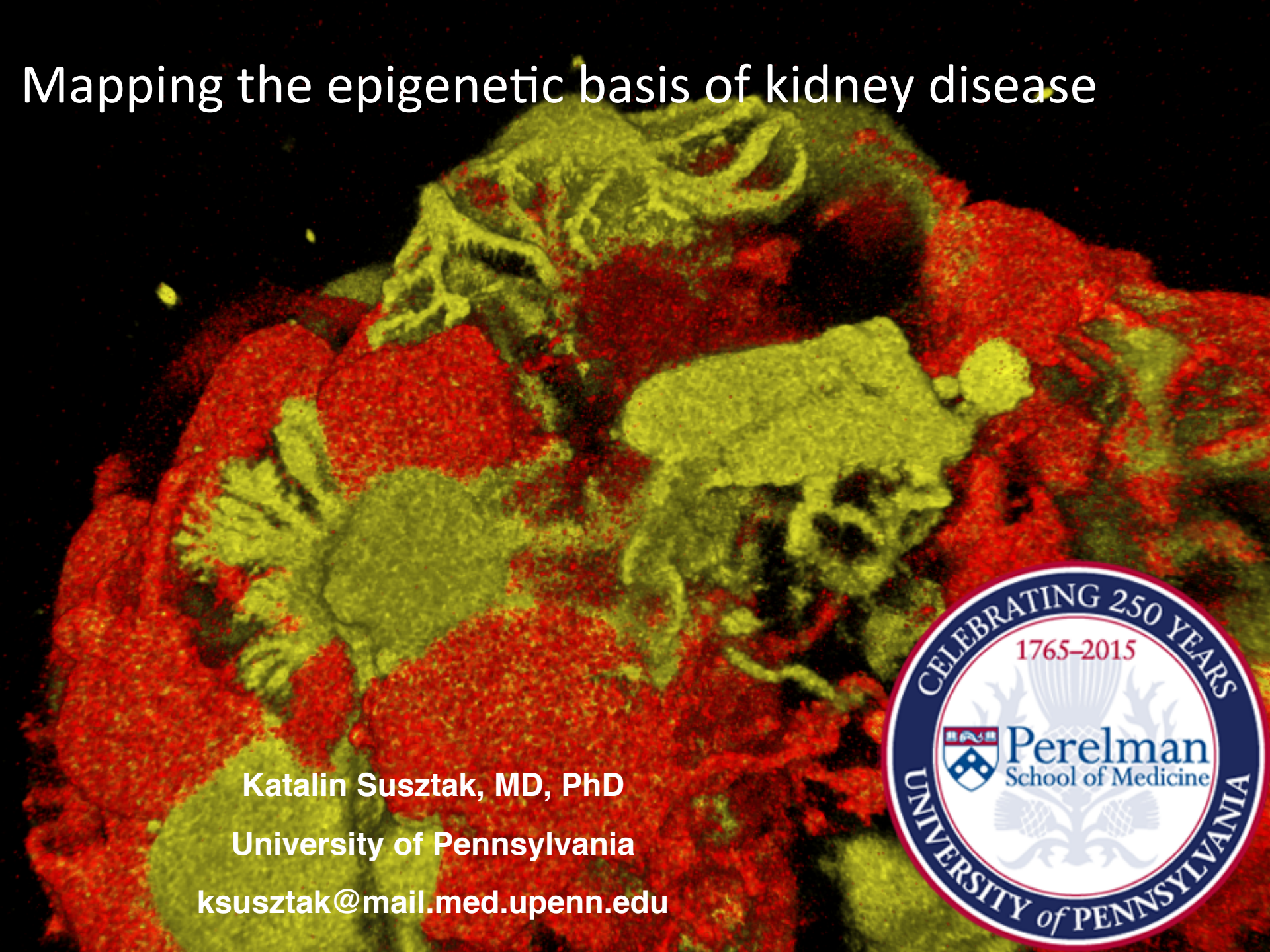
# Mapping the epigenetic basis of kidney disease



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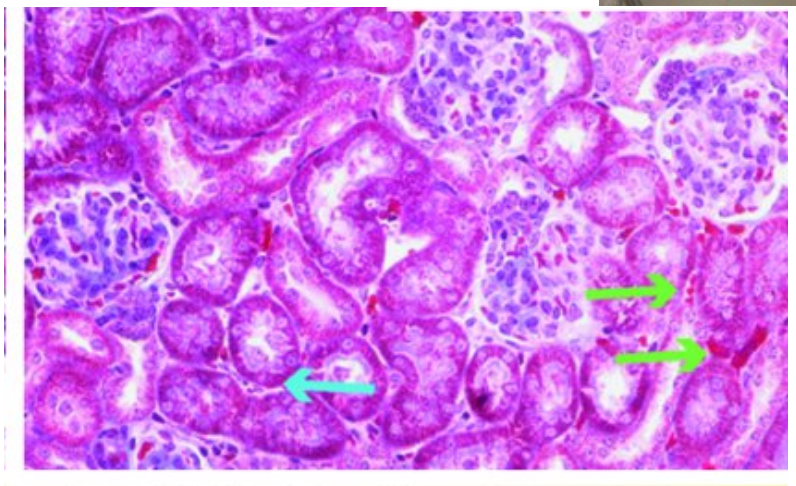
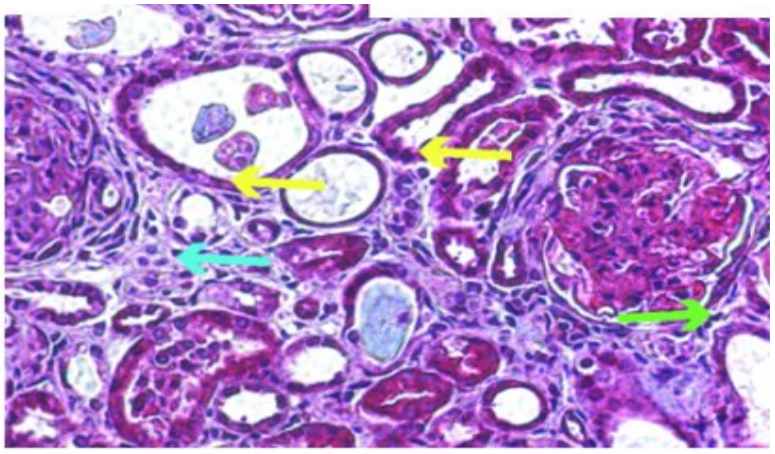
**University of Pennsylvania**

