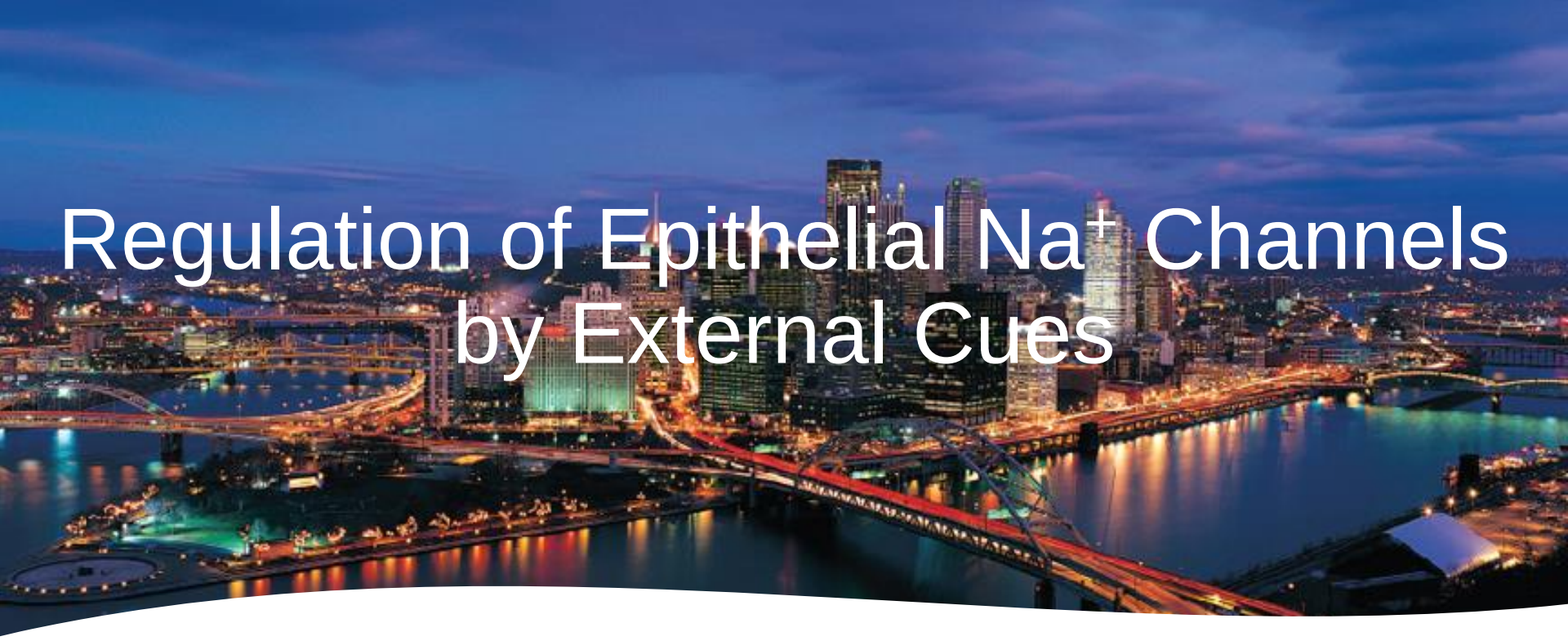


Regulation of Epithelial Na^+ Channels by External Cues

Thomas Kleyman, MD

Chief, Renal-Electrolyte Division

