

# Chronic Kidney Disease of Unknown Etiology: Biomarkers of Kidney Damage in Farmworkers

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ENVIRONMENT

## Climate Change, the Kidney Killer

Is chronic kidney disease the canary in the coal mine in terms of feeling the health impacts of climate change?

<https://www.thinkglobalhealth.org/article/climate-change-kidney-killer>

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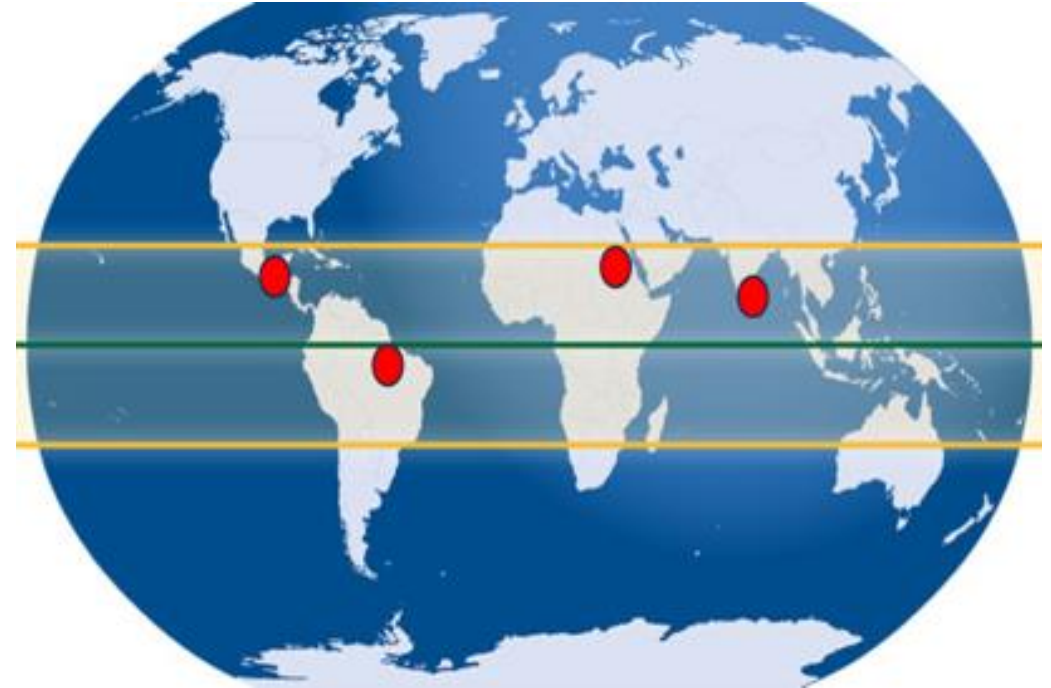


**Southeastern  
Coastal Center**

for Agricultural Health and Safety

# Chronic Kidney Disease of Unknown Etiology (CKDu)

- **Progressive loss of kidney function**
  - Asymptomatic rise in Creatinine
  - Variable: Low grade or absent proteinuria
  - Variable: fatigue, fever, acute presentation
  - Blood pressure, blood sugar normal
  - Late diagnosis with severe morbidity and mortality
- **Geographic/Occupational clusters of CKDu**
  - Increasingly identified in past 3 decades
  - Hot / humid conditions
  - Agricultural communities – e.g. sugarcane & rice
- **Distinct population at risk**
  - Predominance of male farm workers
  - Young Age of Onset – 20 to 40 yrs old
- **Unknown cause(s) unrelated to common risk factors**
  - Not diabetes
  - Not hypertension
- **Unique histopathology on kidney biopsy**
  - Early evidence of glomerular/vascular damage
  - Late disease characterized by interstitial/tubular fibrosis



## CKDu Hotspots

Impacting farmworkers in regions  
with climates similar to  
Southeastern US /Florida

# Cause of CKDu? Many hypotheses – no answers

1. Heat stress and/or dehydration
2. Environmental exposures (pesticides, metals, particulate matter )
3. Genetics – family history of kidney disease, heat adaptation
4. Other nephrotoxins: medications, tobacco, nutrition, fructose, infections

To date, no *single* hypothesis has been able to explain development of disease

Multi-factorial cause - A “double hit” to the kidneys might be required

Recurrent Heat stress / Dehydration + Environmental exposures --> CKDu

# CKDu in US and/or Southern US Farmworkers? We don't know...

Similar Set of Occupational Stressors

Heat

Strenuous work

Humidity

Agricultural chemicals

**Limited studies in US and Florida in US – and focus on acute kidney injury (AKI)**

33% of 555 participants in Florida Farmworker Study had AKI on at least one workday.

471 California Farmworkers - 36% - core body increased  $>1^{\circ}\text{C}$  , 14.9% AKI after 1 day of agricultural work.

**End-stage renal disease – 2017 U.S. Renal Data Report**

**“hot spots” of ESRD in rural areas and large agricultural tracts in Southeastern California, South Texas and Southeast of U.S.**

Ongoing SCCAHS study of incidence of CKDu in Florida using OneFlorida Database.

PI” William Hogan

<https://www.sccaahs.org/index.php/research/detection-of-chronic-kidney-disease-of-unknown-etiology-in-florida-by-repurposing-a-statewide-data-infrastructure-for-surveillance/>

Chapman CL, Hess HW, Lucas RAI, Glaser J, Saran R, Bragg-Gresham J, Wegman DH, Hansson E, Minson CT, Schlader ZJ. Occupational heat exposure and the risk of chronic kidney disease of nontraditional origin in the United States. *Am J Physiol Regul Integr Comp Physiol*. 2021 Aug 1;321(2):R141-R151. doi: 10.1152/ajpregu.00103.2021. Epub 2021 Jun 23. PMID: 34161738; PMCID: PMC8409908.

Bragg-Gresham J, Morgenstern H, Shahinian V, Robinson B, Abbott K, Saran R. An analysis of hot spots of ESRD in the United States: Potential presence of CKD of unknown origin in the USA?. *Clin Nephrol*. 2020; 93: 113-119. doi: 10.5414/CNP92S120. Pubmed: <https://www.ncbi.nlm.nih.gov/pubmed/31496516>; PMID: 31496516.