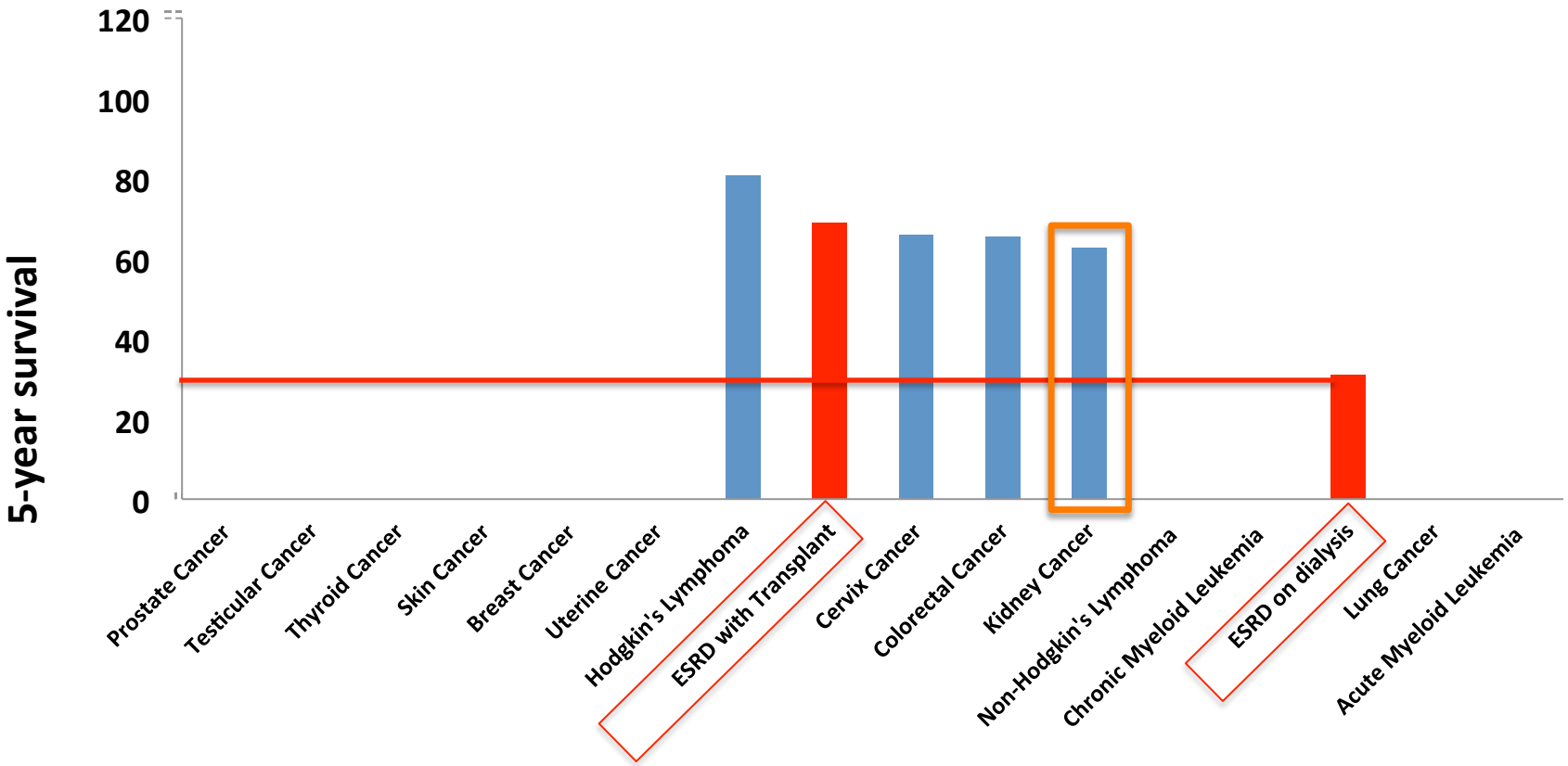
**ksusztak@mail.med.upenn.edu**

# The Problem



# End Stage Kidney Disease




**ESRD care is ~ $30 billion/year ~10% of Medicare budget**

# Why do people develop Kidney Disease ?

# Chronic kidney disease; typical gene environmental disease

ATTCGAGTCA
GTCGGTTCAG
TTTCCCGG

Genome

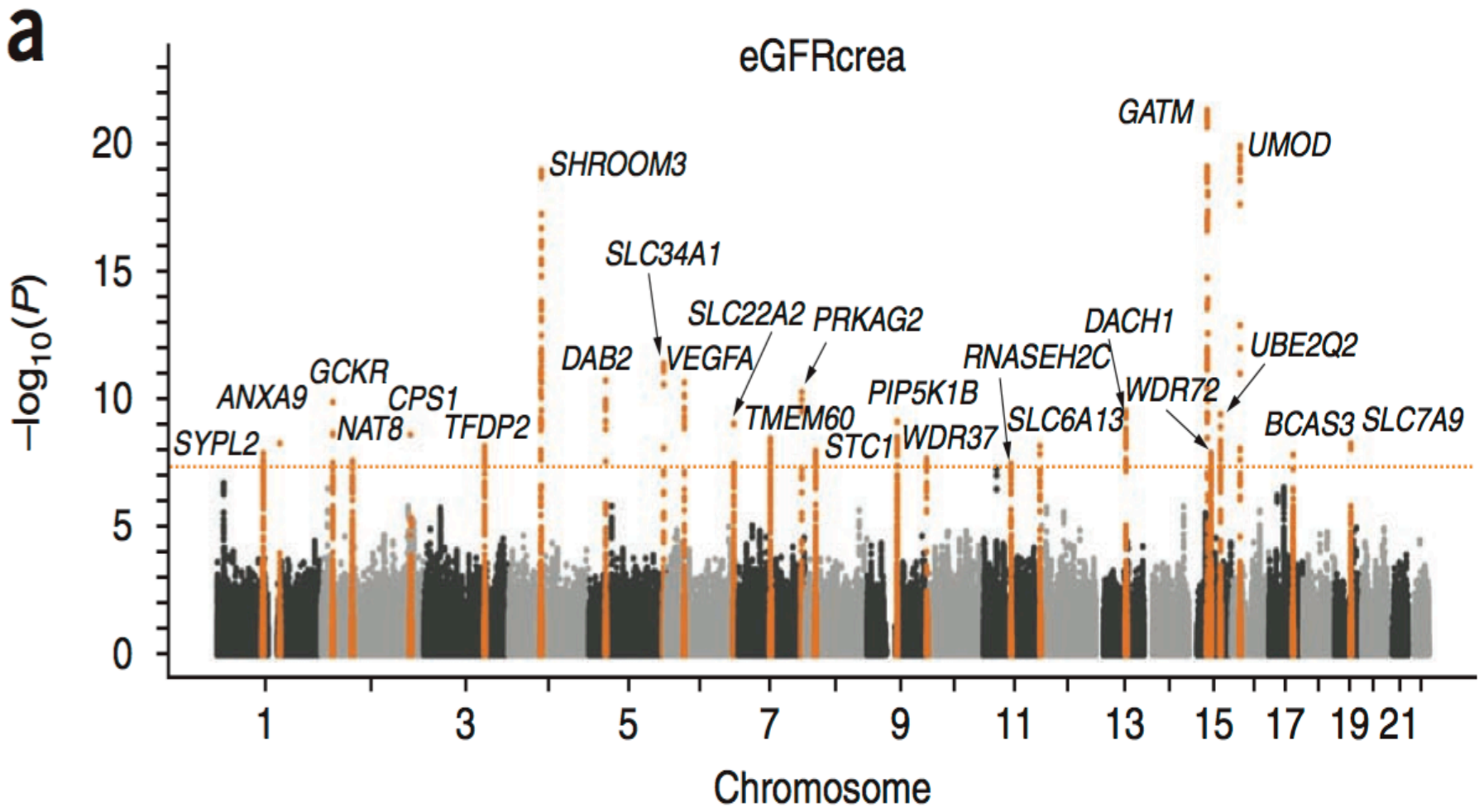
0.33-0.7

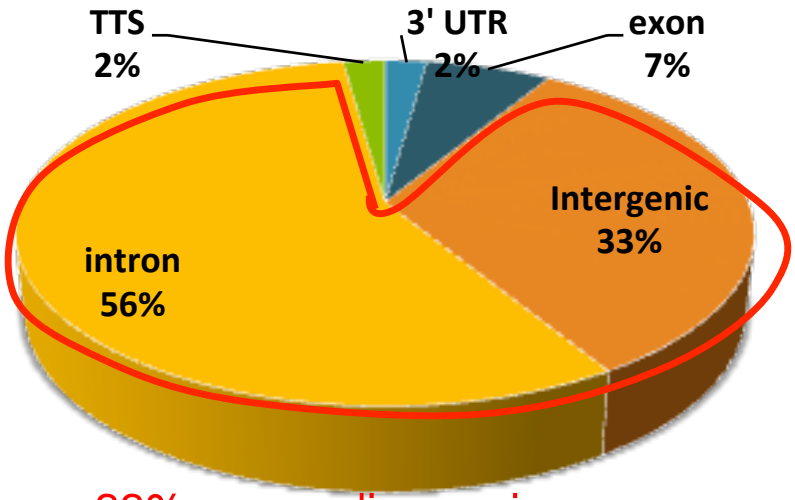
Environment

Transcriptome

Kidney disease

# GWAS for CKD in EUR population






# How do they lead to kidney disease development ?

Causal SNP

Target cell type

Target gene

Mode of dysregulation

Nat Genet. 2009 Jun;41(6):712-7.
Nat Genet. 2010 May;42(5):373-5.
Hum Mol Genet. 2012 Dec 15;21(24):5373-84
Nat Genet. 2011 Jun;43(6):513-8.
PNAS 2009 Jun 9;106(23):9362-7

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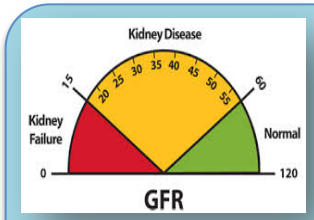
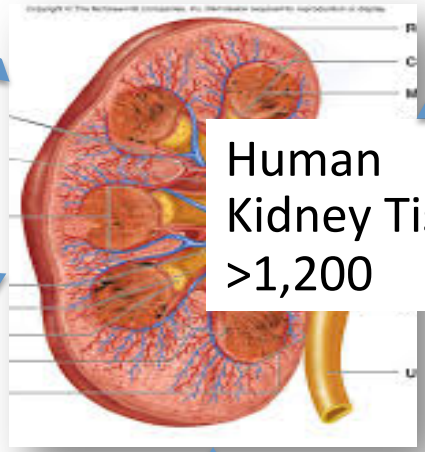
# Integrated translational approach for target identification for chronic kidney disease



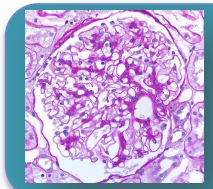

Real time updated
**Clinical data:**

-kidney function
-CVD
-DM

**Transcriptome:**
**Microdissected glomeruli, tubuli**
-RNAsequencing (n=500)



Human
Kidney Tissue
>1,200



**Histopathology:**
Standardized scoring
20 independent parameter

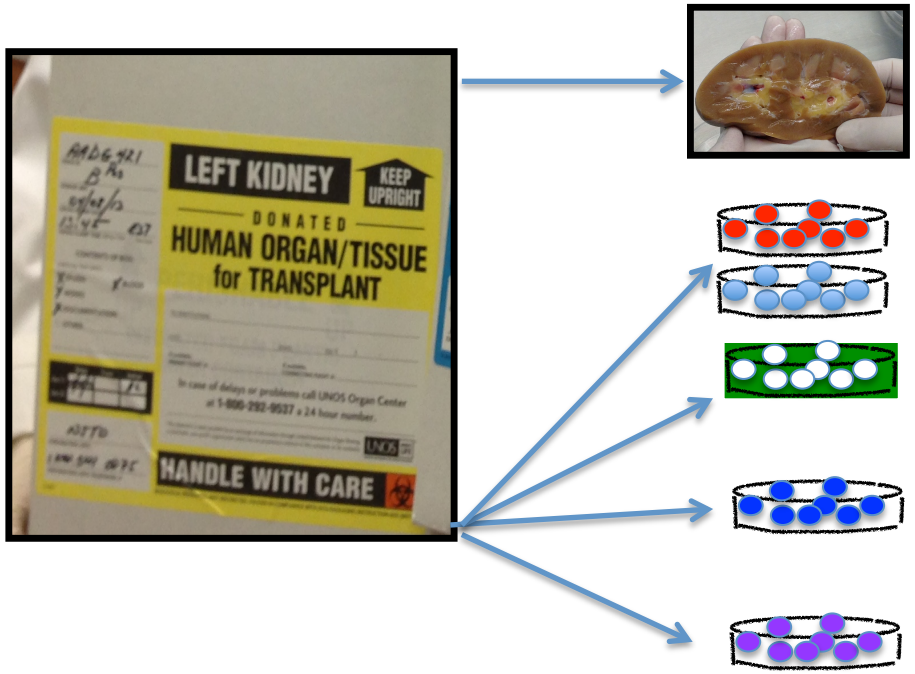
**Epigenome analysis:**

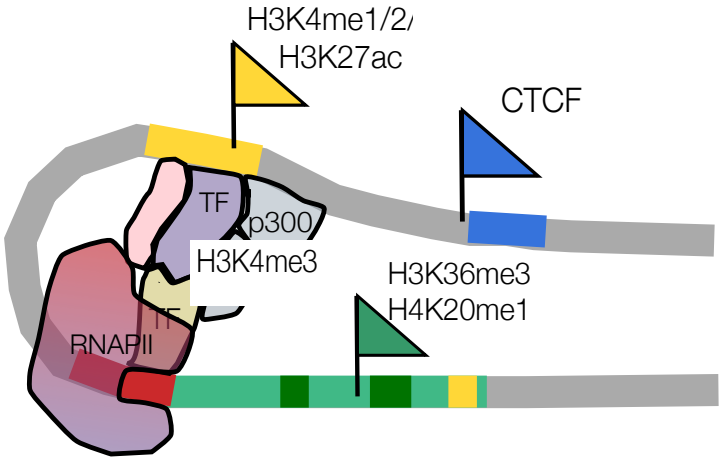
<u>-Cytosine methylation (Infinium arrays)</u>
-500 blood samples
-150 dissected tubule samples

<u>-Histone modification</u>
-Cell type ChIPs (podocytes, endo, mesangial cells, fibroblasts, tubule cells)
-Control vs. CKD tissue samples

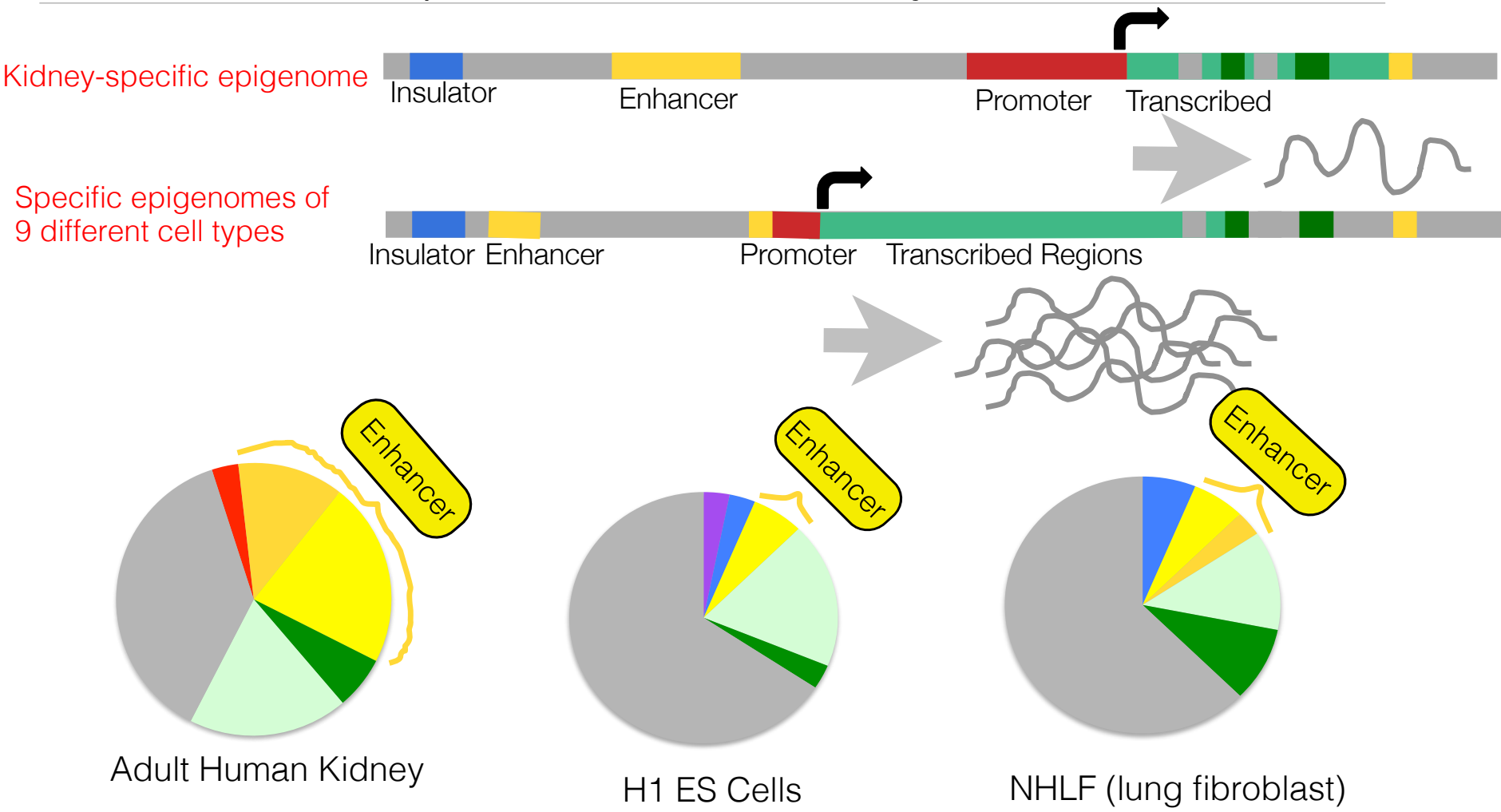
**Genotyping:**
-Affymetrix Axiom Biobank Chips