Adler Professor of Medicine

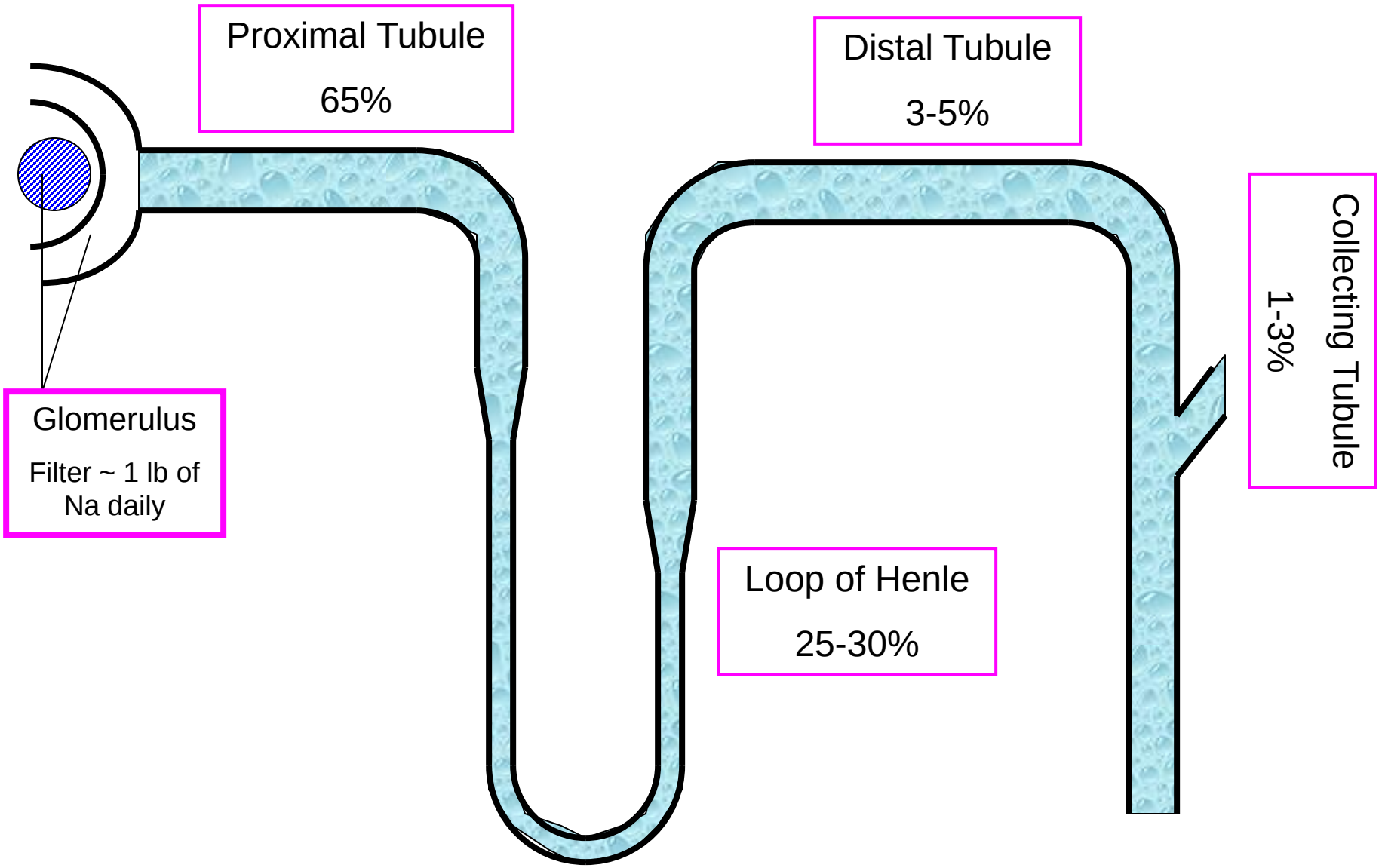
Professor of Cell Biology and of Pharmacology and Chemical Biology