Moyce, S, Armitage, T, Mitchell, D, Schenker, M. Acute kidney injury and workload in a sample of California agricultural workers. *Am J Ind Med*. 2020; 63: 258– 268. <https://doi.org/10.1002/ajim.23076>

Mix, Jacqueline PhD, MPH; Elon, Lisa MS, MPH; Vi Thien Mac, Valerie PhD, RN; Flocks, Joan JD; Economos, Eugenia; Tovar-Aguilar, Antonio J. PhD; Stover Hertzberg, Vicki PhD, FASA; McCauley, Linda A. PhD, RN Hydration Status, Kidney Function, and Kidney Injury in Florida Agricultural Workers. *Journal of Occupational and Environmental Medicine* 60(5): p e253-e260, May 2018. | DOI: 10.1097/JOM.0000000000001261

# Research Goals

## *Current diagnosis of CKDu is inadequate*

- *Symptomatic diagnosis occurs at the later stage of the disease – too late.*
- *Current biomarkers insensitive to kidney disease until progression is severe*
- *Early detection vital for preventing progression and reducing treatment costs.*

## **Goal 1: Identify urinary biomarkers of CKDu to enable intervention and treatment**

### *Causal factors, pathophysiology, and disease progression remain unclear*

- *Complex human exposures in population-based studies*
- *Pathology data from late-stage disease*
- *Difficulty in correlating biomarker data and pathologic injury*

## **Goal 2: Develop an animal model to understand the effects of heat and environmental exposures on kidney function.**

# Pilot study of urine biomarkers in Guatemalan Sugar Cane Workers

## CKDu in Central America

## Sugarcane Field Work in Guatemala



### Cane Cutters

Heavy exertion

Long work hours

Intense sunlight and heat  
(temperatures frequently  
surpass 40°C [104°F])

Manually use machetes

Paid by amount of cane cut (6  
tons/day)

>60,000 cases of CKDu were reported  
in these regions from 1997–2013  
which led to renal failure and death.

