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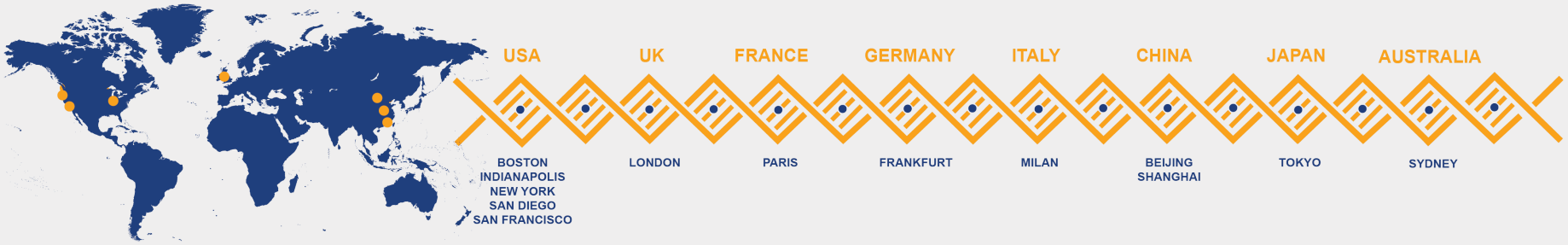
Renal Disease Research Platform

CrownBio Clinically Relevant NHP and Rodent Models and Services



Renal Disease Background

- Renal disease exists in many forms including:
  - genetic diseases of the kidney e.g. Polycystic Kidney Disease (PKD)
  - complications from other disorders such as diabetic nephropathy
- PKD is the fourth leading cause of kidney failure affecting around 600,000 people in the US alone, with currently no cure available
- Nephropathy is the leading cause of dialysis and kidney transplant in developed countries, affecting 40% of people with Type 1 and 2 diabetes
- However, diabetic nephropathy can be delayed or prevented by controlling blood glucose levels and blood pressure
- Further research and treatments are needed for both PKD and diabetic nephropathy, which require evaluation via appropriate preclinical models and study designs

CrownBio Renal Disease Resources

- CrownBio provides a range of models for Renal Disease research including:
  - Induced Renal Disease models
    - Uni-nephrectomy, aldosterone-induced renal disease in Sprague-Dawley rats
  - Genetically Relevant PKD Models
    - *jck* and *pcy* mouse models
    - *PCK* rat model
  - Specialty Translational Models for Diabetic Nephropathy
    - ZDSD rat, polygenic obese and diabetic model
    - Spontaneously diabetic Non-Human Primates (NHPs)
- Customizable study design with main endpoints including:
  - Body weight
  - Plasma BUN, ALT, AST, bilirubin, and creatinine measurement
  - Urinary albumin measurement
  - Kidney weights and histology for fibrosis or cyst volume determination

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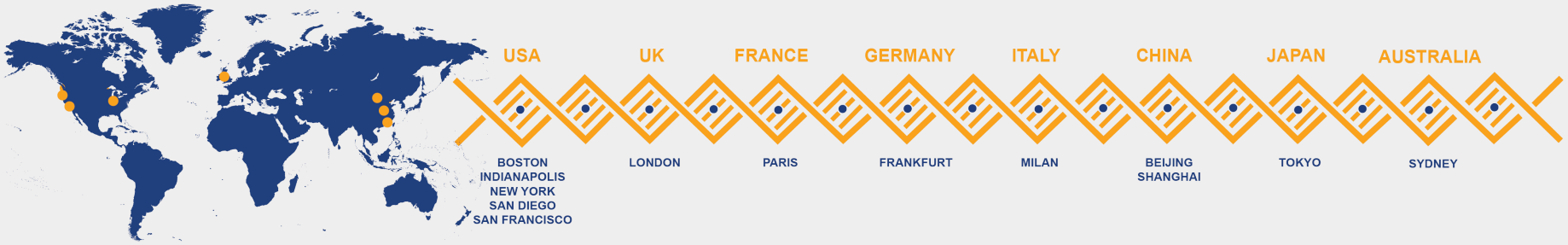
Uni-Nephrectomy, Aldosterone-Induced Renal Disease

