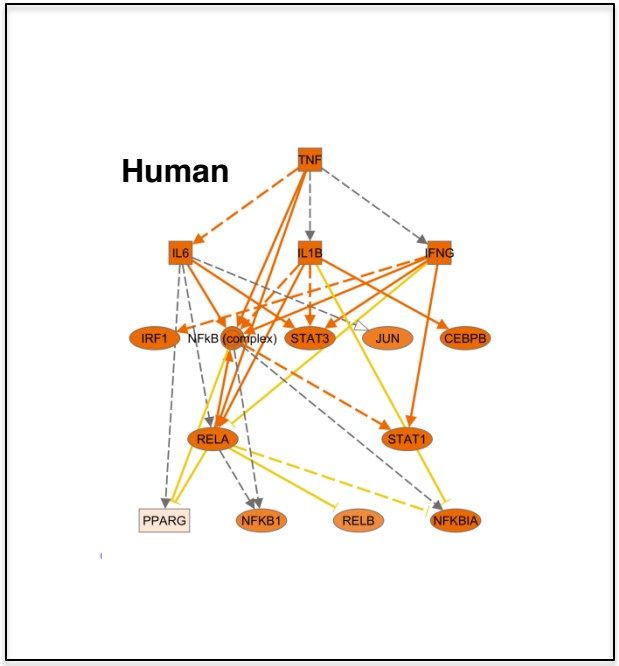




Gurley et al. *Submitted*

TNF-α Downstream Signaling Pathways





Gurley et al. *Submitted*

Project 2.2. To validate novel DN biomarkers individually and after incorporation into basic predictive model.