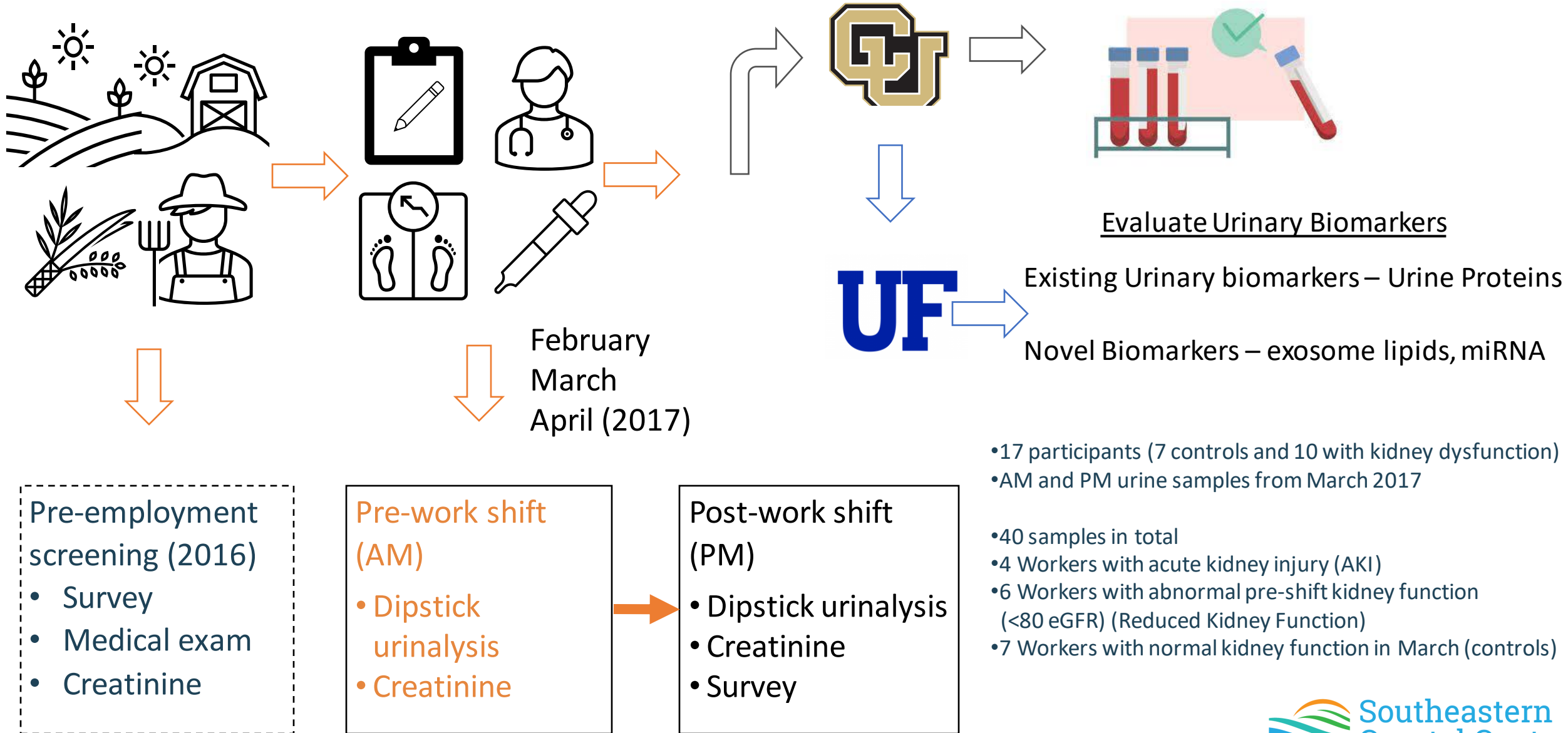
6-month season

4,000 hired annually

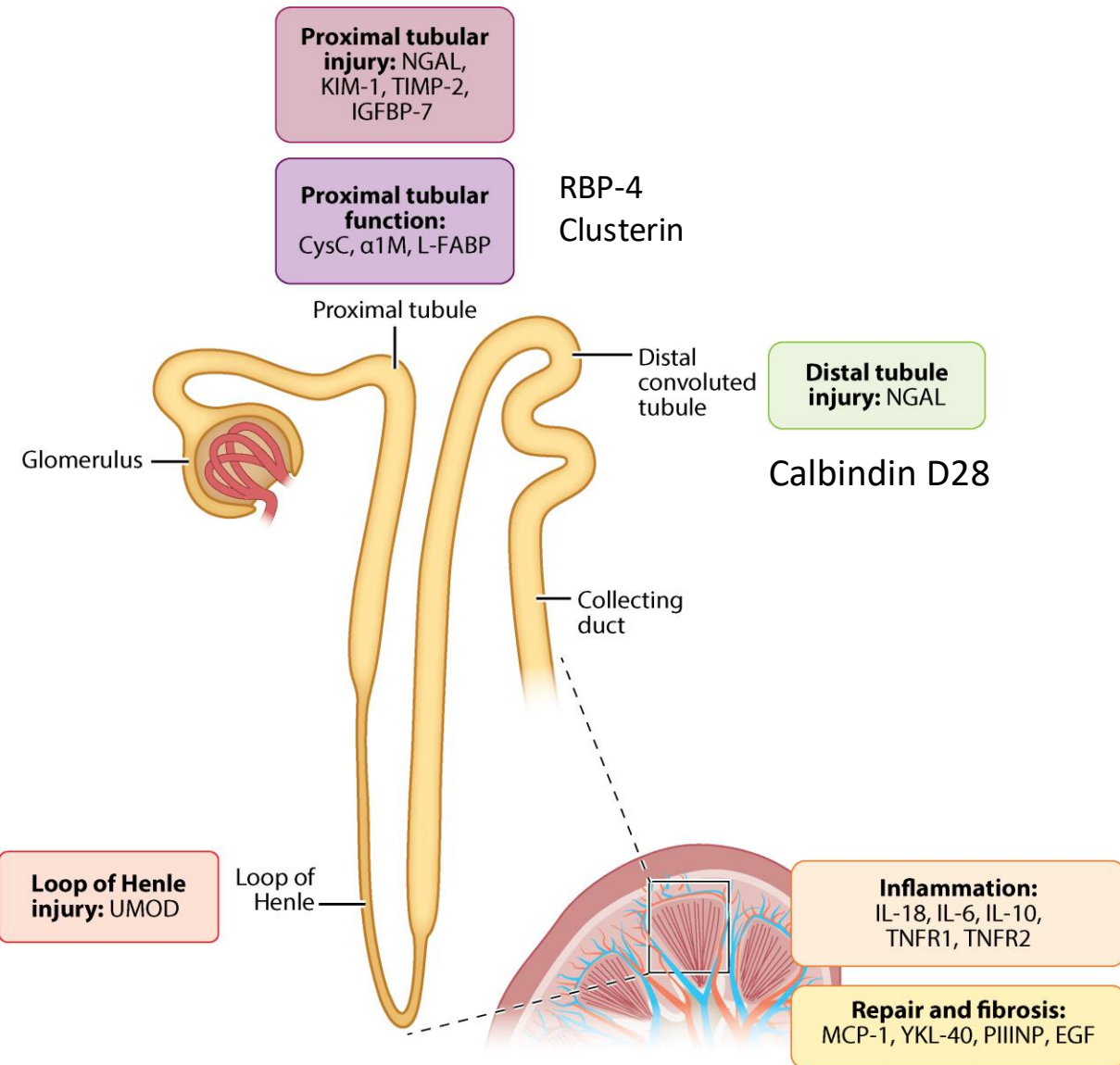
- Local / Highland workers
- Cane / Production workers

# Pilot study of urine biomarkers in Guatemalan Sugar Cane Workers

Stratified random sampling from a cohort of 500 workers in longitudinal study during the 2016-2017 harvest.

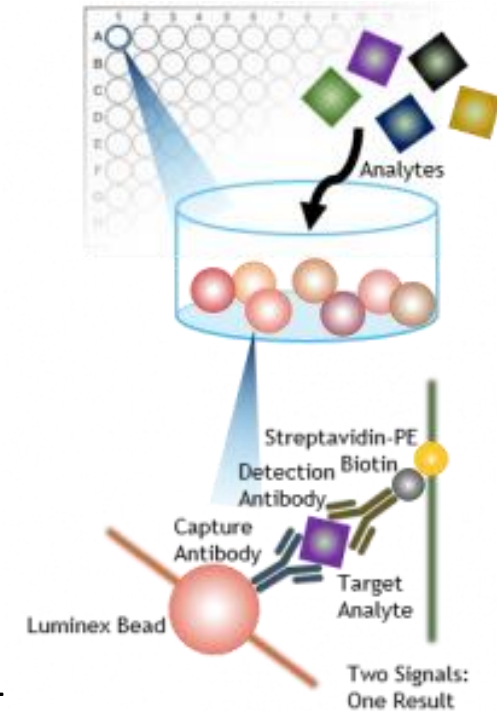


# Urine Protein Biomarkers



- alpha-1-Microglobulin
- beta-2-Macroglobulin
- Calbindin D28
- Clusterin (apoJ)
- Cystatin C
- Epidermal Growth Factor
- Glutathione-S-Transferase alpha-1
- Glycoprotein NMB
- Interferon-induced Protein-10
- Interleukin-18
- Kidney Injury Molecule-1
- Monocyte Chemoattractant Protein-1
- N-acetyl-beta-D-Glucosaminidase
- Neutrophil Gelatinase-Associated Lipocalin N-GAL
- Osteopontin
- Renin
- Retinol Binding Protein 4
- Tissue Inhibitor Metalloproteinase-1
- Trefoil Factor 3
- Uromodulin
- Vascular Endothelial Growth factor A

## Multiplex Luminescence assay





# Urinary Protein Biomarker analysis identifies candidate biomarkers

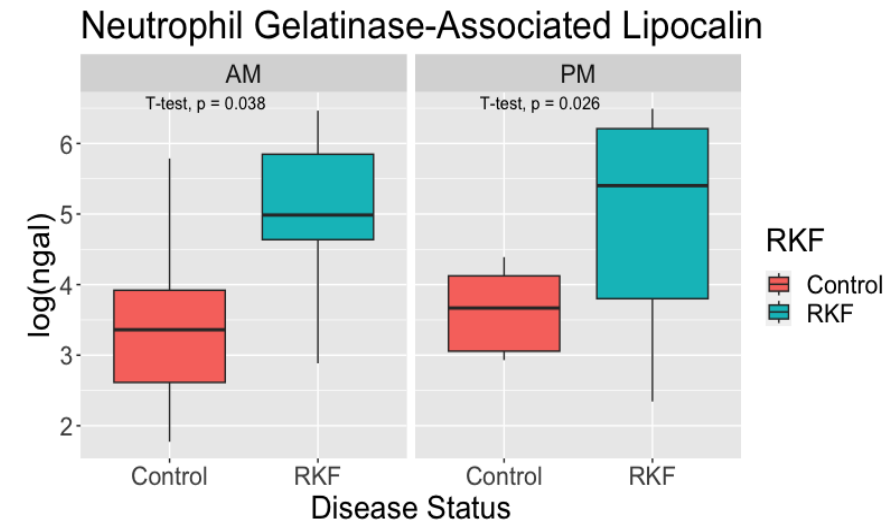
Acute Kidney Injury (AKI): Increase in serum creatinine by  $\geq 0.3$  mg/dL within 48 hrs; OR,  
Increase in serum creatinine to  $\geq 1.5$  times baseline