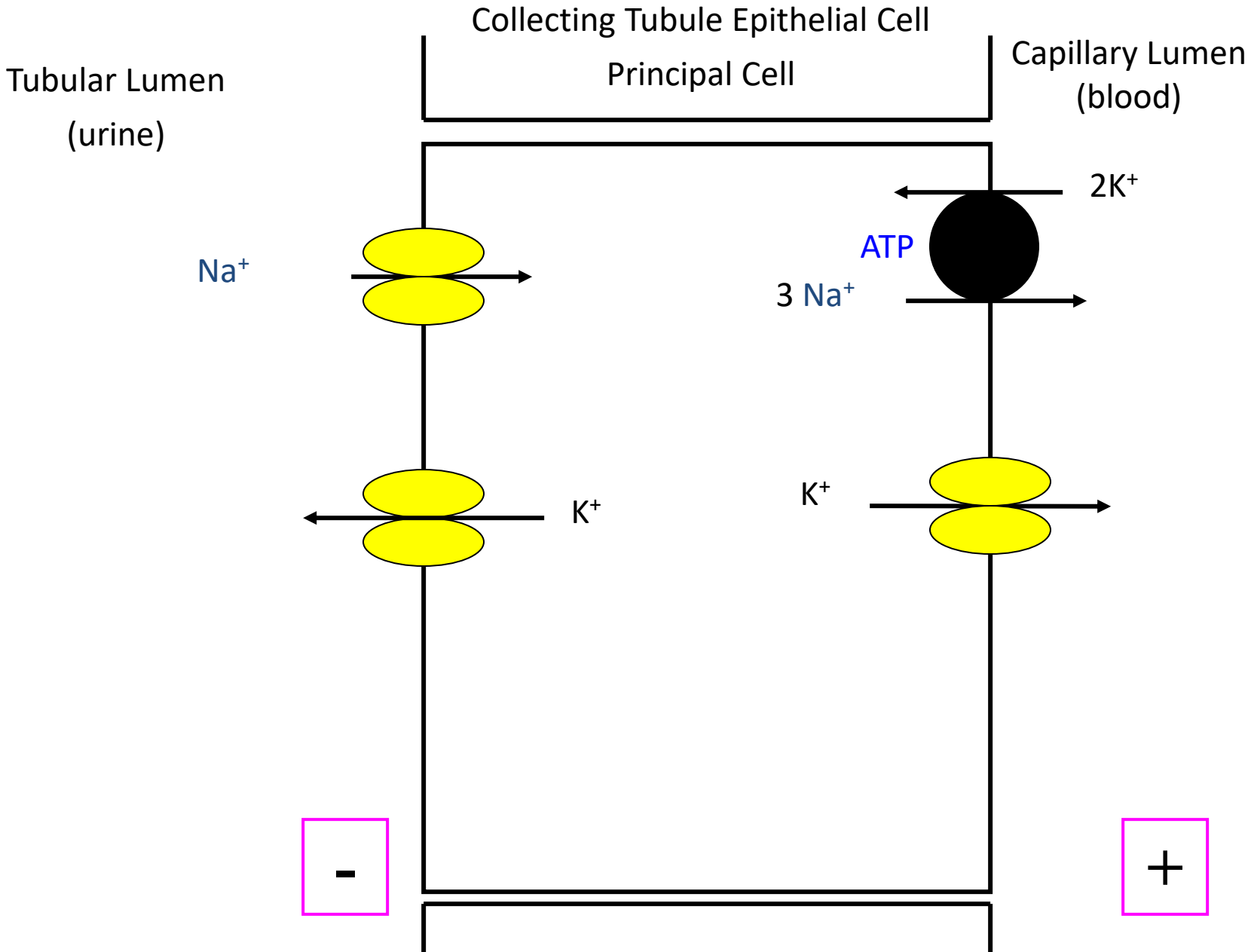
University of Pittsburgh

Kidney - has a major role in fluid and electrolyte homeostasis

- **Extracellular fluid volume homeostasis**
- Potassium homeostasis
- Osmotic homeostasis
- pH homeostasis