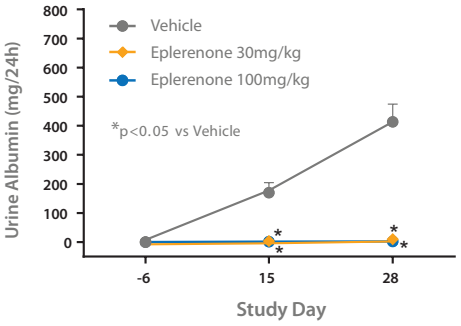
Sprague-Dawley (SD) Rats

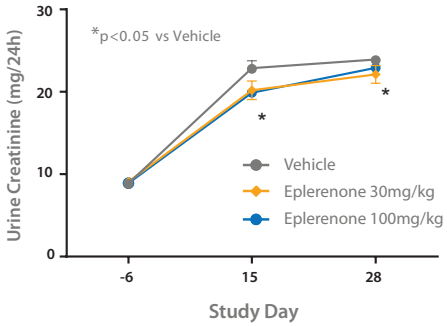


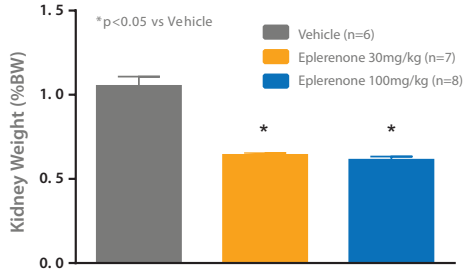
Uni-Nephrectomy, Aldosterone-Induced Renal Disease – Model Development

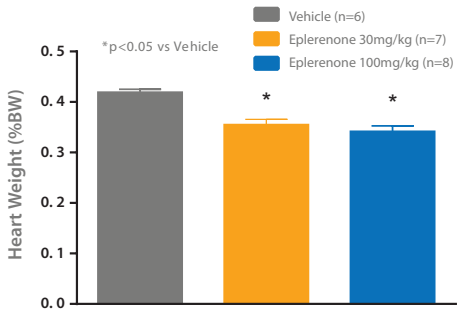
- Renal disease is induced in SD rats by aseptic uni-nephrectomy followed by aldosterone administration (0.75µg/h via Alzet mini-pump) and increased salt intake (6% NaCl in standard diet + 0.3% KCl in drinking water to prevent hypokalemia)
- The regimen results in increased stress on the remaining kidney leading to renal injury and hypertension
- The model has been validated with the administration of the clinically approved, mineralocorticoid receptor antagonist eplerenone

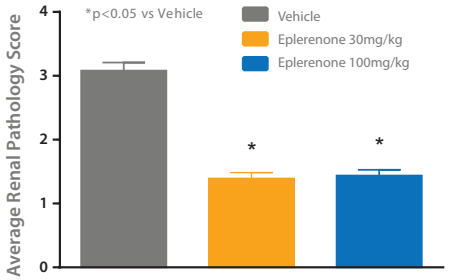
Uni-Nephrectomy, Aldosterone-Induced Renal Disease – Model Validation