# 1. The causal variant is localized to a regulatory region in a disease relevant cell type (kidney)

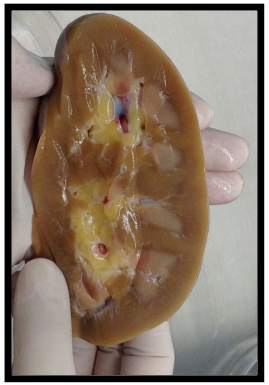


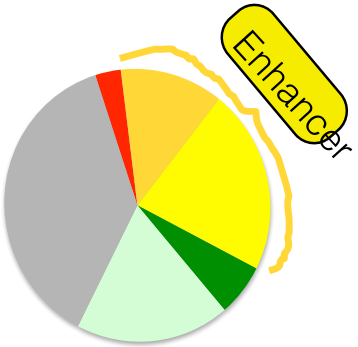



# CKD SNPs are enriched on kidney-specific enhancers in comparison to non-kidney cell lines

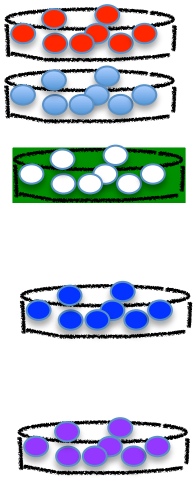


# CKD SNPs are enriched on tubule cell specific enhancers when comparing kidney cell lines

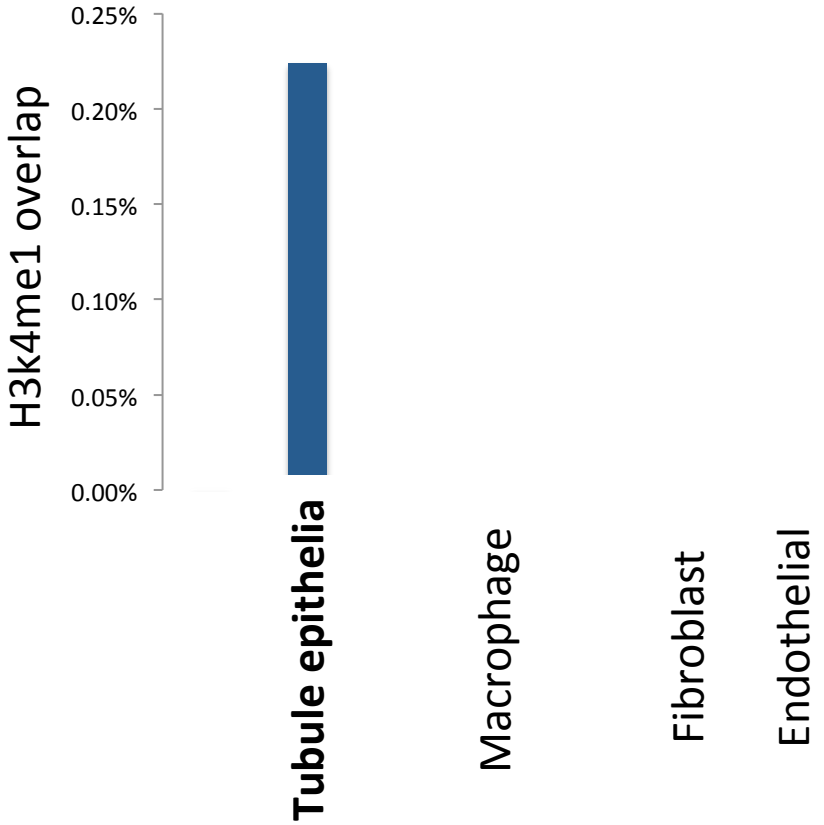




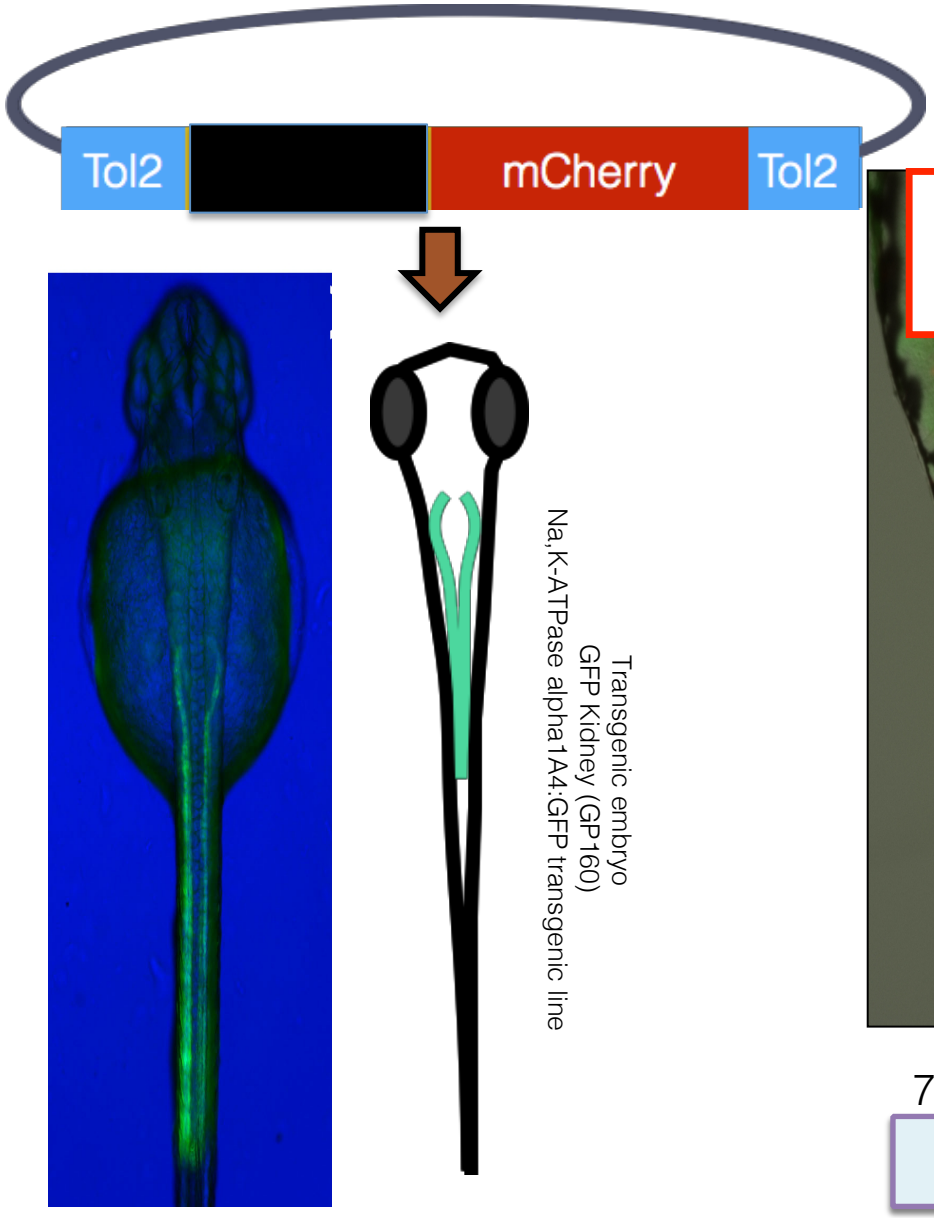

Adult Human Kidney

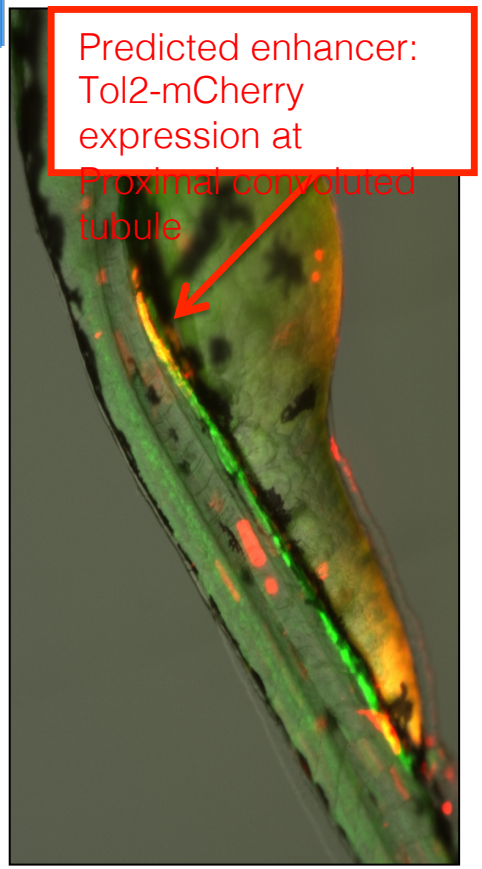


Glomerular
Endothelial
Mesangial
Epithelial (podocyte)
Tubule epithelial
Macrophages
Fibroblasts



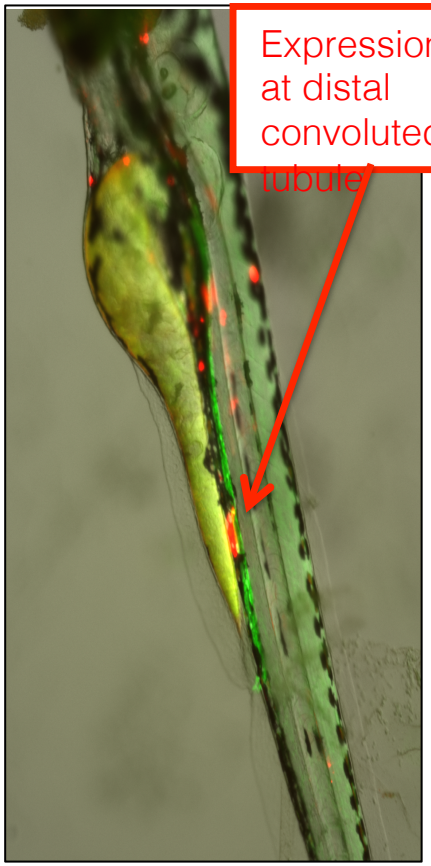
# Experimental validation
# The causal variant is expressed on disease relevant cell type