Reduced Kidney Function (RKF) : pre-shift eGFR < 80

Assessed AM and PM urine samples  
Compared AKI vs Control  
RKF vs Control

Controls: do not meet criteria for AKI or RKF

Biomarker	AKI VS CONTROL		RKF VS CONTROL	
		P-Value		P-Value
B2macroglobulin	AKI AM Higher	0.08	RKF AM higher	0.02
Calbindin	AKI AM Higher	0.1	RKF AM higher	0.093
Calbindin			RKF PM higher	0.042
Clusterin APOJ			RKF AM higher	0.031
EGF			Control AM higher	0.025
EGF			Control PM higher	0.046
GSAT			RKF PM lower	0.095
MCP1			RKF AM higher	0.011
NGAL			RKF AM higher	0.038
NGAL			RKF PM higher	0.026
RBP4	AKI PM Higher	0.09	RKF AM higher	0.042
Tff3			RKF AM higher	0.026
Tff3			RKF PM higher	0.028



## Limitations

- Small sample size
- Controls are farmworkers
- Using existing definitions of kidney disease

# Conclusions

- Several candidate protein biomarkers associated with Acute Kidney Injury in Guatemalan farmworkers
- Distinct set of candidate biomarkers associated with decreased kidney function (eGFR <80) in farmworkers

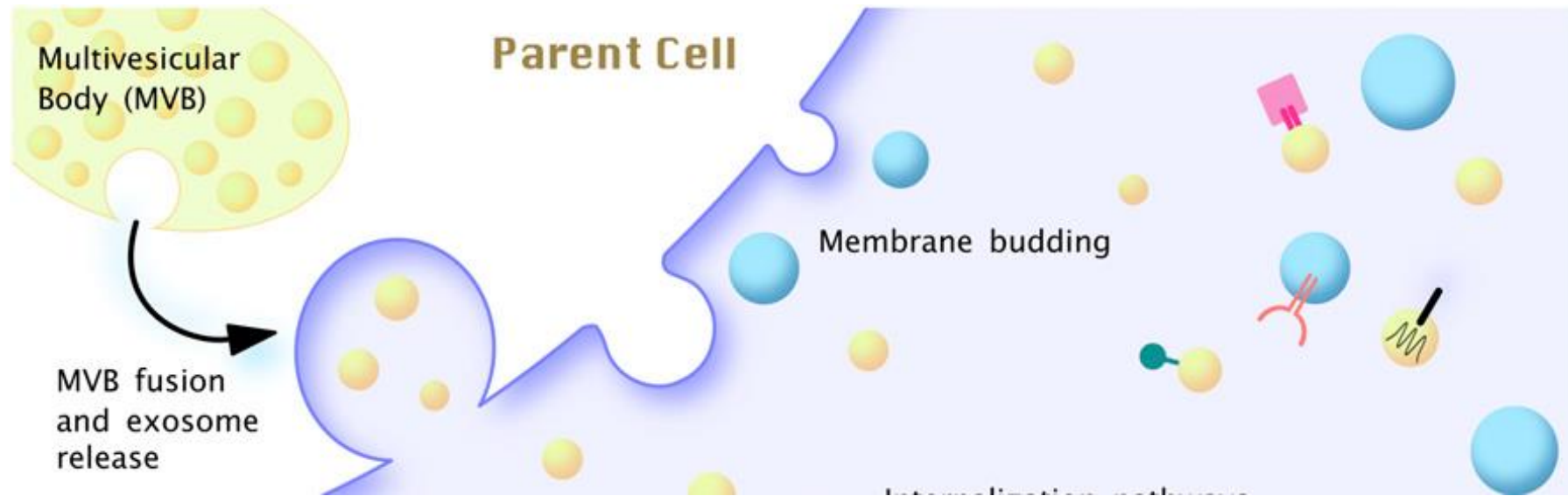
## Next Steps

- Assess biomarkers in larger sample of same cohort
- Assess biomarkers in independent cohort from following year
- Compare biomarkers between human and animal studies

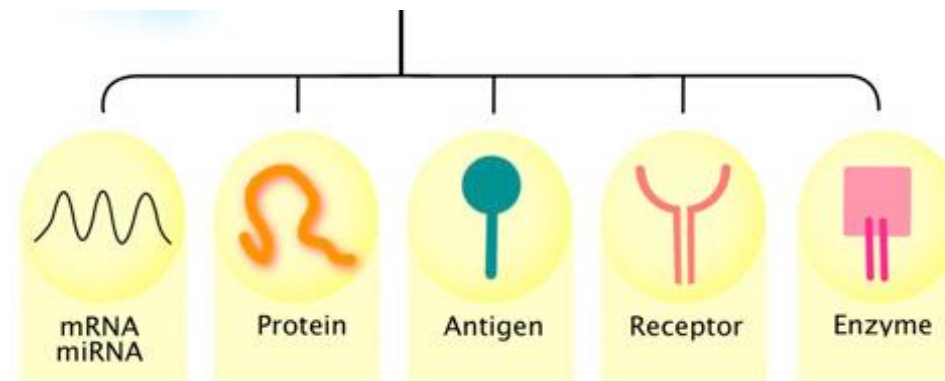
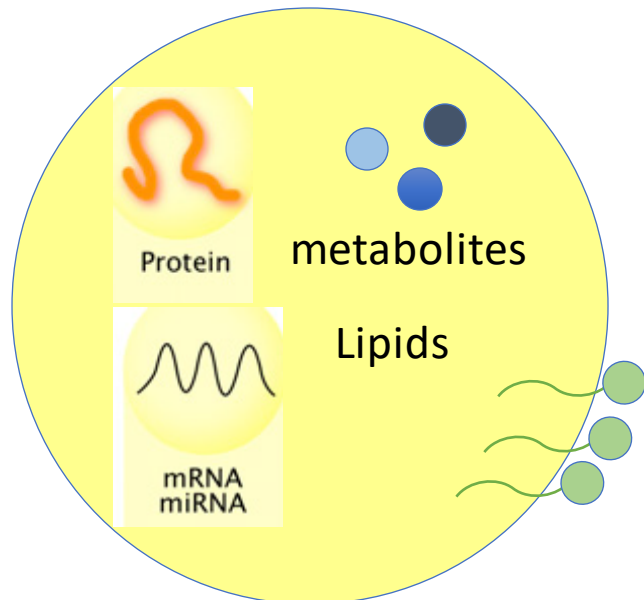
# Ongoing efforts on CKDu biomarkers