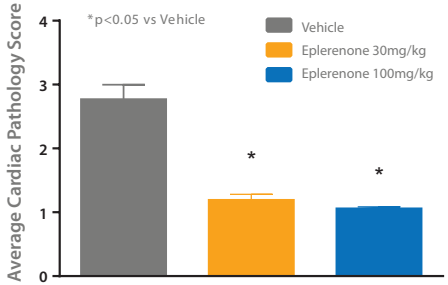












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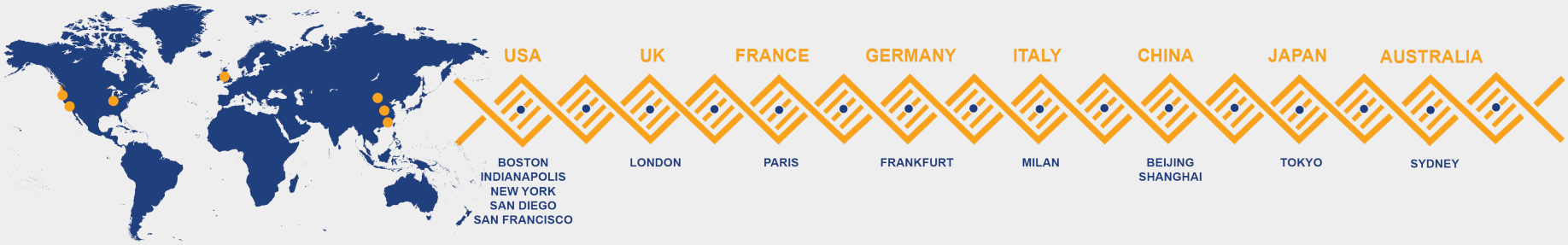
Rodent Models of PKD

jck and *pcy* mouse and *PCK* rat



Summary of CrownBio's PKD Model Features

- Translatable rodent models which closely mirror human PKD
- Develop renal disease associated with the genes that cause human disease; highly relevant for use in novel agent evaluation

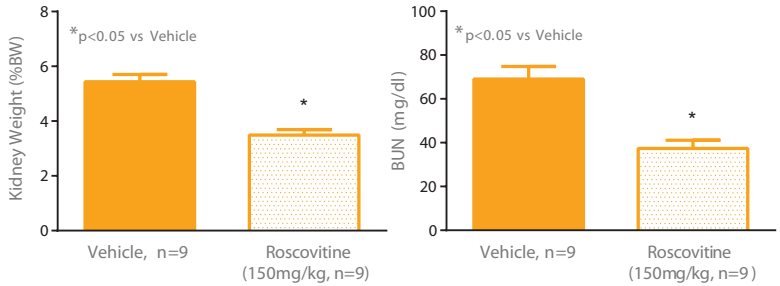
Model	*jck* Mouse	*pcy* Mouse	*PCK* Rat (maintained at CRL)
Rodent Strain	C57Bl/6J-nek8^{jck}	CD-1-pcy^{kusm}	PCK/Crl-Pkhd1pck/Crl
Gene Affected	Associated with the same gene that causes human nephronophthisis type 9	Associated with the same gene that causes human nephronophthisis type 3	Mutated in the same gene that causes human autosomal recessive PKD (ARPKD; PKHD1)
Disease Symptoms and Progression	Slowly progressing renal cystic disease. Cysts develop in multiple regions of the nephron and are more severe in male mice	Slowly progressive renal cystic disease. Cysts develop in the collecting tubules, other segments of the nephron become cystic as disease progresses. Male and female mice are similarly affected by the disease	Slowly progressing form of ARPKD. Displays significant kidney and liver cyst development similar to most humans with PKD

Study Design Overview for PKD Research

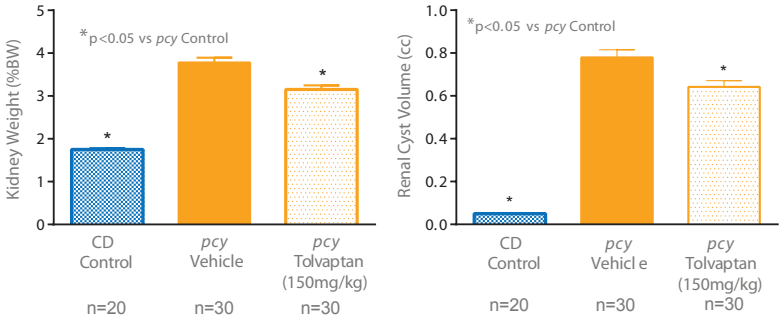
Model	*jck* Mouse	*pcy* Mouse	*PCK* rat (maintained at CRL)
Rodent Age	5 weeks	5 weeks	4 weeks
Test Substance Administration Route	Oral gavage or admixed in diet	Admixed in diet	Oral gavage or admixed in diet
Typical Dosing Length	5 weeks	15 weeks	12 weeks
Endpoints	Cyst volume and fibrosis in kidney, including histological verification Kidney weight/%body weight Serum BUN Concentration of test article in the blood Other analyses relevant to cellular mechanisms may be included Body weight and food intake recorded weekly	Cyst volume and fibrosis in kidney, including histological verification Kidney weight Serum BUN Concentration of test article in the blood Other analyses relevant to the target may be included Body weight and food intake recorded weekly	Cyst volume and fibrosis in kidney and liver, including histological verification Kidney and liver weight Serum BUN, ALT, AST, and bilirubin Concentration of test article in the blood Other analyses relevant to cellular mechanisms may be included Body weight and food intake recorded weekly
Positive Control	CDK inhibitor roscovitine	Vasopressin receptor-2 (V2) antagonist tolvaptan	PPARγ agonist pioglitazone