72 hpf

Chr16
region A




72 hpf

Chr 16
region C

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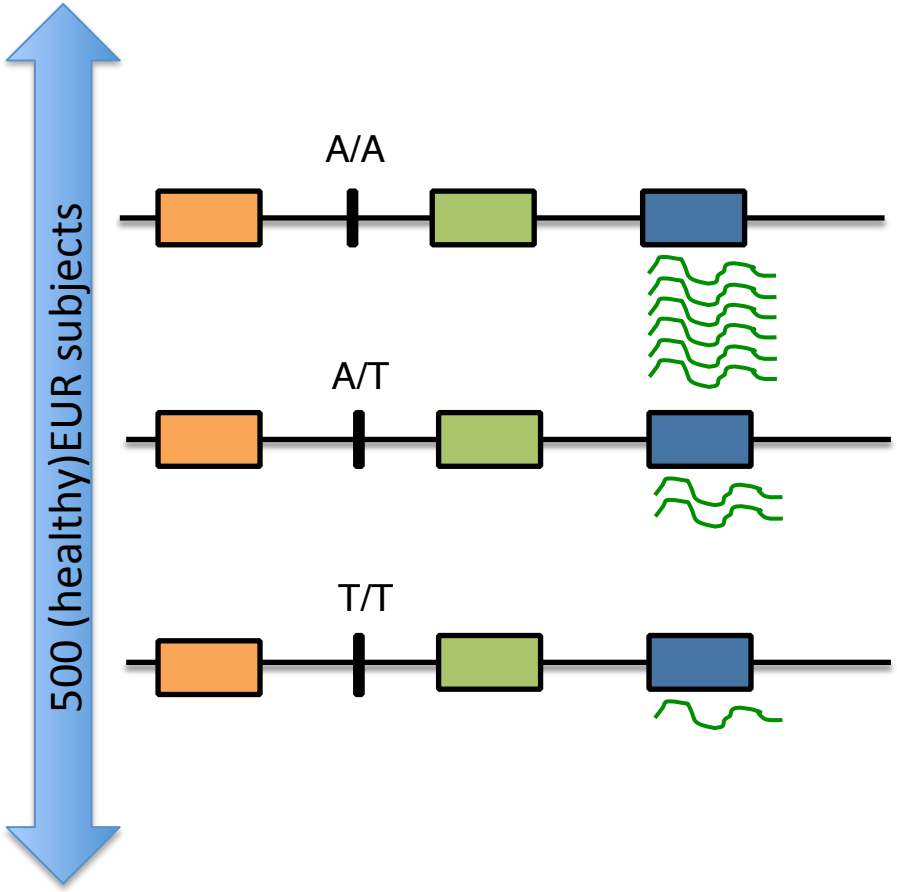
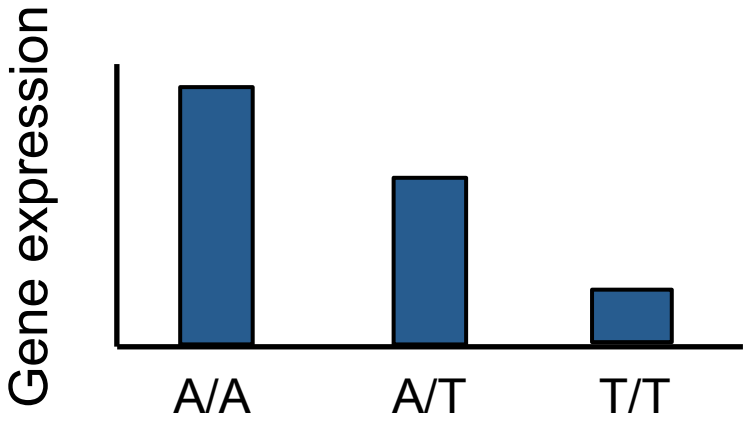
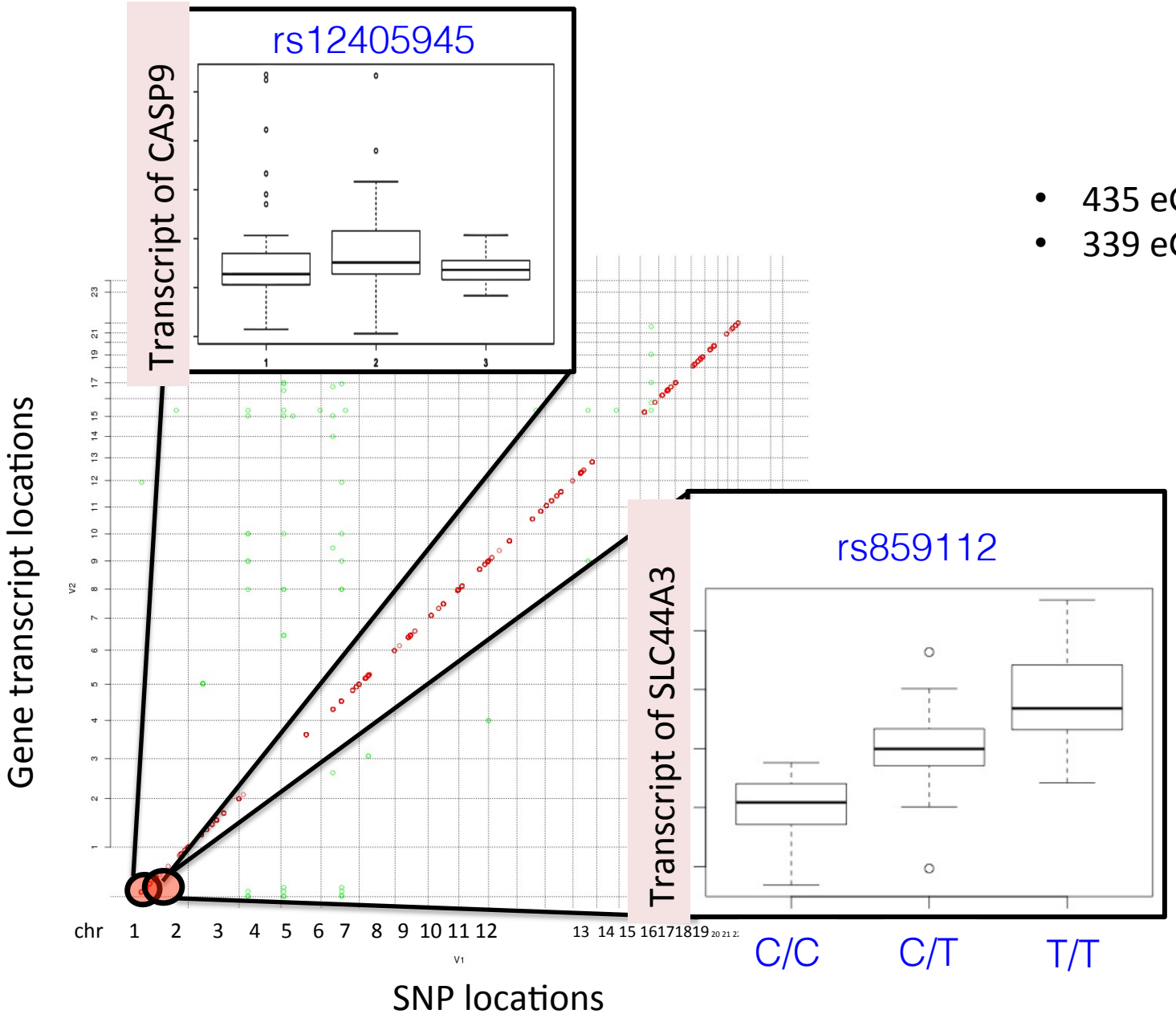
# *The variant alters target expression in disease relevant tissue (the kidney)*




expression quantitative trait locus (eQTL)

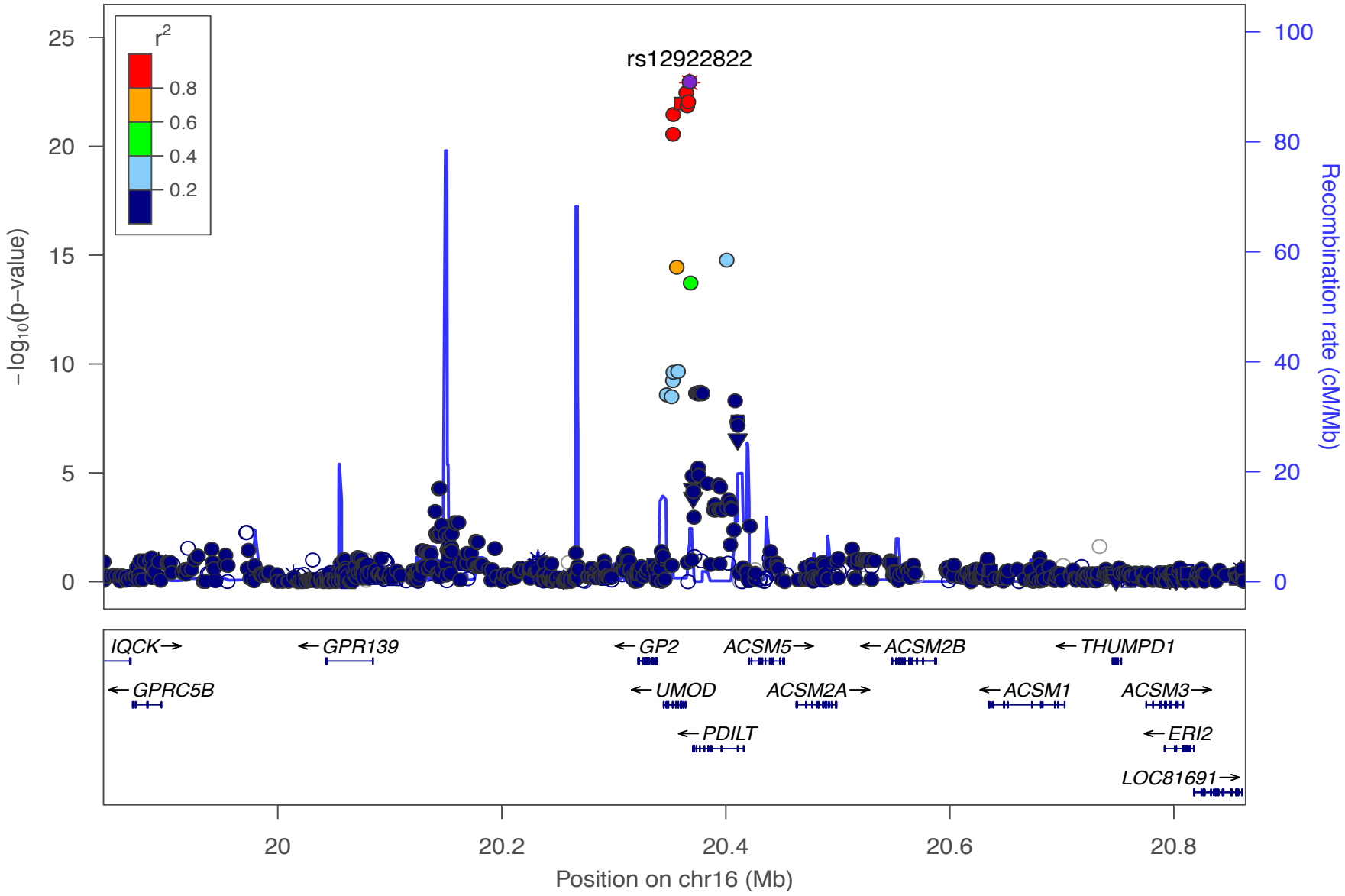
# Genotype driven gene expression changes (99 CEU kidneys)




- 435 eGenes (p-value < $10^{-8}$ )
- 339 eGenes (p-value < $10^{-9}$)

Red spot: *cis*-eqtl
Distance: 1 Mb
*P*-value: 4.0E-10

# *Which gene is the target of the polymorphism?*



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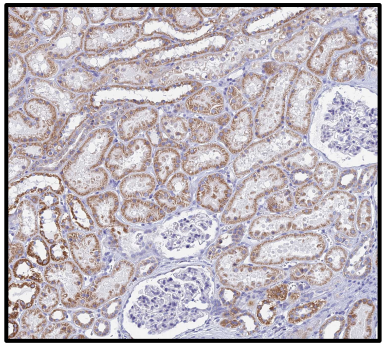
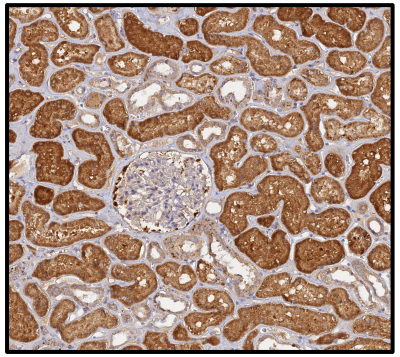
# Which gene is expressed in the kidney ?

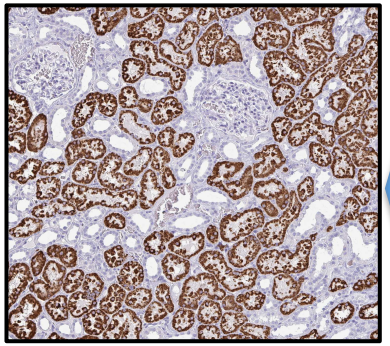
ACSM1



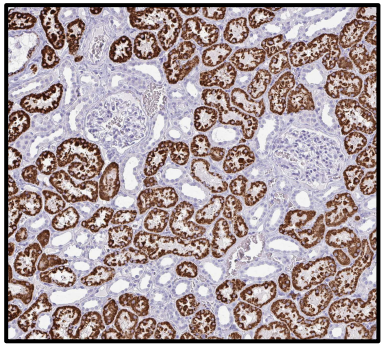
ACSM5



ACSM2A



ACSM2B



| Gene Name | kidney |
|---|---|
| GP2 | 3 |
| UMOD | 1362 |
| PDILT | |
| ACSM5 | 4 |
| ACSM2A | 106 |
| ACSM2B | 119 |
| ACSM1 | 0.7 |

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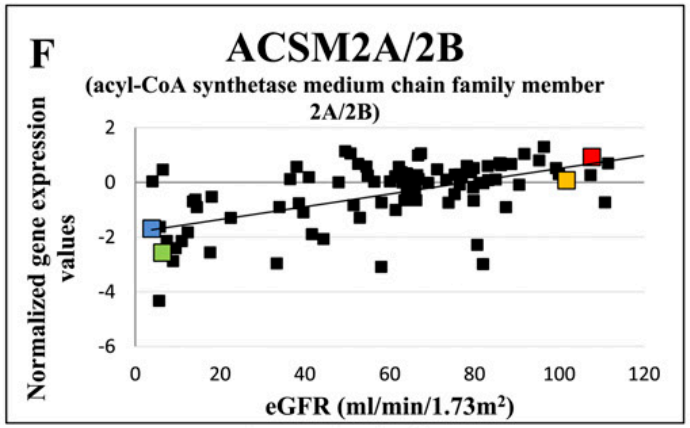
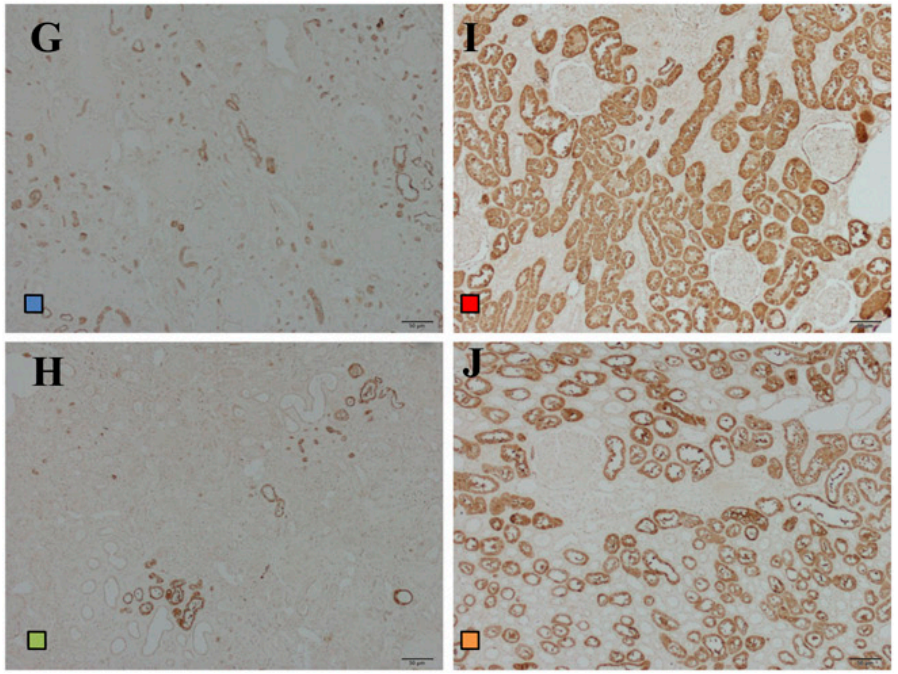
# Expression of the target correlates with kidney function