- Follow up urinary protein biomarkers in Guatemalan Farmworkers
- Novel Biomarker development using urinary exosomes
- Animal Model development to develop biomarkers and assess role of heat and pesticide exposure in pathophysiology

# Novel biomarkers - Exosome based Biomarkers



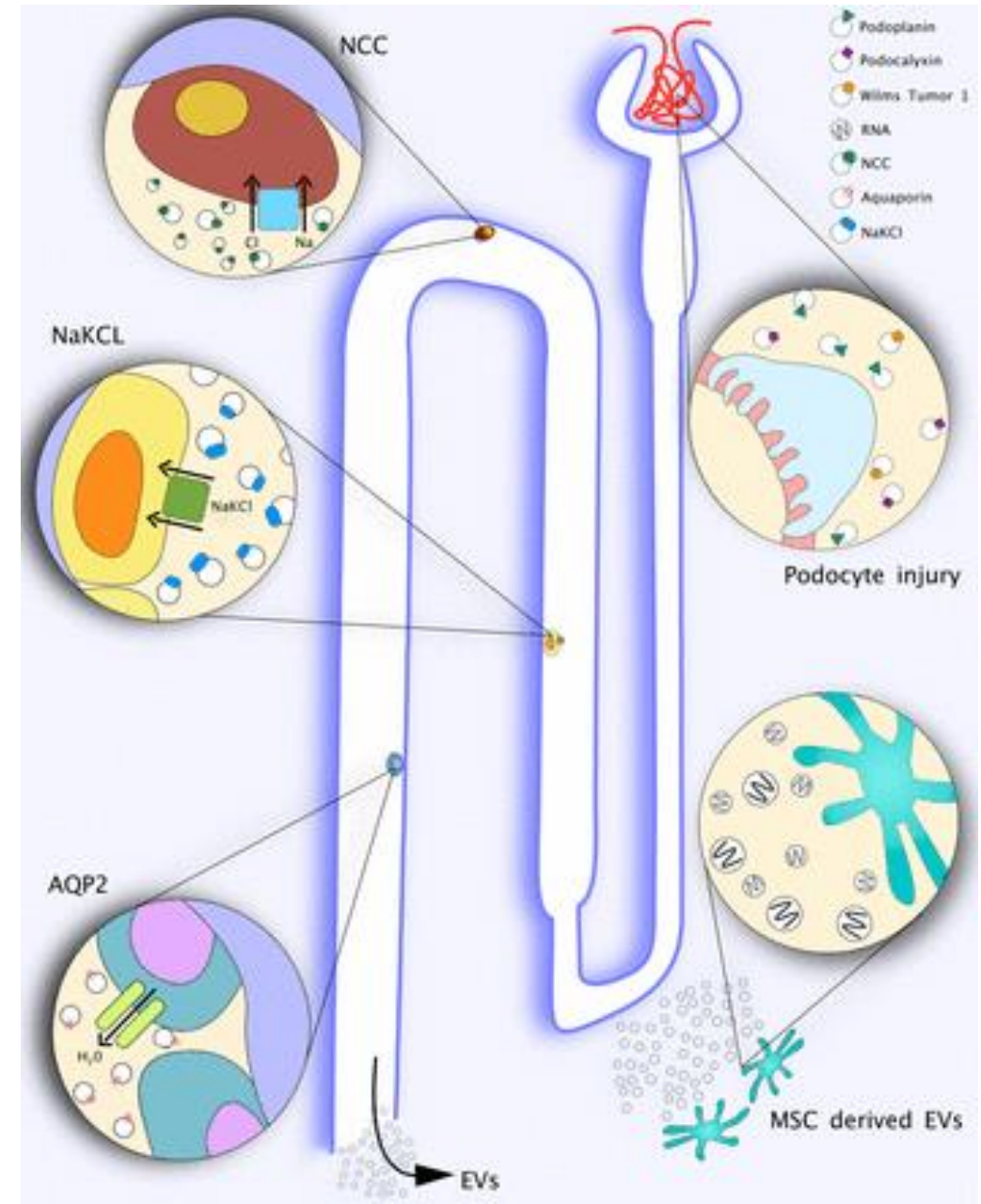
30 to 100 nm



Adapted from [J Am Soc Nephrol](#). 2016 Jan;27(1):12-26.

# Urinary Exosome Biomarkers of Kidney Function

- Direct reflection of urothelial & kidney cell function
- Reflect cell type of origin – can distinguish multiple cell types and kidney regions
- Markers not present/detectable in soluble fraction of urine



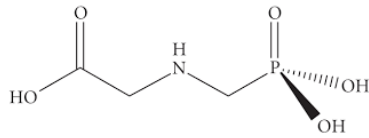
# Urinary exosome biomarkers efforts

- Isolate and characterize urinary exosome from urine samples of farmworkers and from animal model
- Evaluate lipidomics – the lipid content of the exosomes – to identify potential individual or sets of lipids which could represent biomarkers. Lipid composition reflects cell of origin and biological response in kidney cells
- Evaluate miRNA – micro RNA –very small regulatory RNA contained in exosomes – miRNA type and number can reflect the cell of origin and biological response in kidney cells.