PKD Models
Response to Treatment

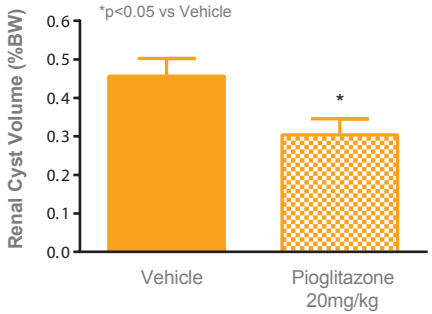
The *jck* Mouse Responds to Roscovitine Treatment



The *pcy* Mouse Responds to Tolvaptan Treatment



The *PCK* Rat Responds to Pioglitazone Treatment



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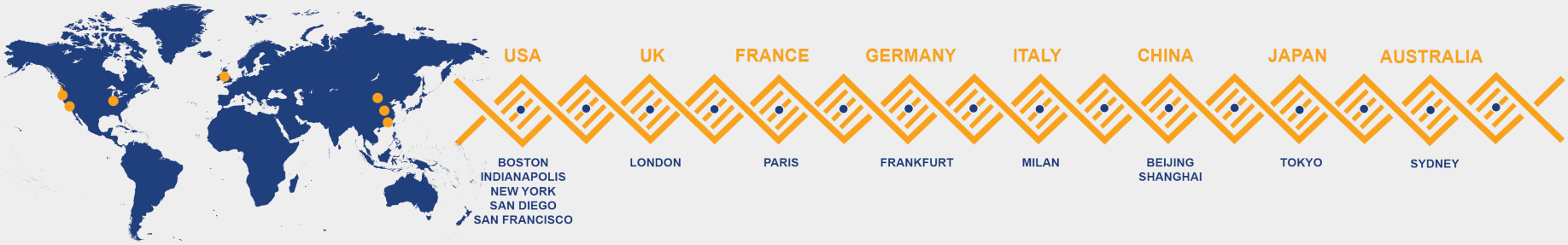
Type 2 Diabetic Nephropathy

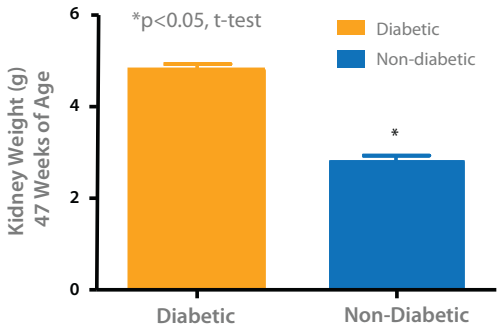
The ZDSD Rat and Spontaneously Diabetic NHP Models