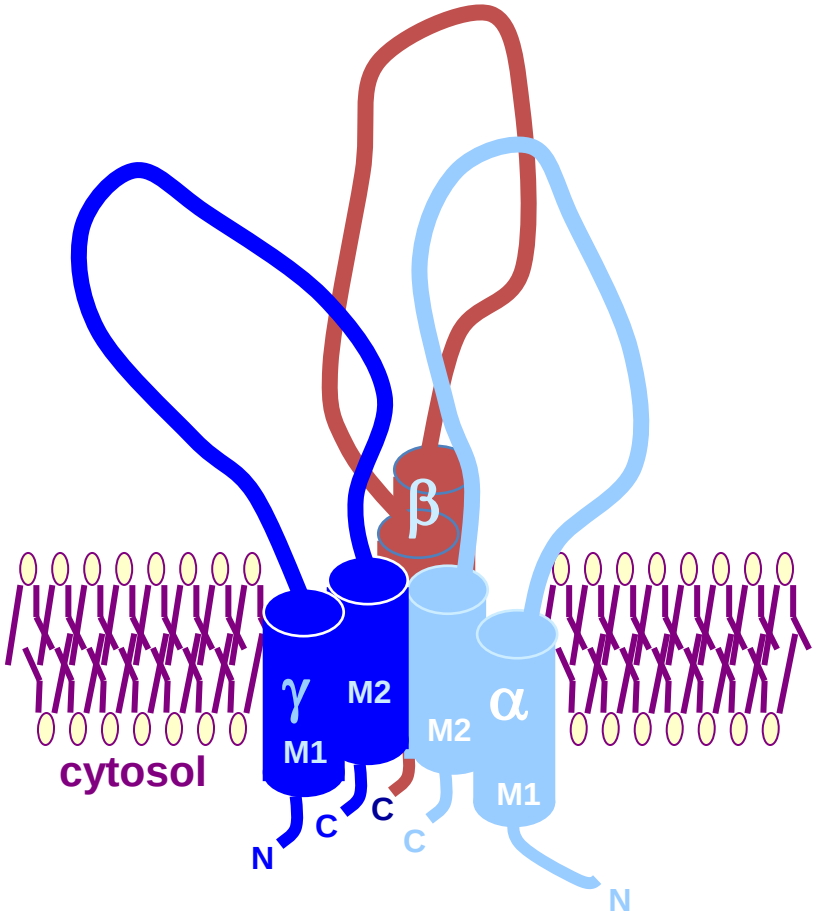
Tubular reabsorption of Na




Collecting Tubule Epithelial Cell
Principal Cell
Tubular Lumen
(urine)
Capillary Lumen
(blood)
2K+
ATP
3 Na+
Na+
K+
K+
-
+

Epithelial Na^+ Channels

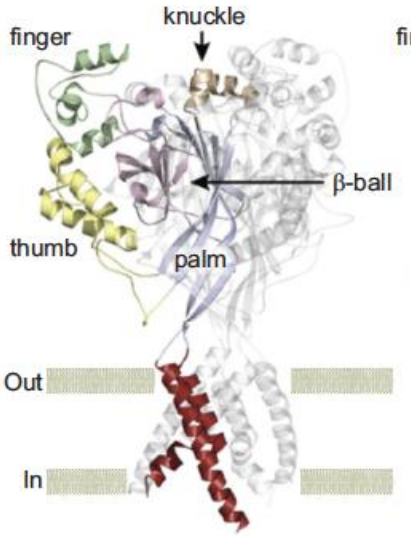
- Na^+ channels are composed of three structurally related subunits
  - α, β and γ
- Trimeric complex
  - 1α (or δ) / 1β / 1γ
- Members of the ENaC/degenerin family
- Found in epithelia lining:
  - Aldosterone-sensitive distal nephron
  - Airway and alveoli
  - Distal colon
- Liddle Syndrome
  - Early onset hypertension
  - Gain-of-function mutations

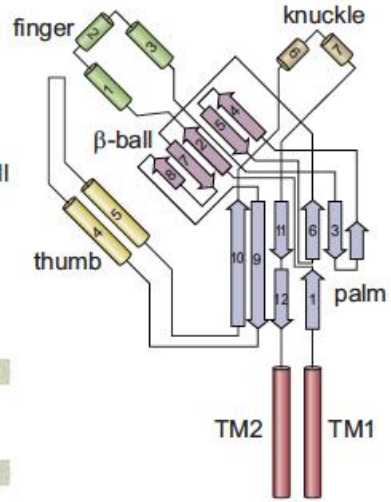


Epithelial Na^+ Channels

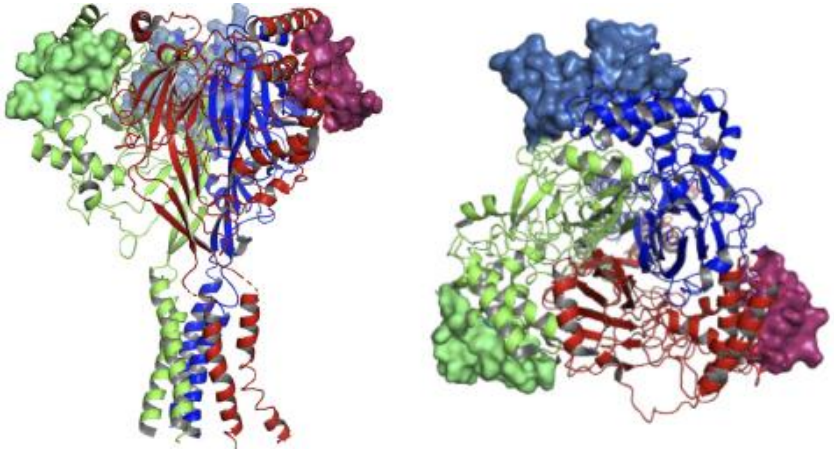
Expressed in tissues that affect total body Na^+ stores, extracellular fluid volume, vascular tone and blood pressure