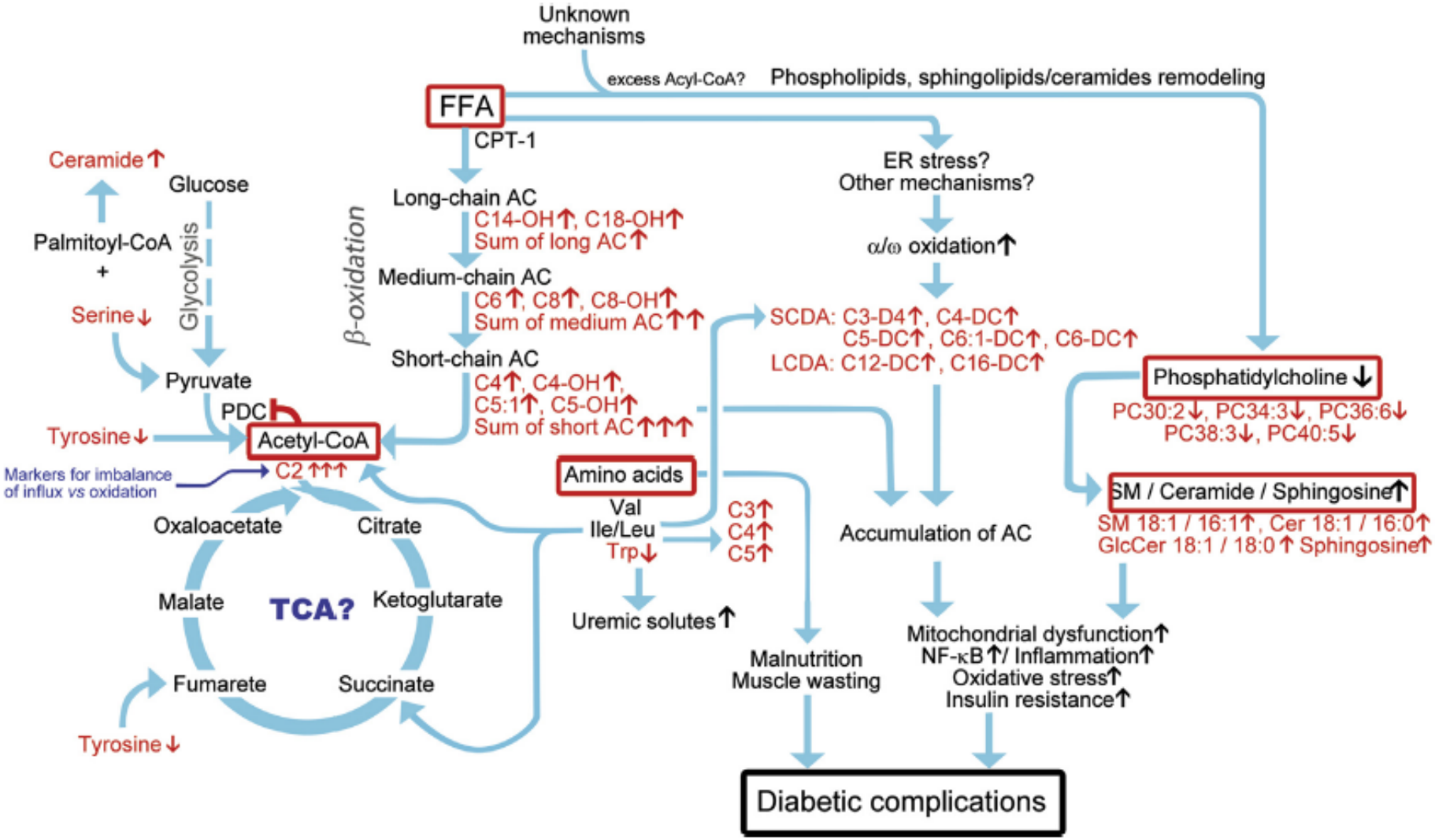
Tai E Shyong
Lim Su Chi

Table 4. Clinical and biochemical characteristics of T2DM participants in validation study

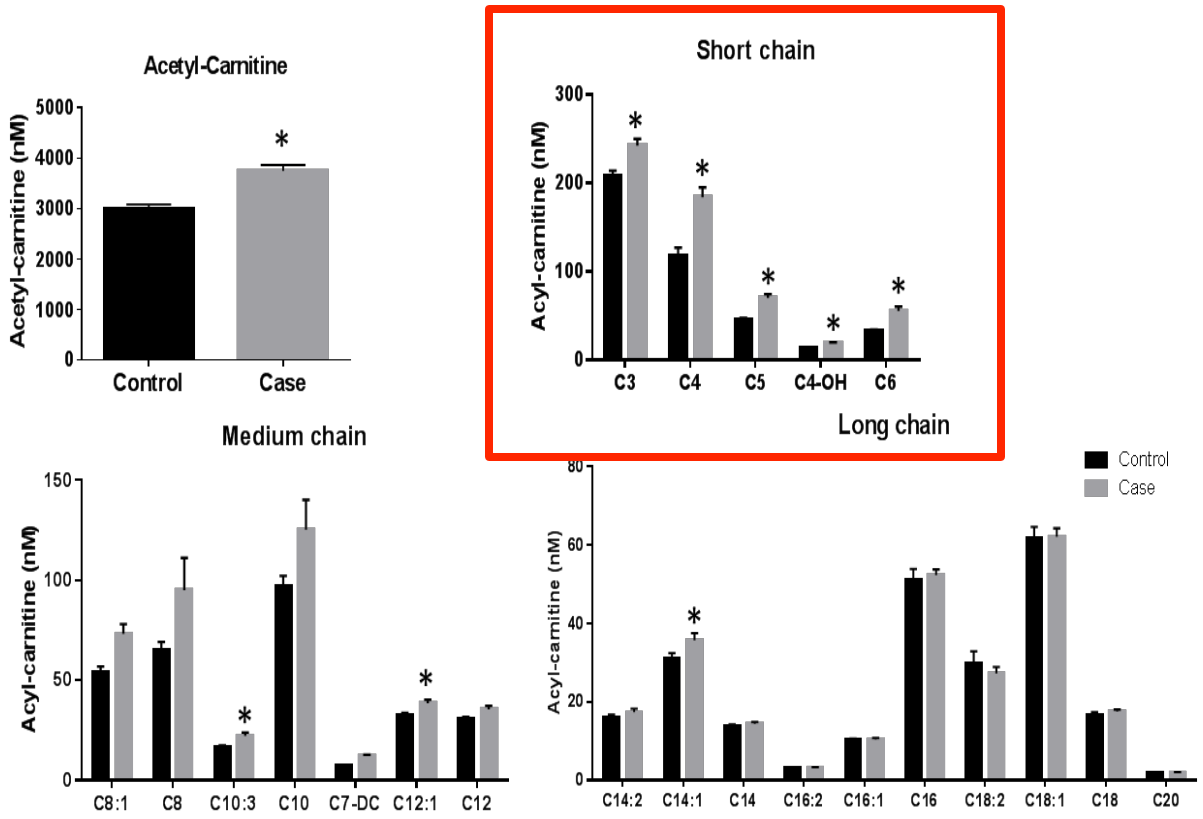
	T2DM Control (n = 149)	Macroalbuminuric DKD (n = 149)	*P* value[a]
Age (yr)	57.0 ± 10.2	57.0 ± 10.3	NA
Male sex (%)	62.4	62.4	NA
Diabetes duration (yr)	10.8 ± 7.1	10.9 ± 6.9	NA
Ethnicity (%)			NA
Chinese	71.1	71.1	
Malay	16.1	16.1	
South Asian	12.8	12.8	
Current smoker (%)	11.9	17.2	0.241
BMI (kg/m^2)	26.2 ± 4.4	27.4 ± 5.5	0.061
HbA_{1c} (%)	8.1 ± 1.8	8.8 ± 2.2	0.004
HbA_{1c} (mmol/mol)	65 ± 14	73 ± 18	0.004
Systolic BP (mm Hg)	131 ± 17	144 ± 21	<0.0001
Diastolic BP (mm Hg)	77 ± 10	79 ± 12	0.120
HDL cholesterol (mmol/l)	1.29 ± 0.37	1.19 ± 0.31	0.009
LDL cholesterol (mmol/l)	2.75 ± 0.83	3.09 ± 1.10	0.003
Triacylglycerol (mmol/l, IQR)	1.34 (0.98–1.93)	1.95 (1.28–2.95)	<0.0001
eGFR (ml/min/1.73 m^2)	94 ± 21	62 ± 32	<0.0001
Urinary ACR (mg/g, IQR)	9 (6–18)	861 (463–2098)	<0.0001
Statin use (%)	73.3	78.9	0.312
RAS blocker use (%)	53.1	83.9	<0.0001
Insulin use (%)	24.5	35.6	0.038