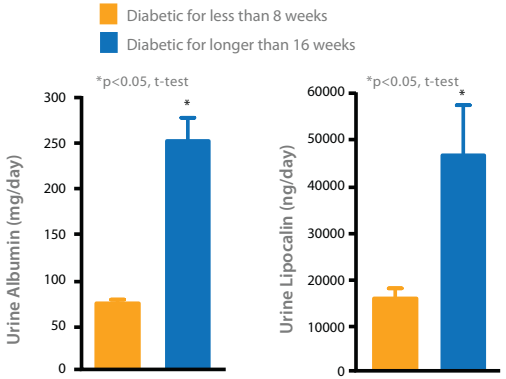


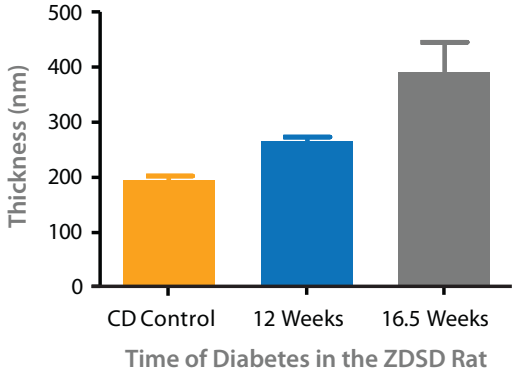
The ZDSD Rat Model

- Inbred polygenic model for metabolic syndrome, obesity, diabetes, and diabetic complications with functional leptin signaling
- Displays diabetes progression similar to human disease:
  - Prediabetes (8-16 weeks of age)
  - Overt diabetes (>16 weeks of age)
  - Diabetic complications (24- weeks of age) including nephropathy
- At CrownBio we have evaluated
  - Morphological changes affecting the kidney glomeruli in ZDSD rats
  - Biomarkers for renal dysfunction (IL6, TNF-α, NGAL, KIM-1, VEGF, serum albumin, free fatty acids, and oxidative stress levels)
- We tested response to ACE inhibitors in the ZDSD rat, showing the model to be highly translatable to the human condition