Pearson R = 0.526
$R^2$ = 0.2768
Pcorr = 2.45 x $10^{-6}$



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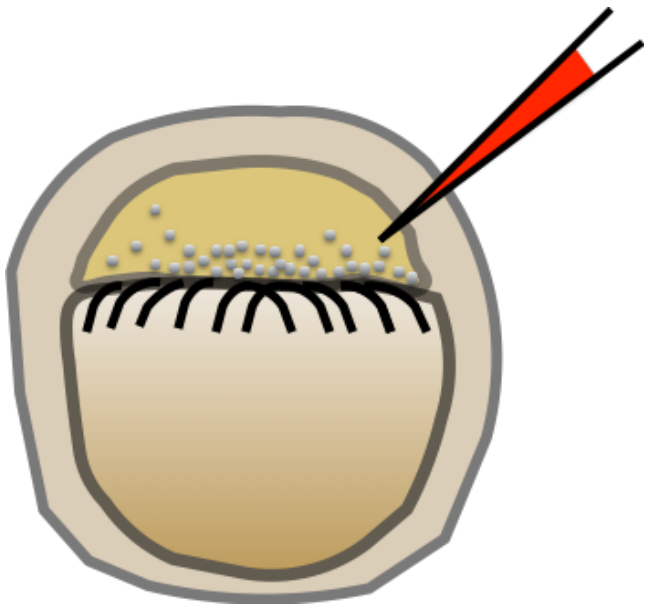
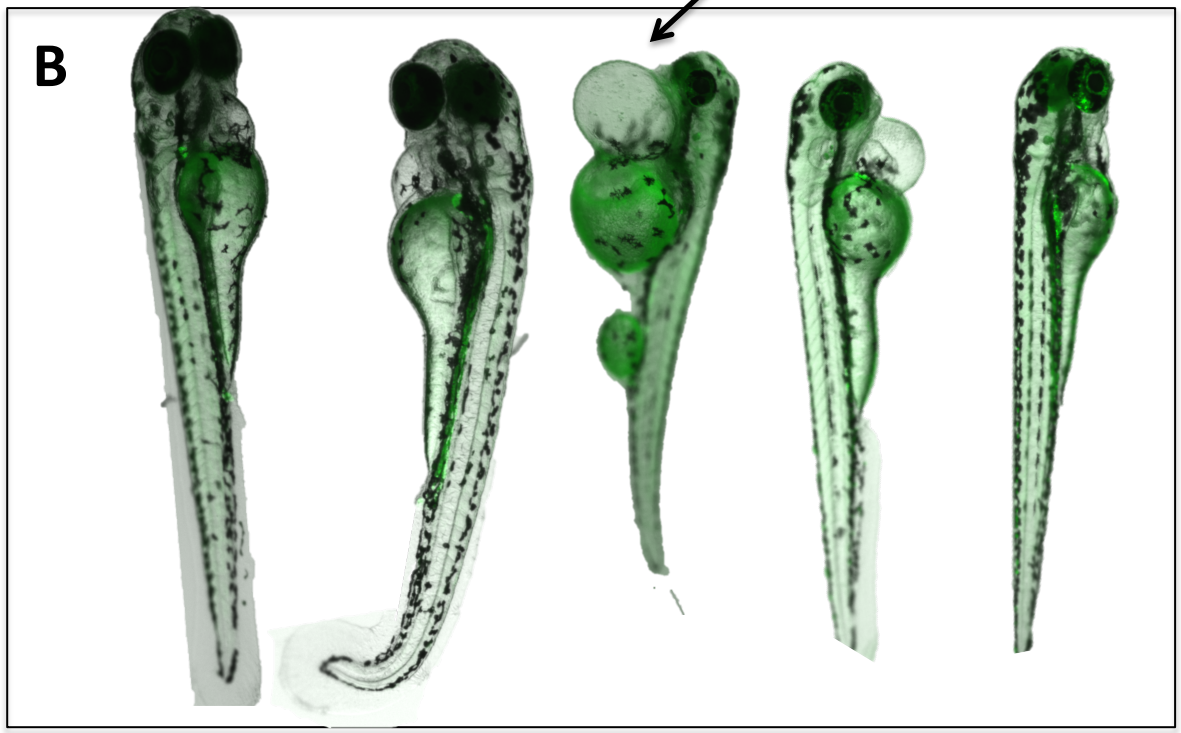
# Functional studies in model organism



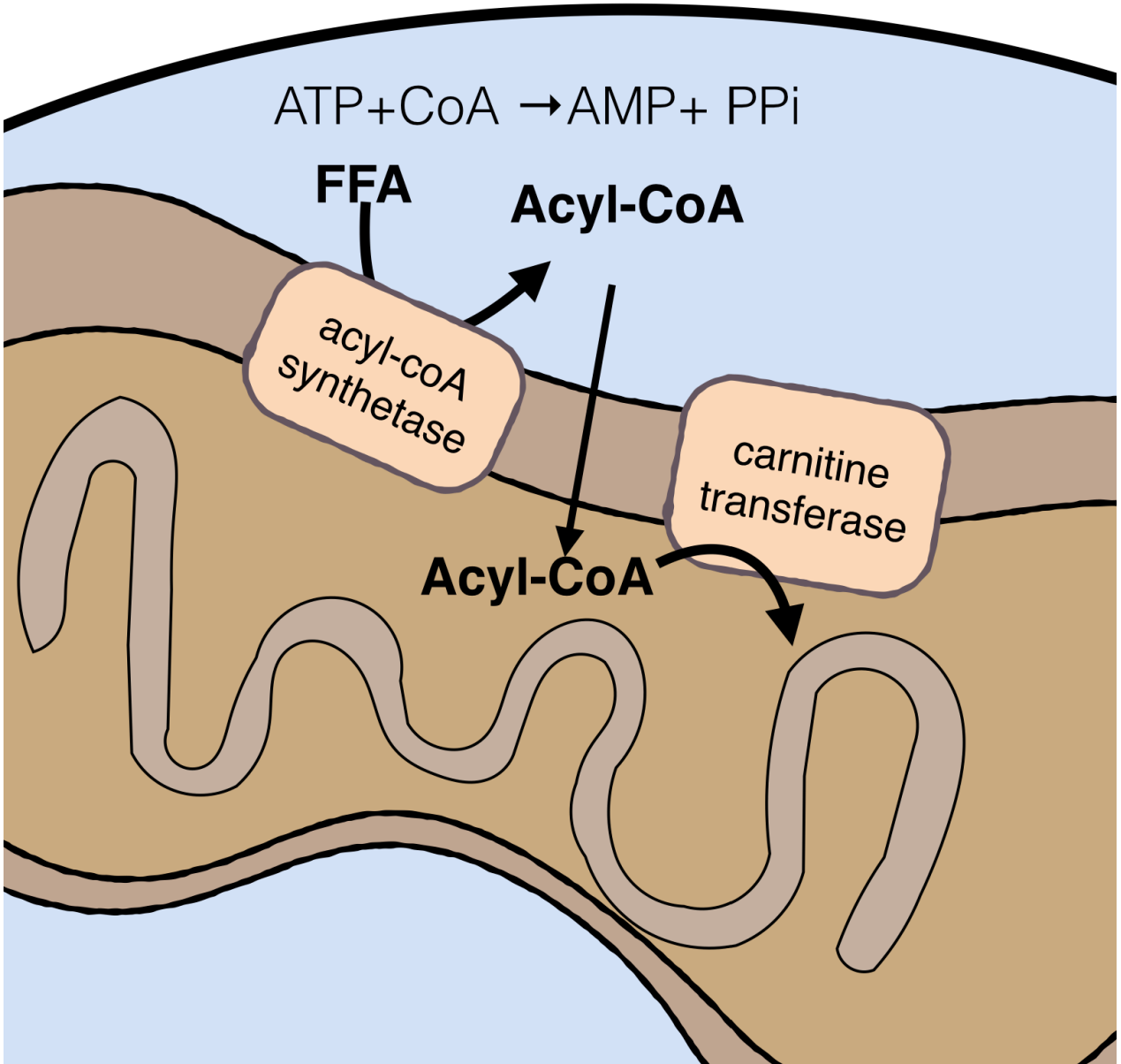
Morpholino knock down
in zebrafish embryo

Acsm3 200 uM

Pericardial edema
(sign of kidney damage)



# Functional studies in model organism



# CONCLUSION 1.

Our Roadmap to understand GWAS associated hits

-Human tissue samples are needed

-Epigenome maps to identify regulatory DNA

-Model organisms to validate validate causal variant

-eQTL maps to identify target genes

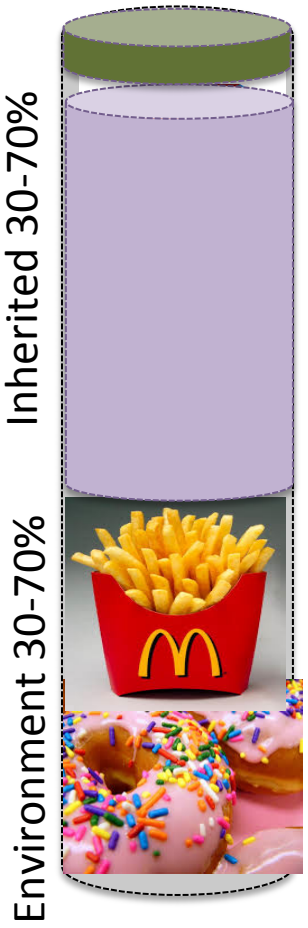
-Examine kidney expression, correlation with kidney function

-Model organisms to validate gene function (zfish to mouse)

Our analysis indicate that ACSM gene family are likely targets of a common CKD GWAS hit on Chr16

*Fatty acid metabolism might be the target of common CKD associated GWAS variant*

# These variants explain small fraction of heritability




Can be explained by sequence variants (SNP)

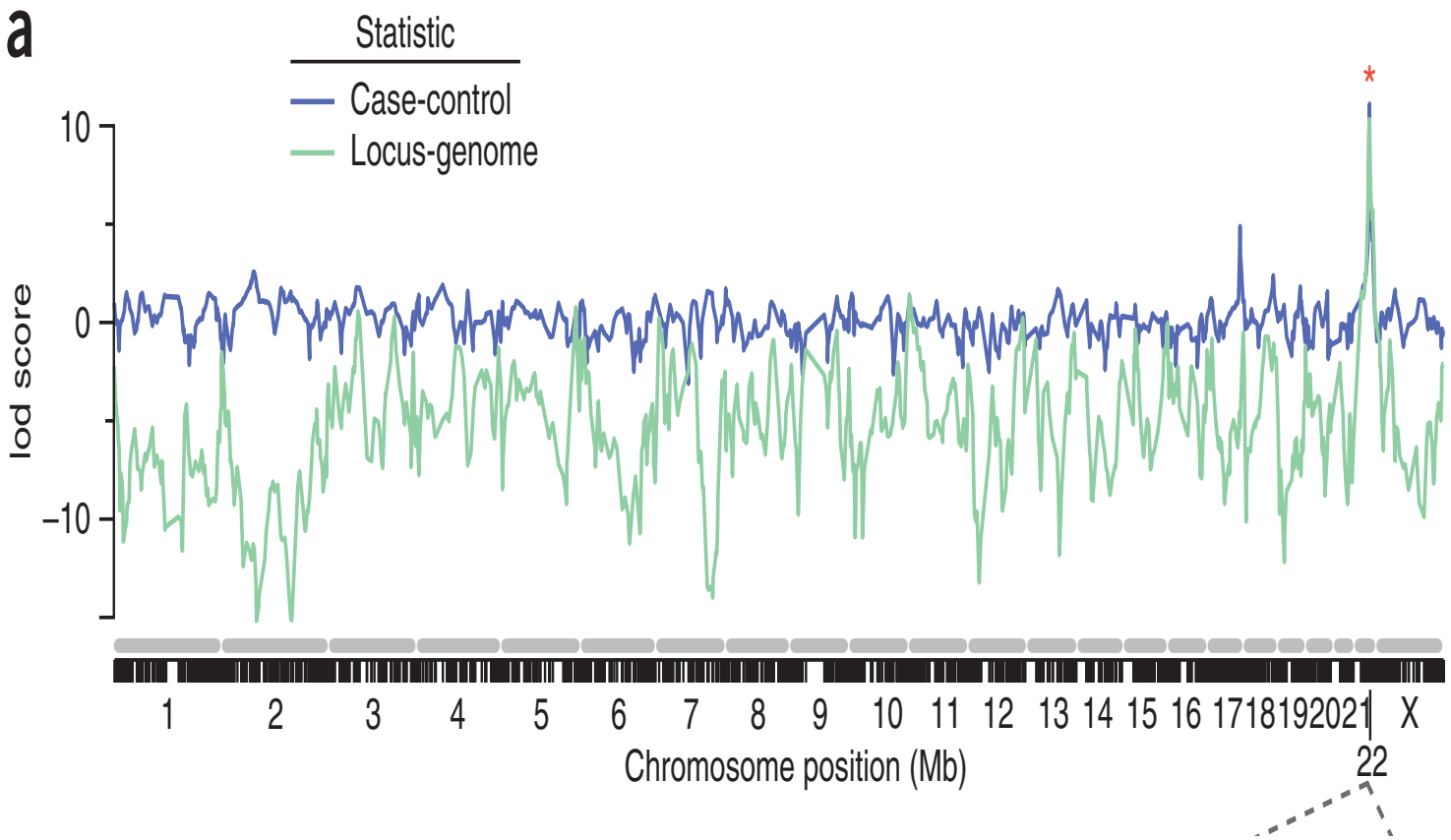
Inherited but we can not identify DNA sequence variation