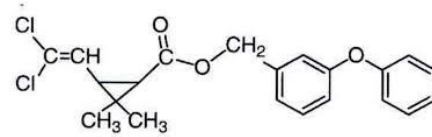
# Animal model of Heat & Pesticide exposure to assess effect on kidney



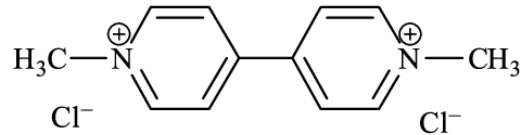
Rat Model



Glyphosate



Permethrin



Paraquat



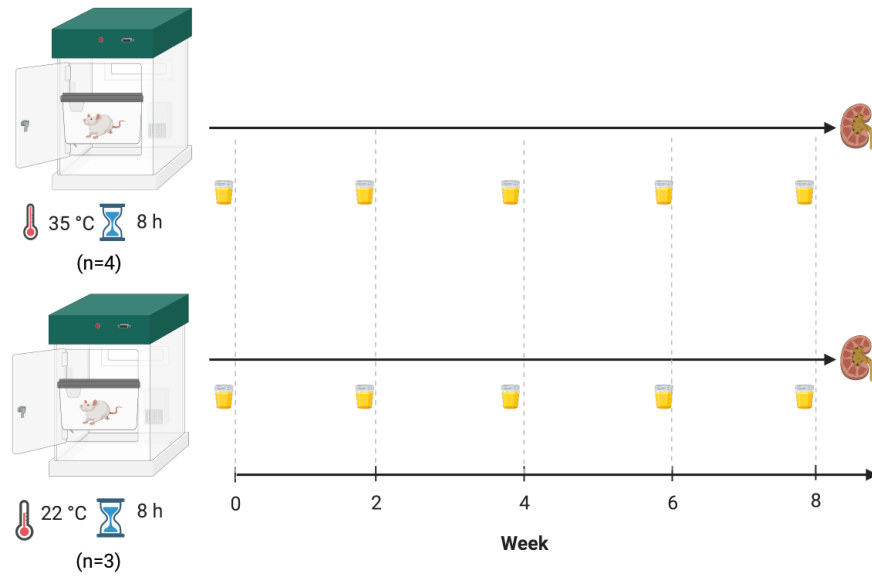
Kidney Disease

OR

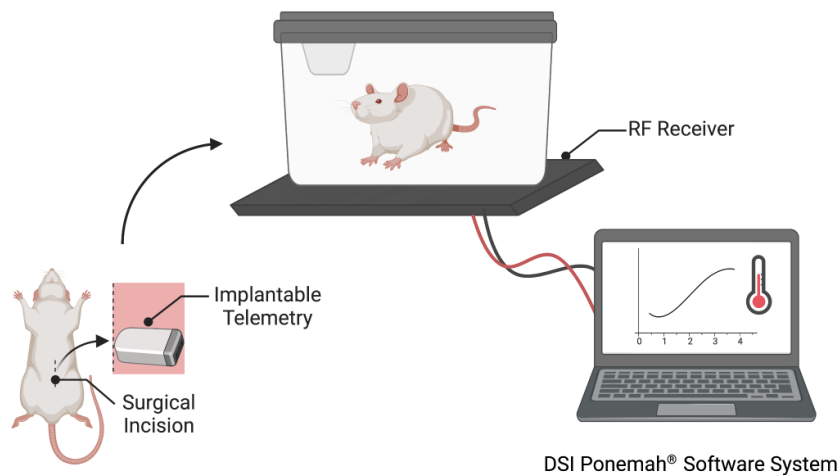
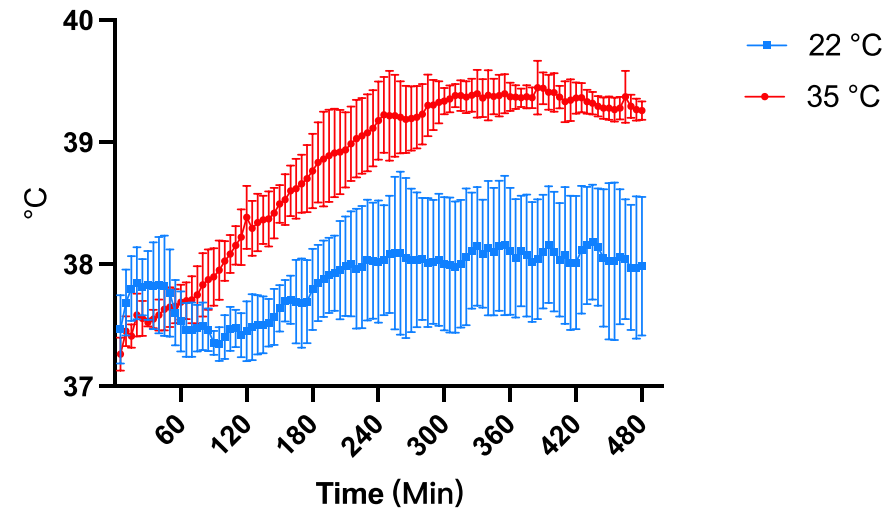
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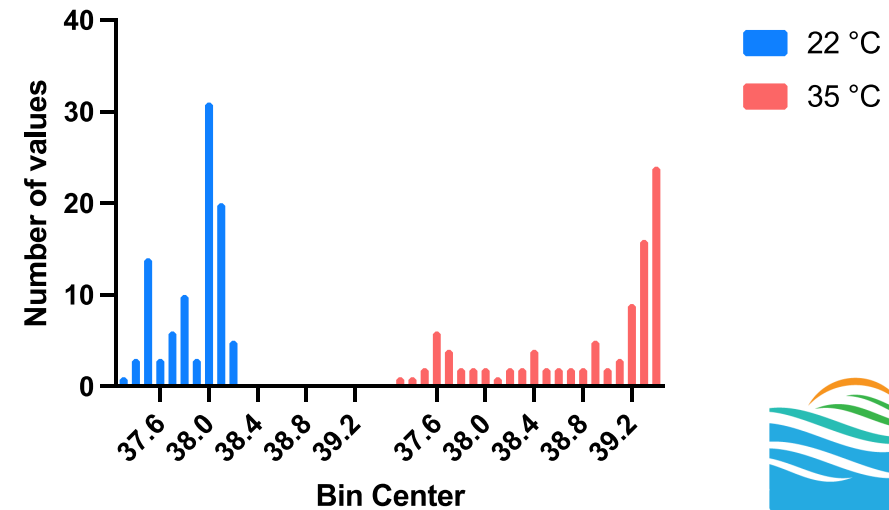
# Heat exposure body temperature monitoring