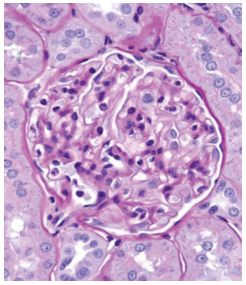
Diabetic Nephropathy in the ZDSD Rat



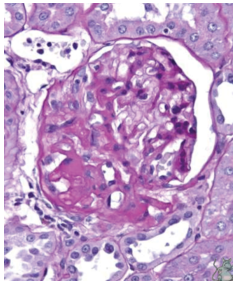


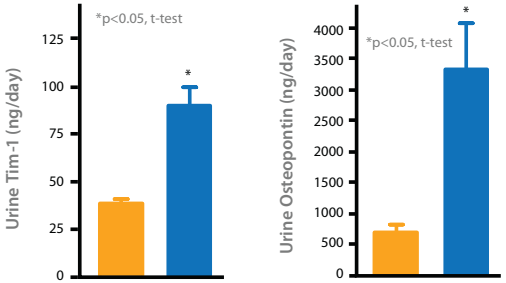


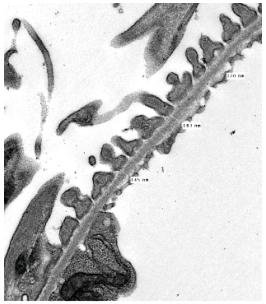
Age-Matched Control



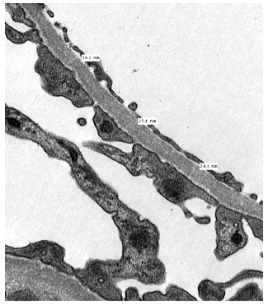
Diabetic, 27 Weeks







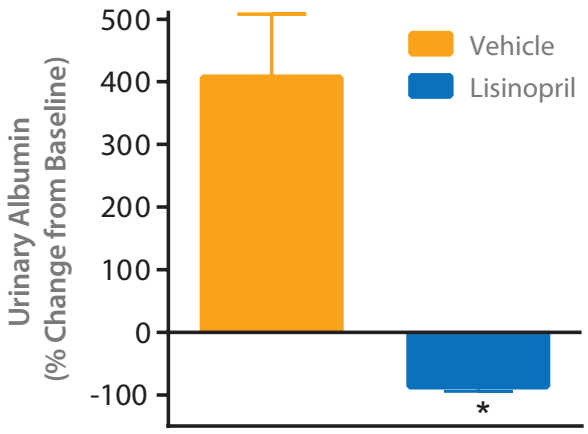
Age-Matched Control

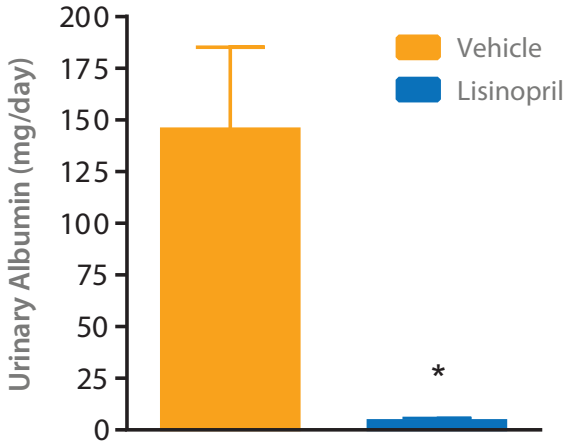


Diabetic, 12 Weeks

ZDSD Rat Response to ACE Inhibitor Lisinopril

- Lisinopril significantly decreases urinary albumin



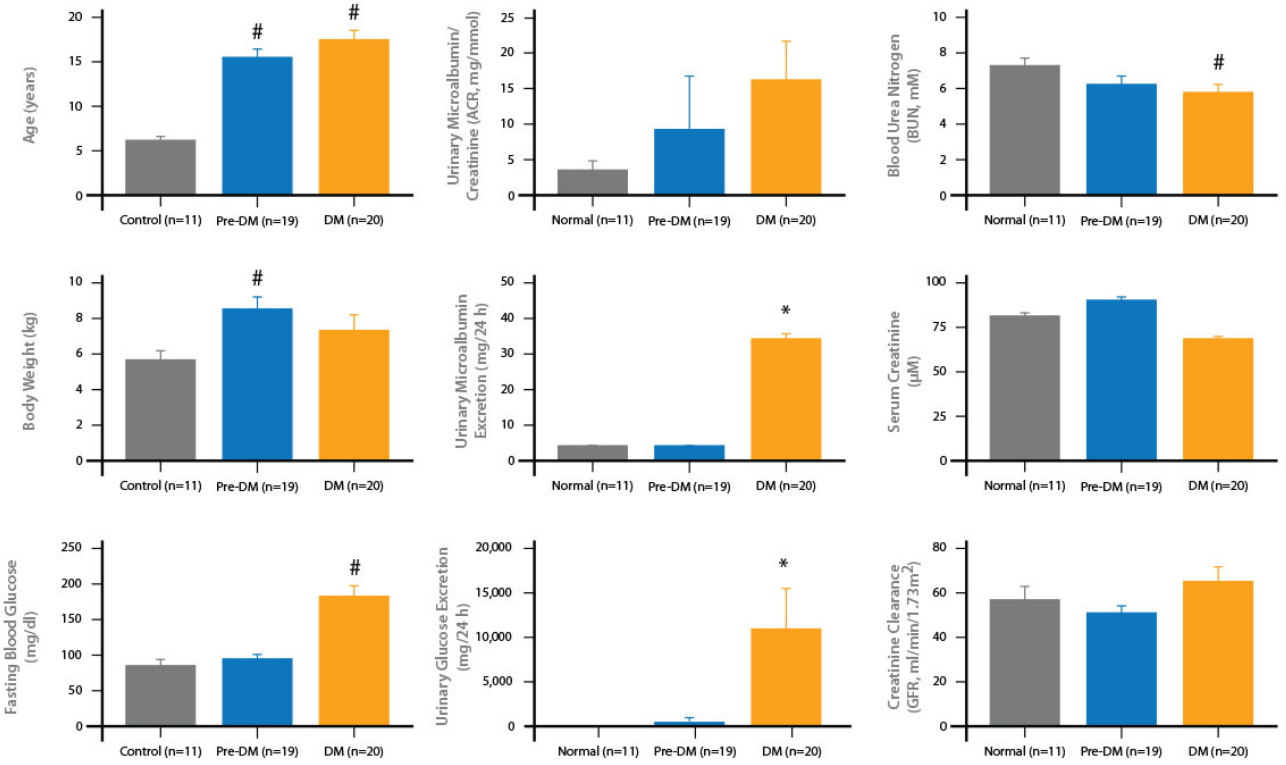


Spontaneously Diabetic NHPs

- Spontaneously diabetic NHPs mirror all aspects of human diabetes progression including microvascular complications such as nephropathy
- CrownBio has characterized a cynomolgus model of diabetic nephropathy which has the same features observed in diabetic nephropathy patients including:
  - Hypergylcemia
  - Glucosuria
  - Albuminuria
  - Proteinuria
- Renal function surrogate biomarkers including BUN and serum creatinine have been characterized in our model
- A transcriptome analysis was performed on kidney biopsy specimens to identify genes differentially expressed during disease progression

Diabetic Nephropathy NHP Models

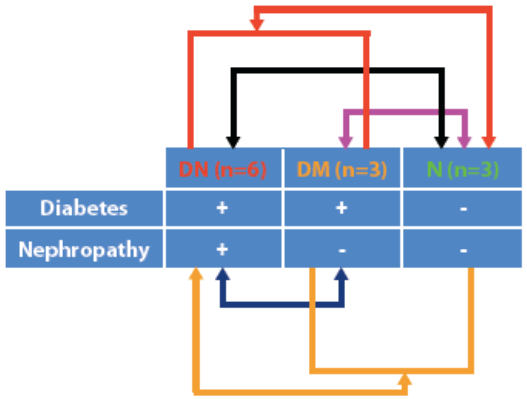
- Phenotypes observed in the Diabetic Nephropathy NHP model



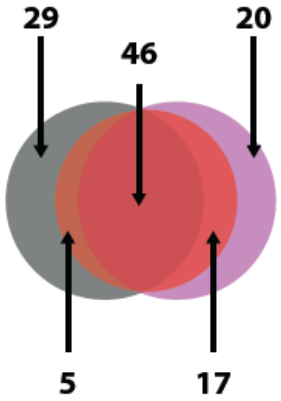
*p<0.05, #p<0.01.

Transcriptome Analysis Identifies Kidney Disease Related Genes

- 95 Differentially Expressed Genes (DEGs) between normal, pre-diabetic, and diabetic NHPs detected (>4 fold up/down)