**Missing heritability**

Larger sample size
**Different ethnic groups**
Deeper sequencing
**Epigenetics**

# Different ethnic groups…

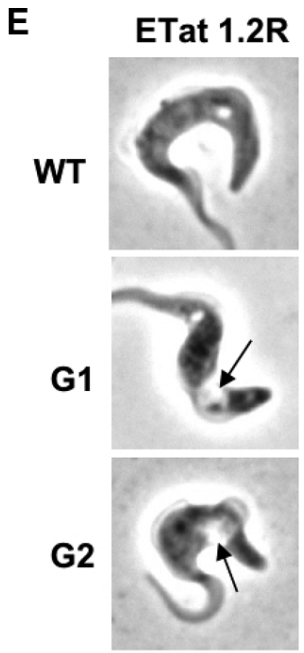
## …admixture study in YRB for kidney disease…




Kopp et al Nature Genetics

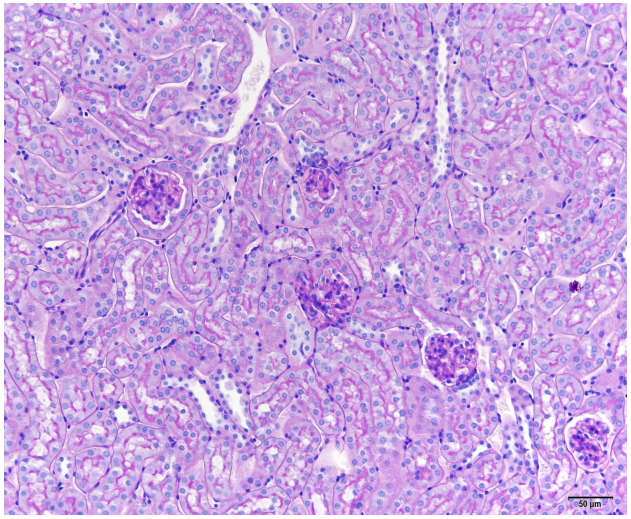
# Association of Trypanolytic ApoL1 Variants with Kidney Disease in African Americans

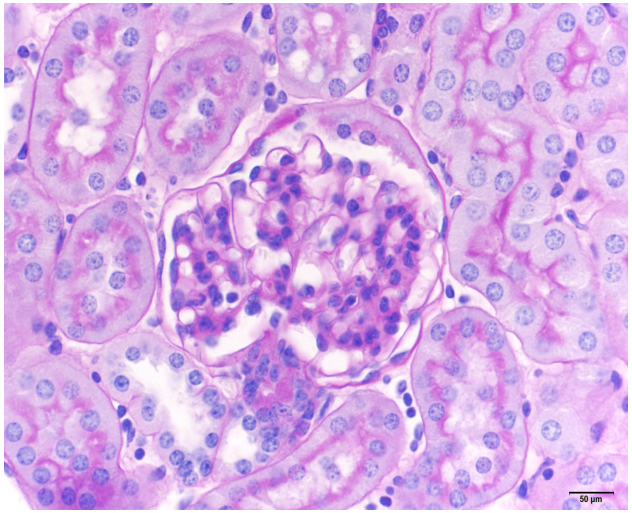
Giulio Genovese,[1,2]* David J. Friedman,[1,3]* Michael D. Ross,[4] Laurence Lecordier,[5] Pierrick Uzureau,[5] Barry I. Freedman,[6] Donald W. Bowden,[7,8] Carl D. Langefeld,[8,9] Taras K. Oleksyk,[10] Andrea L. Uscinski Knob,[4] Andrea J. Bernhardy,[1] Pamela J. Hicks,[7,8] George W. Nelson,[11] Benoit Vanhollebeke,[5] Cheryl A. Winkler,[12] Jeffrey B. Kopp,[11] Etienne Pays,[5]† Martin R. Pollak[1,13]†



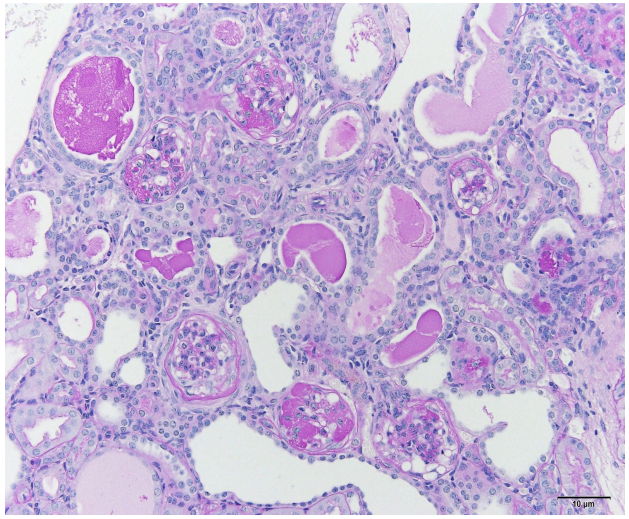
# APOL1 variants cause kidney disease in mice

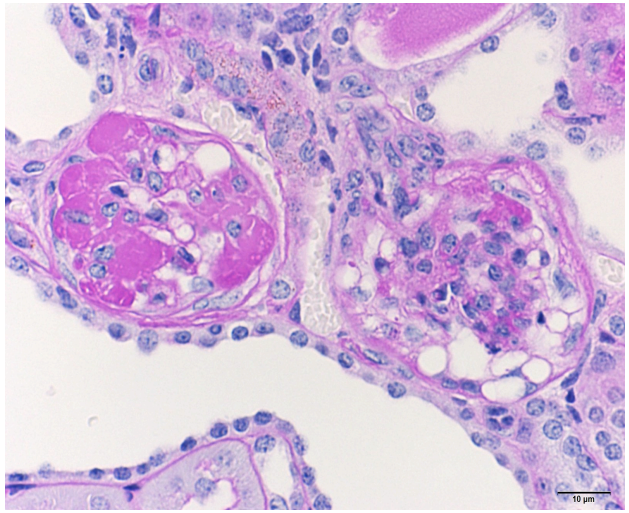
NephrinrtTA/TREG0APOL1



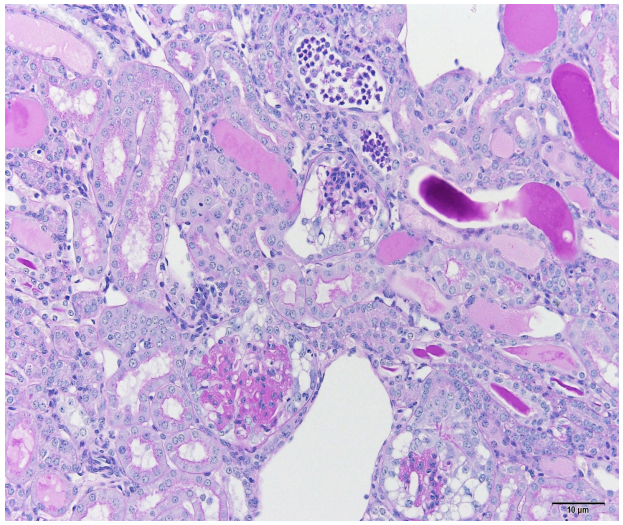


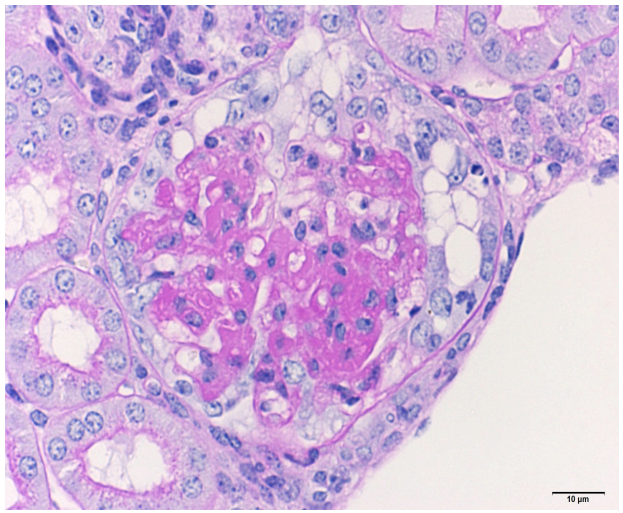
NephrinrtTA/TREG1APOL1





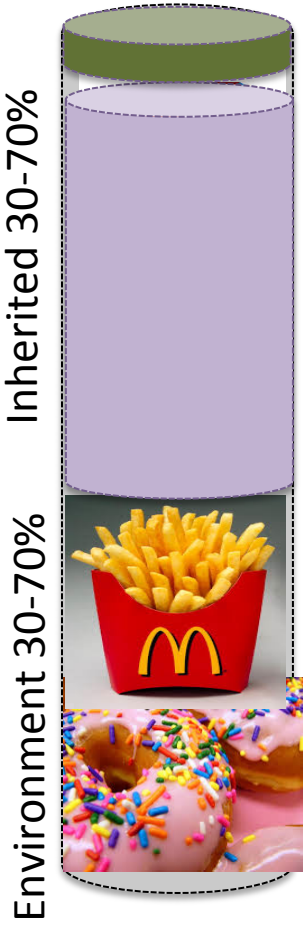
NephrinrtTA/TREG2APOL1





Beckerman, Park and Susztak unpublished

# These variants explain small fraction of heritability




Can be explained by sequence variants (SNP)