### Core Body Temperature

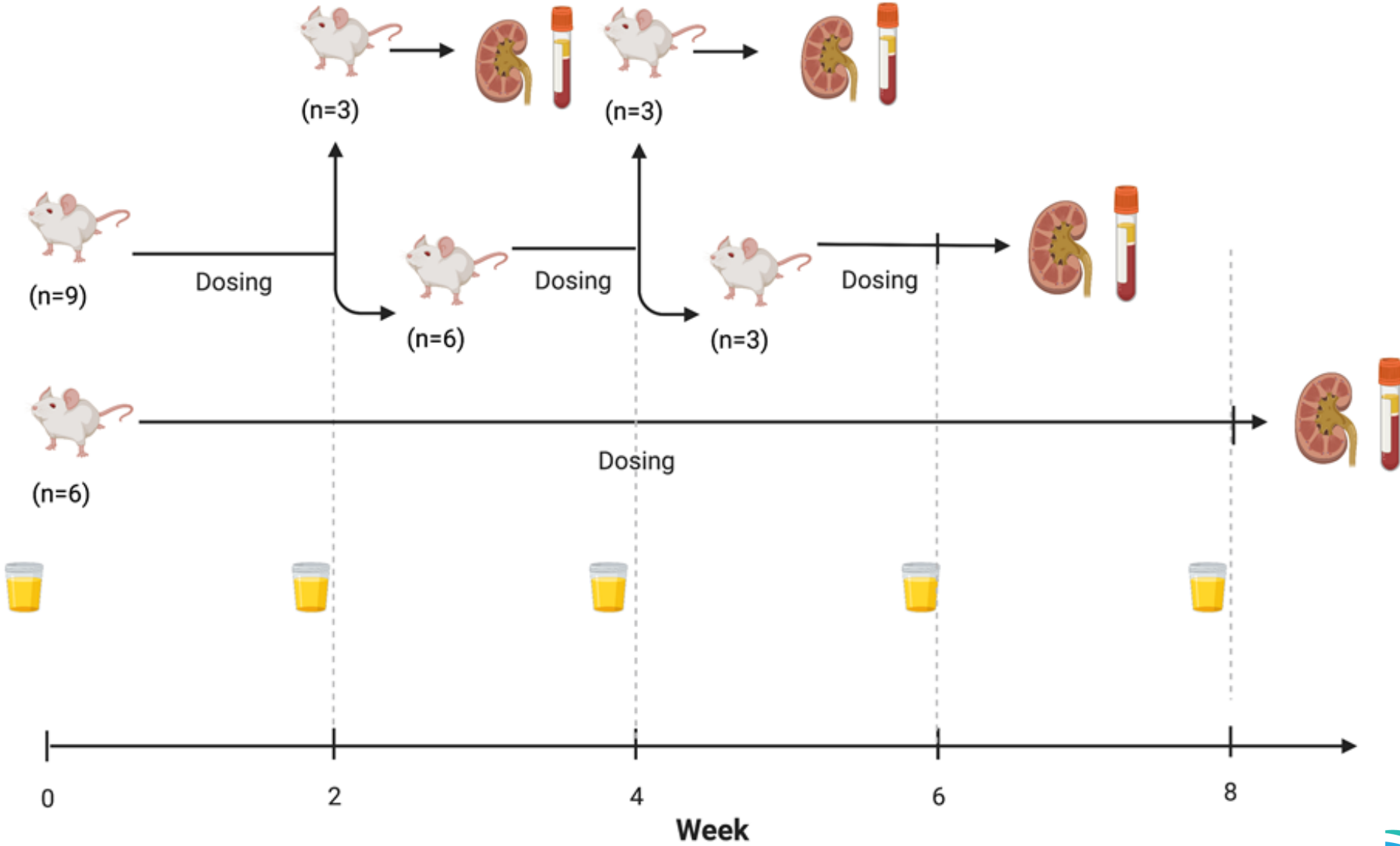


### Frequency distribution





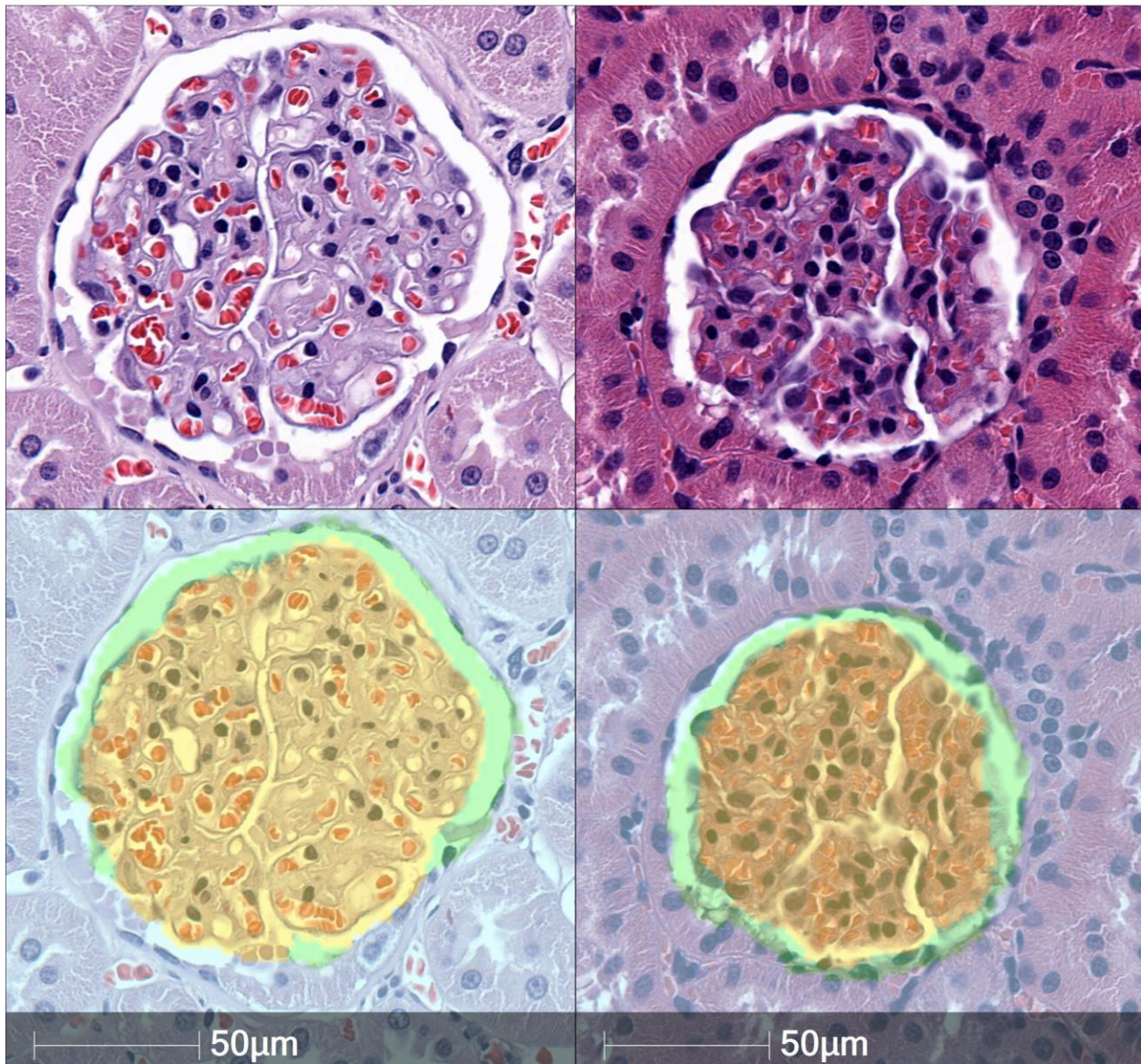
# Chronic intermittent Pesticide exposure study