- 4 DEGs identified by Gene Set Enrichment Analysis that specifically contribute to nephropathy:
  - lactase (LCT)
  - matrix metalloproteinase-7 (MMP7)
  - secreted phosphoprotein 1 (SPP1)
  - hepatitis A virus cellular receptor 1 (HAVCR1)
- These genes form a kidney failure, renal, urological, and inflammatory disease related network, responding to tumor necrosis factor (TNF)



Comparison	Effect	#DEG
1. N vs. DN	Diabetes & nephropathy	75
2. N v.s DM	Diabetes without nephropathy	66
3. N vs. DN & DM	Diabetes	68
4. DN vs. DM	Nephropathy in presence of diabetes	1
5. DN vs. N & DM	Nephropathy	4



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Summary

- Appropriate preclinical models and study designs are needed to design new treatments for Renal Disease
- CrownBio provides a range of models for Renal Disease research including:
  - Uni-nephrectomy, aldosterone-induced renal disease models
  - *jck* and *pcy* mice, and *PCK* rat genetically relevant models of PKD
  - ZDSD rat and Spontaneously diabetic NHP translational models of diabetic nephropathy
- Customizable study design with main endpoints including:
  - Body weight
  - Plasma BUN, ALT, AST, bilirubin, and creatinine measurement
  - Urinary albumin measurement
  - Kidney weights and histology for fibrosis or cyst volume determination
- Expertise and proven track record

Contact CrownBio

- Discover our unique spontaneously diabetic NHPs, our highly disease relevant PKD models, or our translational polygenic obese ZDSD rat models
- Contact CrownBio at **busdev@crownbio.com** to discover the full range of models and services for your renal disease research