Inherited but we can not identify DNA sequence variation

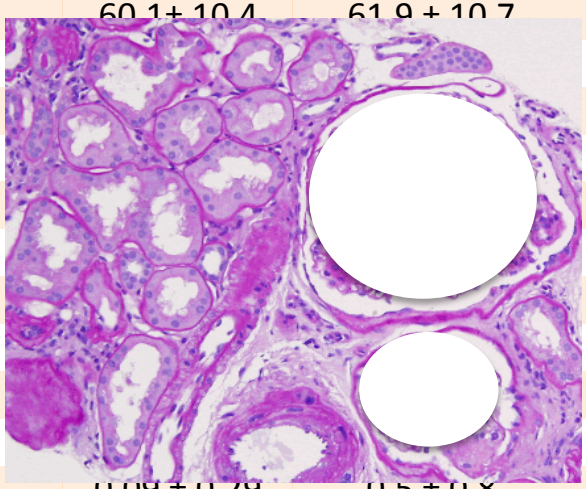
**Missing heritability**

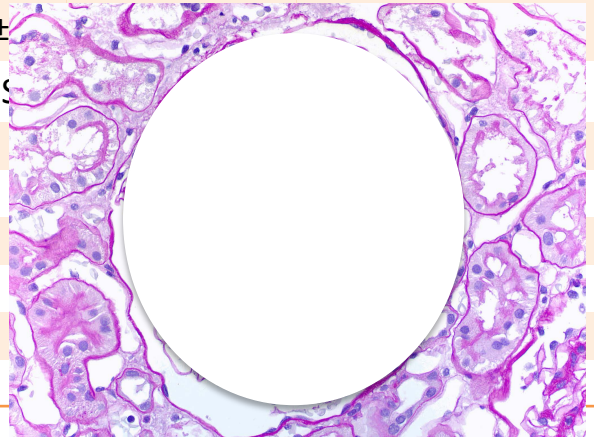
**EPIGENETICS**

# Epigenetic studies in patients with kidney disease

**Demographics of the research participants**

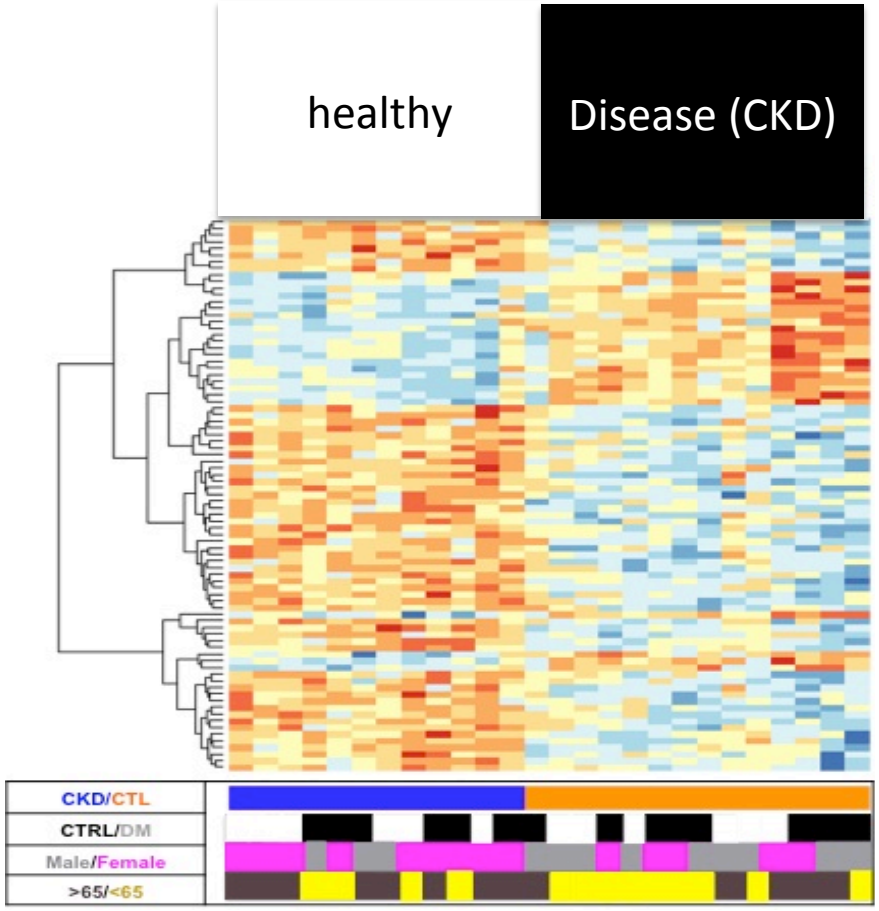
| Characteristics | Control | Hypertension | DM | DKD |
|---|---|---|---|---|
| n | 23 | 23 | 20 | 21 |
| Age (years) Mean ± SD | 60.1± 10.4 | 61.9 ± 10.7 | 65.3±12.1 | 65.8± 12.3 |
| Ethnicity | | | | |
| Asian, Pacific Islander | | | 1 | 0 |
| White, non-Hispanic | | | 2 | 5 |
| Black, non-Hispanic | | | 6 | 7 |
| Hispanic | | | 2 | 3 |
| Other&Unknown | | | 9 | 6 |
| BMI (kg/m2) Mean ± SD | | | 28.9±5.53. | 32.5 ± 7.76 |
| Diabetes | | | 20 | 21 |
| Hypertension | | | 17 | 19 |
| Proteinuria (dipstick) | 0.09 ± 0.29 | 0.5 ± 0.8 | 0.74 ± 1.19 | 3.0 ± 1.7 |
| Serum BUN (mg/dL) Mean ± SD | 15.30 ± 5.38 | 15.56 ± 7.23 | 17.25 ± 4.66 | 35.20 ± 15.82 |
| Serum creatinine (mg/dL) Mean ± | | | 0.98 ± 0.19 | 3.18 ± 3.22 |
| eGFR (ml/min/1.73 $m^2$) Mean ± S | | | 74.47 ± 16.26 | 30.32 ± 17.21 |
| Histology | | | | |
| Tubular atrophy (%) | | | 4.5 ± 4.02 | 31.8 ± 22.2 |
| Interstitial fibrosis (%) | | | 5.5 ± 4.18 | 29.6 ± 19.2 |
| Glomerulosclerosis (%) | | | 7.13 ± 7.52 | 31.11 ± 32.25 |
| Mesangial matrix Expansion | | | 0.32 ± 0.40 | 1.38 ± 1.18 |
| Arteriosclerosis Intima | | | 1.0 ± 0.7 | 2.0 ± 0.8 |

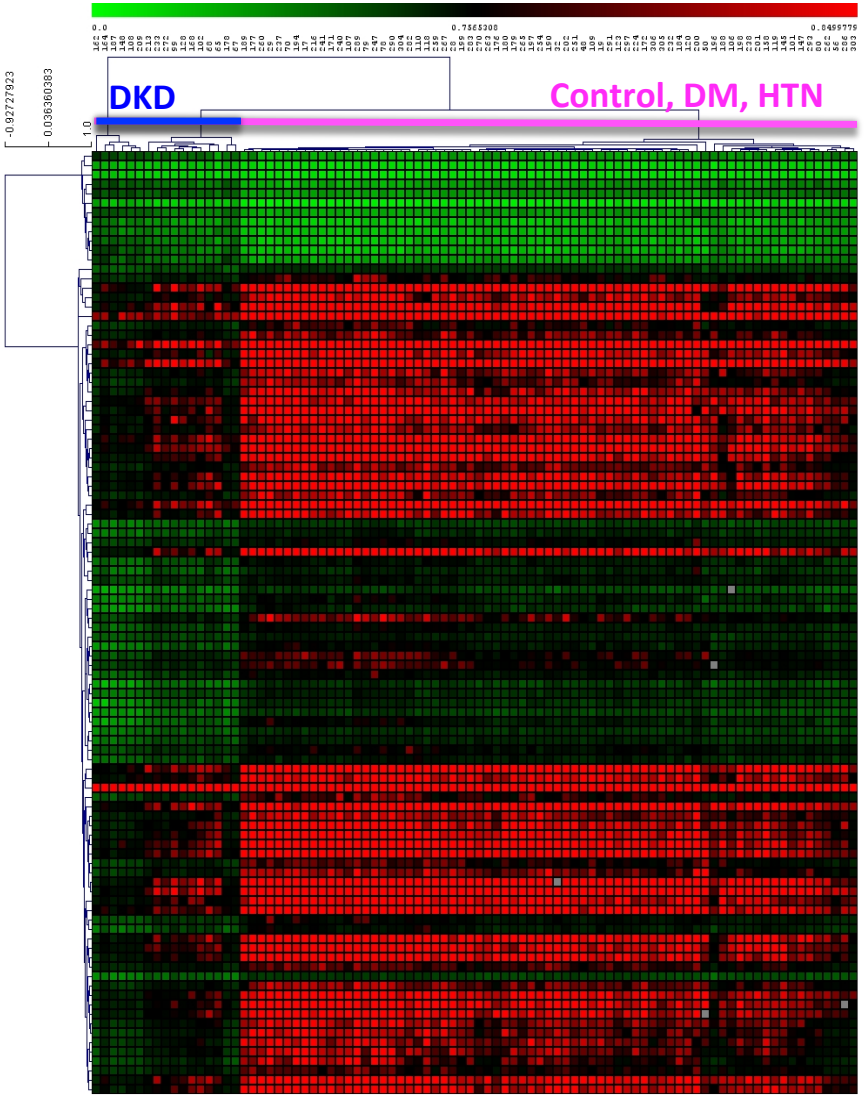




Genome wide
Cytosine methylation analysis
MRE-Chip
Illumina 450K

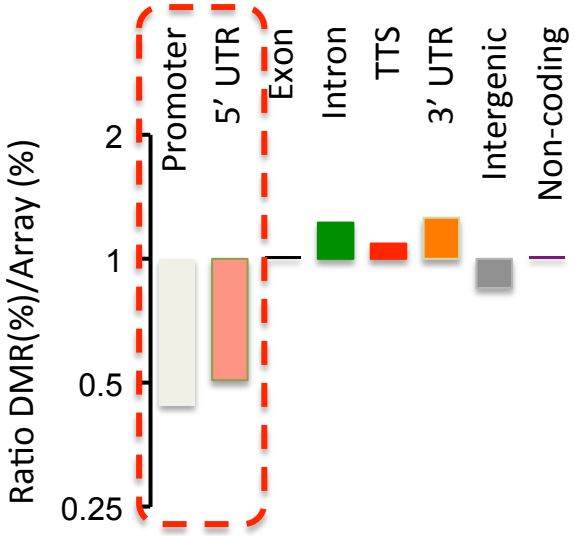
# Distinct Cytosine Methylation Profiles tubule cells obtained from patients with Diabetic kidney Disease

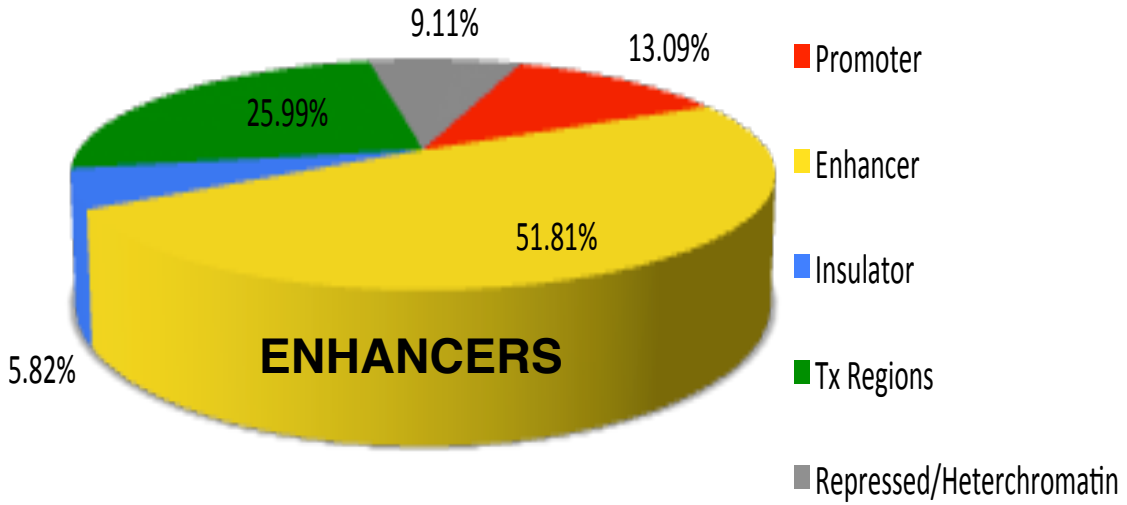



Cytosine methylation ($p<10^{-16}$ $\Delta>13\%$)

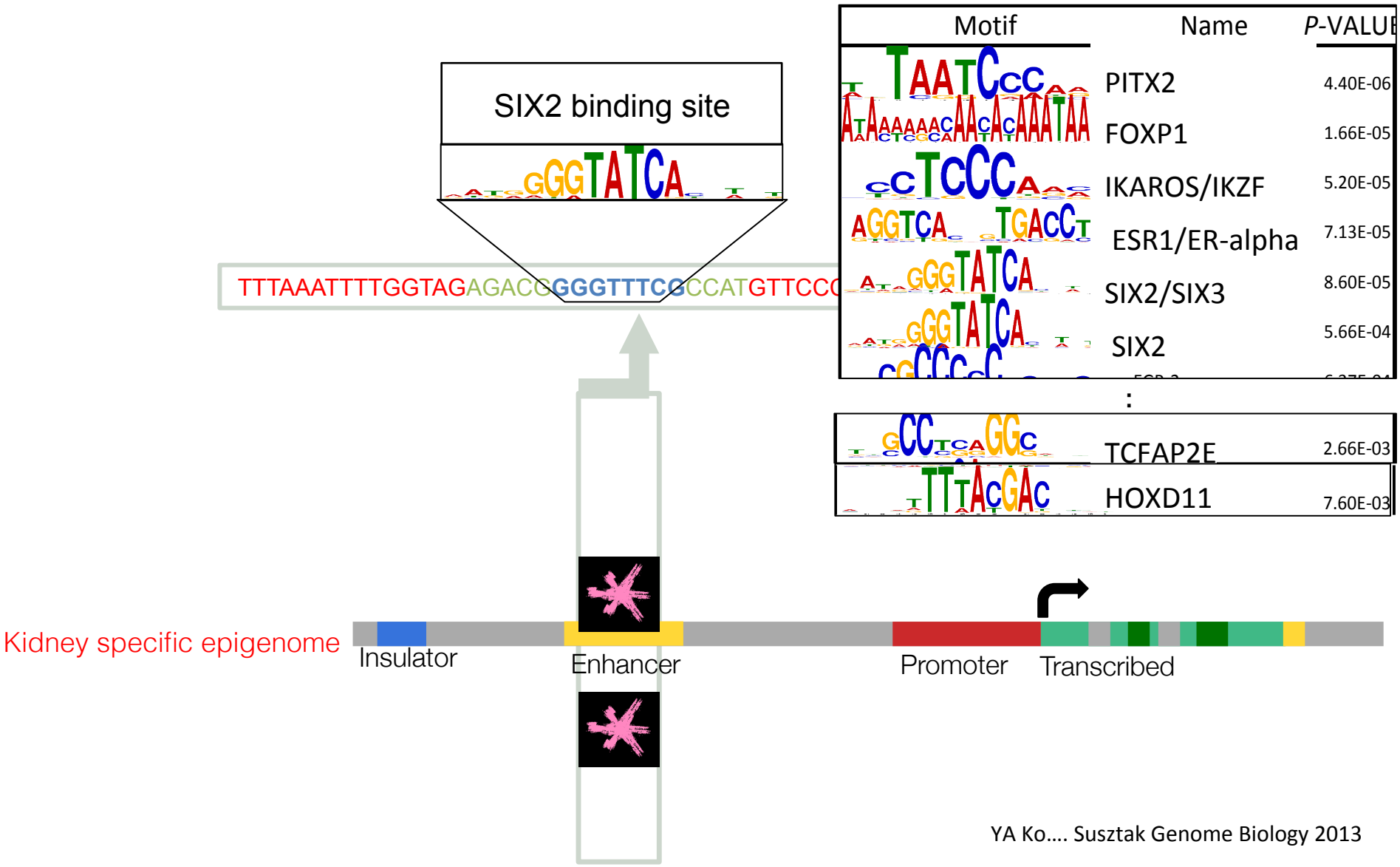
# Differential methylation occurs on kidney specific enhancers