ACR, albumin-to-creatinine ratio; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; NA, not applicable; RAS, renin-angiotensin system; T2DM, type 2 diabetes mellitus.

[a]Student *t* test or χ^2 test where appropriate.

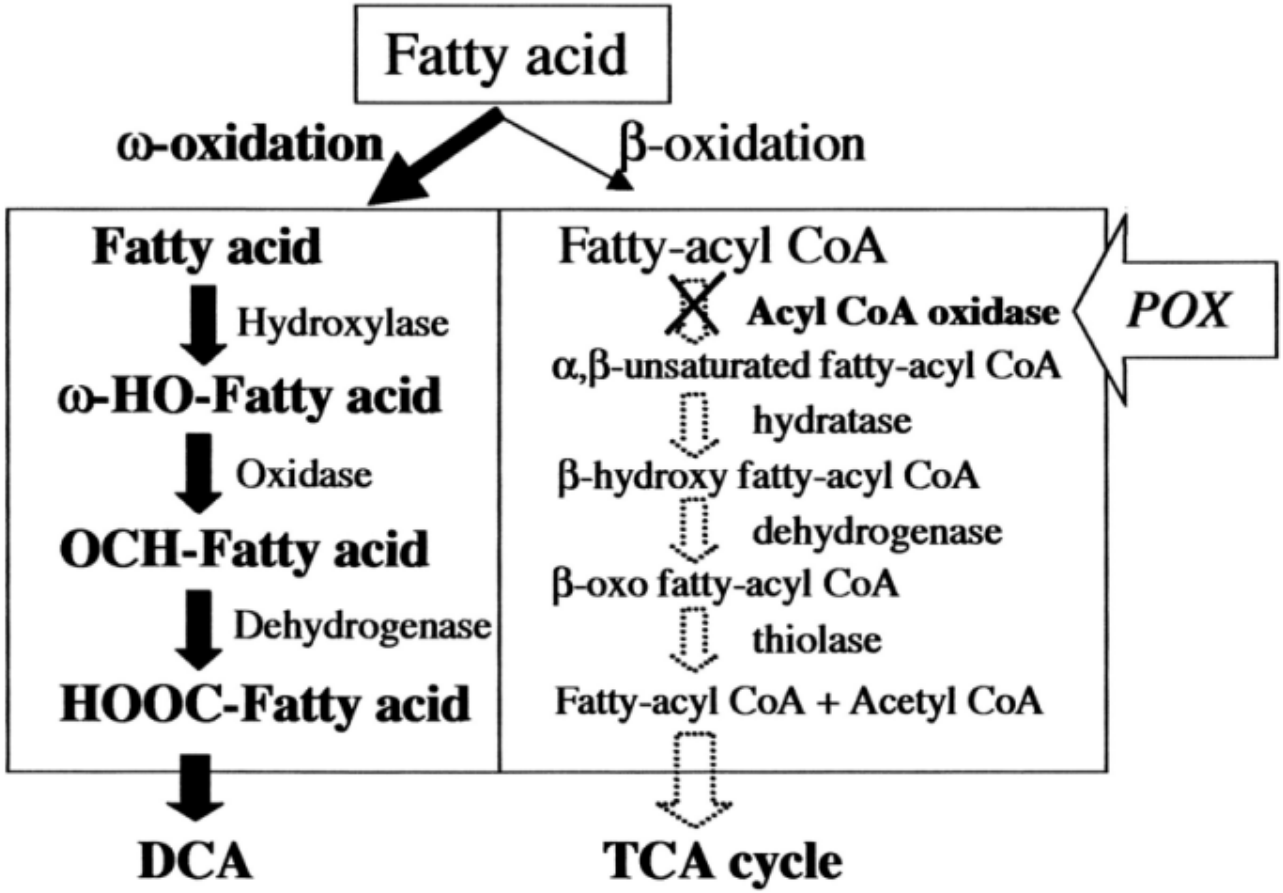

Unknown mechanisms
excess Acyl-CoA?
Phospholipids, sphingolipids/ceramides remodeling
FFA
CPT-1
ER stress?
Other mechanisms?
α/ω oxidation↑
Ceramide↑
Glucose
Palmitoyl-CoA
+
Serine↓
Glycolysis
β-oxidation
Long-chain AC
C14-OH↑, C18-OH↑
Sum of long AC↑
Medium-chain AC
C6↑, C8↑, C8-OH↑
Sum of medium AC↑↑
Short-chain AC
C4↑, C4-OH↑,
C5:1↑, C5-OH↑
Sum of short AC↑↑↑
SCDA: C3-D4↑, C4-DC↑
C5-DC↑, C6:1-DC↑, C6-DC↑
LCDA: C12-DC↑, C16-DC↑
Phosphatidylcholine↓
PC30:2↓, PC34:3↓, PC36:6↓
PC38:3↓, PC40:5↓
Pyruvate
PDC
Tyrosine↓
Acetyl-CoA
C2↑↑↑
Markers for imbalance of influx vs oxidation
Amino acids
Val
Ile/Leu
Trp↓
C3↑
C4↑
C5↑
Accumulation of AC
SM / Ceramide / Sphingosine↑
SM 18:1 / 16:1↑, Cer 18:1 / 16:0↑
GlcCer 18:1 / 18:0↑ Sphingosine↑
Oxaloacetate
Citrate
Malate
TCA?
Ketoglutarate
Fumarete
Succinate
Tyrosine↓
Uremic solutes↑
Malnutrition
Muscle wasting
Mitochondrial dysfunction↑
NF-κB↑/ Inflammation↑
Oxidative stress↑
Insulin resistance↑
Diabetic complications

Accumulation of Short Chain Acyl-Carnitines in Humans with Diabetic Nephropathy



Liu et al. *Kidney Int Rep* (2017) 2, 470–480

Defect in Mitochondrial Fuel Oxidation in Diabetic Nephropathy?



Diversion of FA Carbon from β− to ω− Oxidation

DYNAMO

Diabetes stud**Y** in **N**ephropathy **A**nd other **M**icrovascular c**O**mplications