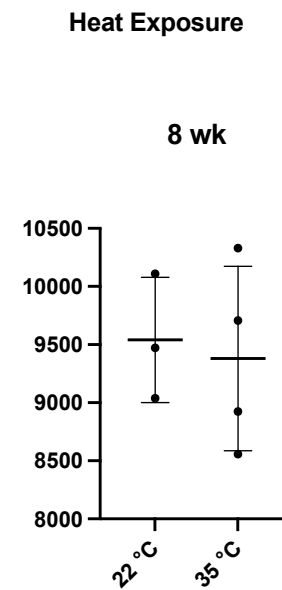
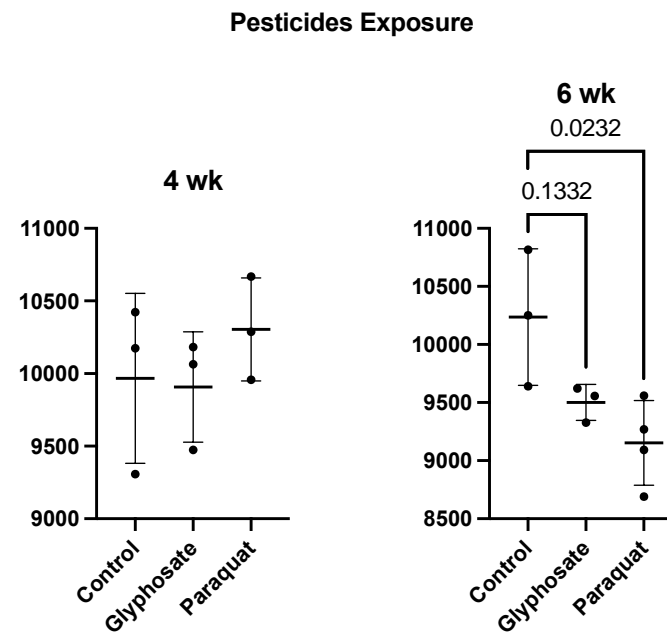
# Animal models allow direct assessment of kidney damage over time by histopathology

## Control

## Treated



**Kidney glomeruli shrinkage with pesticide exposure**



# Next steps in CKDu biomarker study

- Continue existing and novel biomarker studies in farmworkers
  - Populations of study
    - Existing cohorts of Guatemalan farmworkers
    - Establish Florida farmworker cohort
  - Urinary Biomarkers
    - Existing Urine protein biomarkers
    - Exosome based biomarkers
      - Lipid biomarkers
      - miRNA
- Animal Model of heat and pesticide exposure
  - Chronic Intermittent Heat exposure
    - 5 day 8 hr  $>1^{\circ}\text{C}$  core body temperature, two day recovery, 8 weeks
  - Chronic Intermittent individual pesticide exposures
    - Glyphosate, Paraquat, 2,4 D
    - 5 day exposure, relevant dosing, two day recovery, 8 weeks
  - Combined Heat and Pesticide exposure
    - Acute and chronic intermittent exposures
  - Pathology anchored biomarker analysis
    - Kidney pathology – Halo AI analysis
    - Urine biomarkers – existing protein biomarkers and exosome biomarkers

# Acknowledgements



Andres F. Manrique



Jie Zhou



Junli Zhang



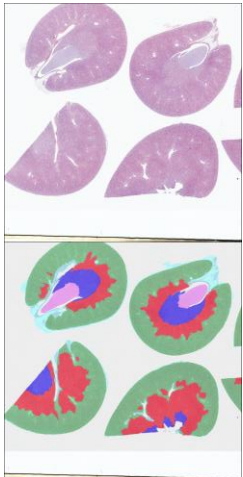
Nancy Denslow



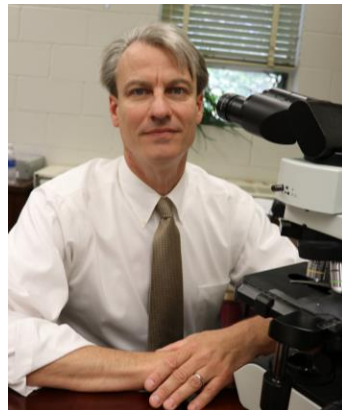
Jamie Butler-Dawson



Abdel Ali



Daniel Verdugo



John Roberts

Funding: NIOSH and NIEHS (Dr. Dawson)



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<u>Marker</u>	<u>Significance/Region</u>	<u>Reference (PMID)</u>
Calbindin D28	highly sensitive to injury to the distal nephron	33161787
Neutrophil gelatinase-associated lipocalin	Early biomarker of AKI caused by various etiologies	24200764
Neutrophil gelatinase-associated lipocalin	Glomeruli, proximal tubules, distal nephron	19148153
Kidney Injury Molecule-1	proximal & distal tubule injury biomarker	34337593
Uromodulin	thick ascending limb; biomarker of kidney tubular health	35948365
$\beta$ 2-macroglobulin	Predicting renal dysfunction	34233325
$\beta$ 2-macroglobulin	Glomeruli	4978446
Osteopontin	Predicting overall survival and renal outcome	20732925
Osteopontin	mainly present in the loop of Henle and distal nephron	11703581
Cystatin C	freely filtered in the glomeruli & mainly reabsorbed by proximal tubules; biomarker of renal function	10369108
glutathione transferase	proximal tubular damage	10463390
alpha-1-Microglobulin	reflects acute and chronic dysfunctions of the proximal tubule	1283528