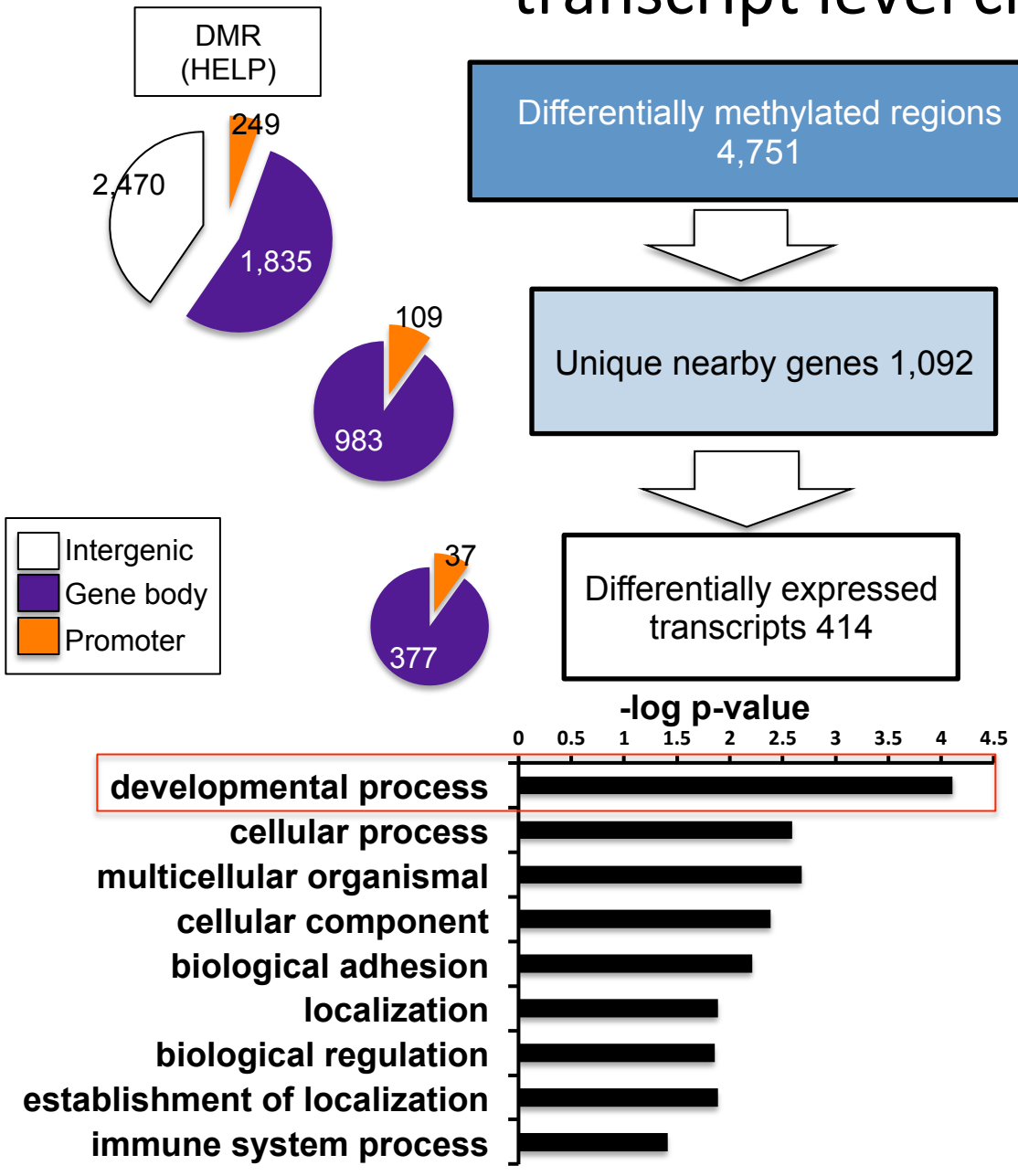

YA Ko…. Susztak Genome Biology 2013

# Differentially methylated regions affect kidney specific transcription factor binding



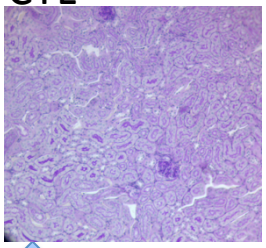

YA Ko…. Susztak Genome Biology 2013

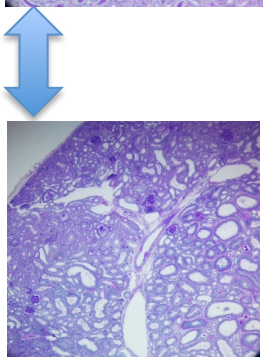
# Cytosine methylation differences correlate with transcript level changes



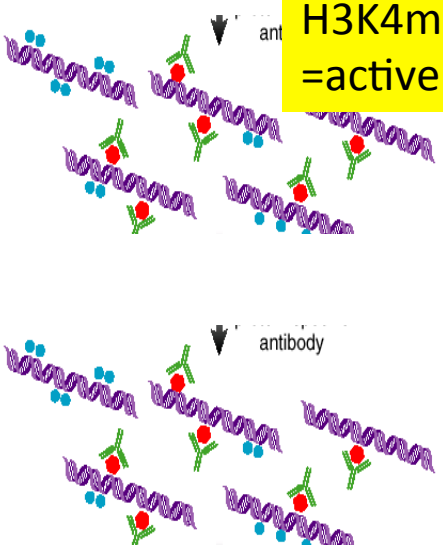
# Are there differences in histone tail modifications in CKD ?

CTL





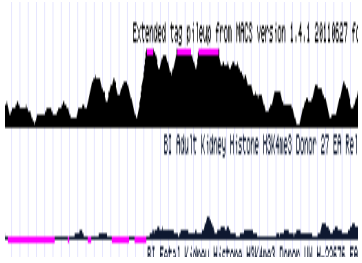
CKD




H3K4me1
=active enhancer

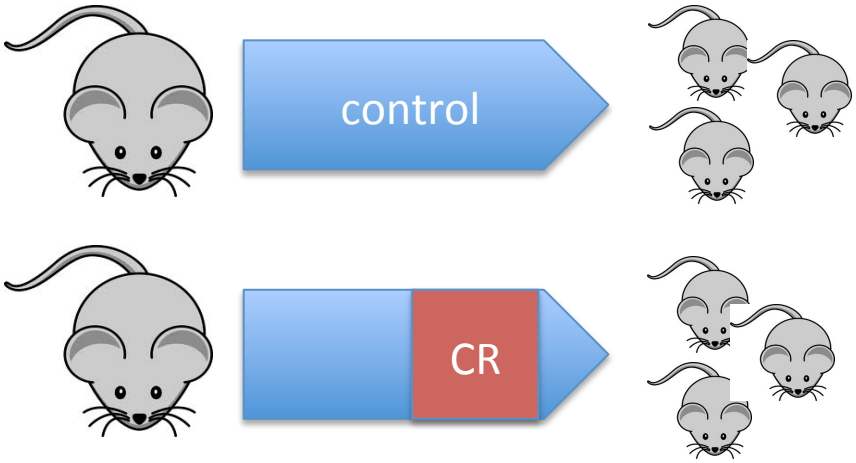
CKD

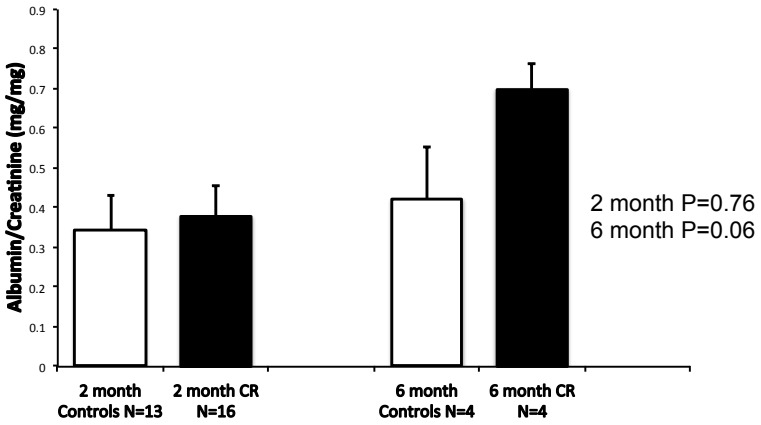
CTL

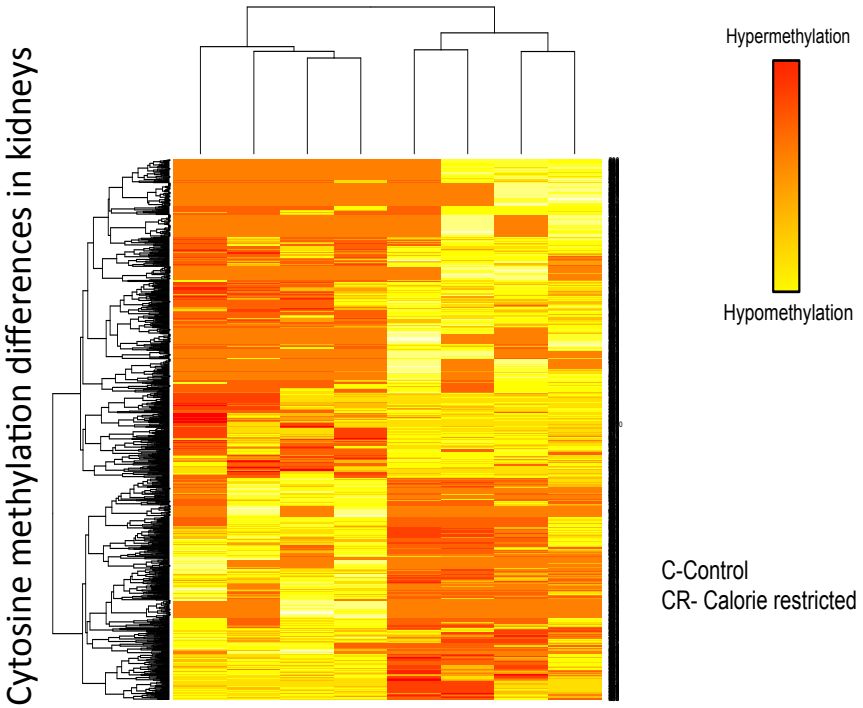


| 2501 AK H3K4me3 | | | |
|---|---|---|---|
| Term | % | P-Value | Corrected |
| developmental process | 23.4 | 3.50E-20 | 7.60E-19 |
| biological adhesion | 6.4 | 1.80E-11 | 2.00E-10 |
| multicellular organismal process | 27.2 | 3.50E-08 | 2.50E-07 |
| cellular process | 59 | 1.70E-04 | 9.60E-04 |
| reproduction | 5 | 1.90E-02 | 8.20E-02 |
| reproductive process | 4.9 | 2.10E-02 | 7.60E-02 |
| cellular component organization | 14.7 | 2.60E-02 | 8.00E-02 |

# Maternal calorie restriction in rats causes low nephron number, HTN, albuminuria and distinct epigenetic changes




**Poster by**
**The Epigenetics of Kidneys Is Altered in Offspring of Maternal Caloric Restriction**
**Howard Slomko, DO[1], Hye Heo, MD[2], Fabien Delahaye, PhD[2], Yongmei Zhao[2], Zhongfang Du MD[1] Kimberly J Reidy, MD[1] and Francine H Einstein, MD[2]**

# Conclusion

# CONCLUSION 2.

Small but highly consistent cytosine methylation changes in CKD tubule samples

Methylation changes are enriched on kidney specific enhancer regions

Fibrosis and developmental genes are more affected by methylation changes

Kidney disease might have a “developmental” origin

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**Collaborators:**
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Anna Kottgen, Freiburg

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