- Lingual epithelium: salt taste receptors
- Adlosterone-sensitive distal nephron
  - “coupled” to K secretion
- Distal colon
- Organum vasculosum of the lamina terminalis (OVLT)
  - rostral wall of the third ventricle
  - lacks a complete blood–brain barrier
  - ENaC in the OVLT has a role sensing the extracellular $[Na^+]$
    - increase in sympathetic tone and BP in response in an increase in $[Na^+]$
- Vascular endothelium
  - suggested to blunt NO release
    - more complicated
- Vascular smooth muscle
  - pressure-induced myogenic response
- Dendritic Cells
  - immune system activation and increase BP in response in an increase in $[Na^+]$
  - collaboration with Annet Kirabo (Vanderbilt)



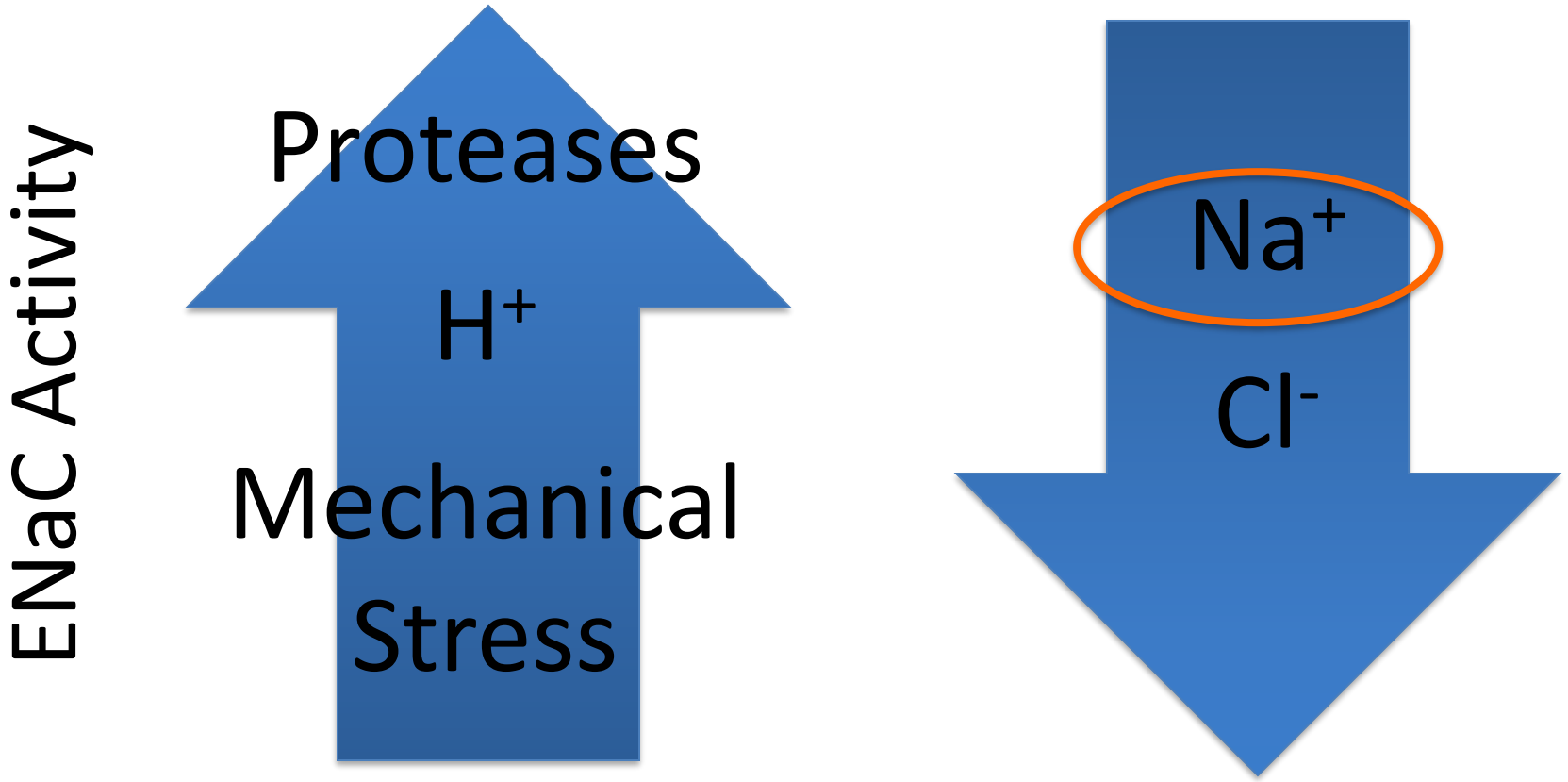


Kashlan and Kleyman AJP 2011

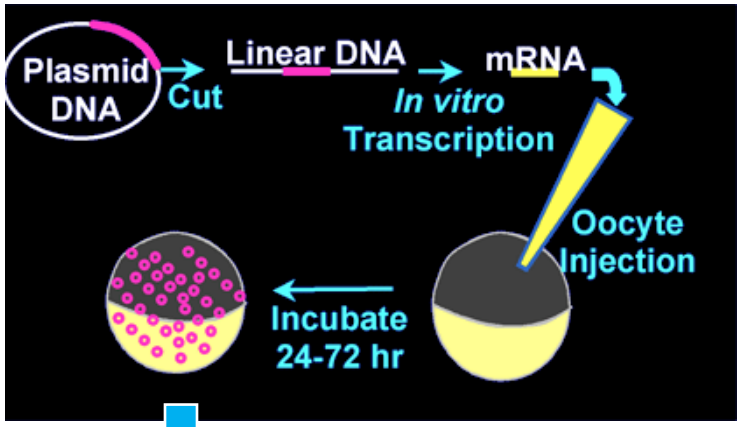


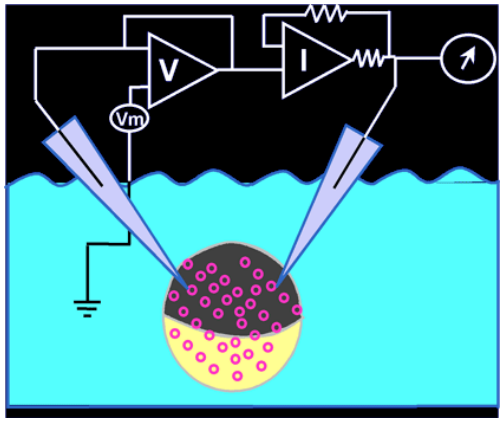
Noreng et al eLife 2018

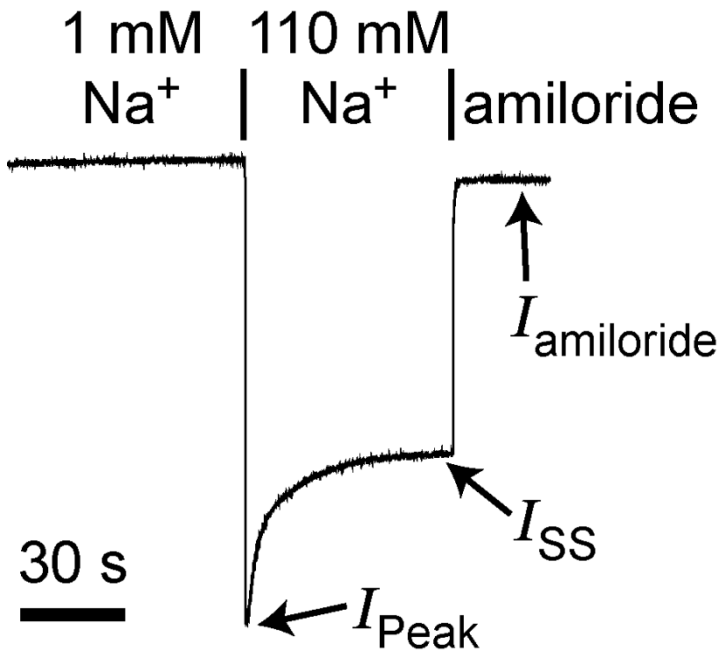
ENaC senses its environment



Na^+ self-inhibition





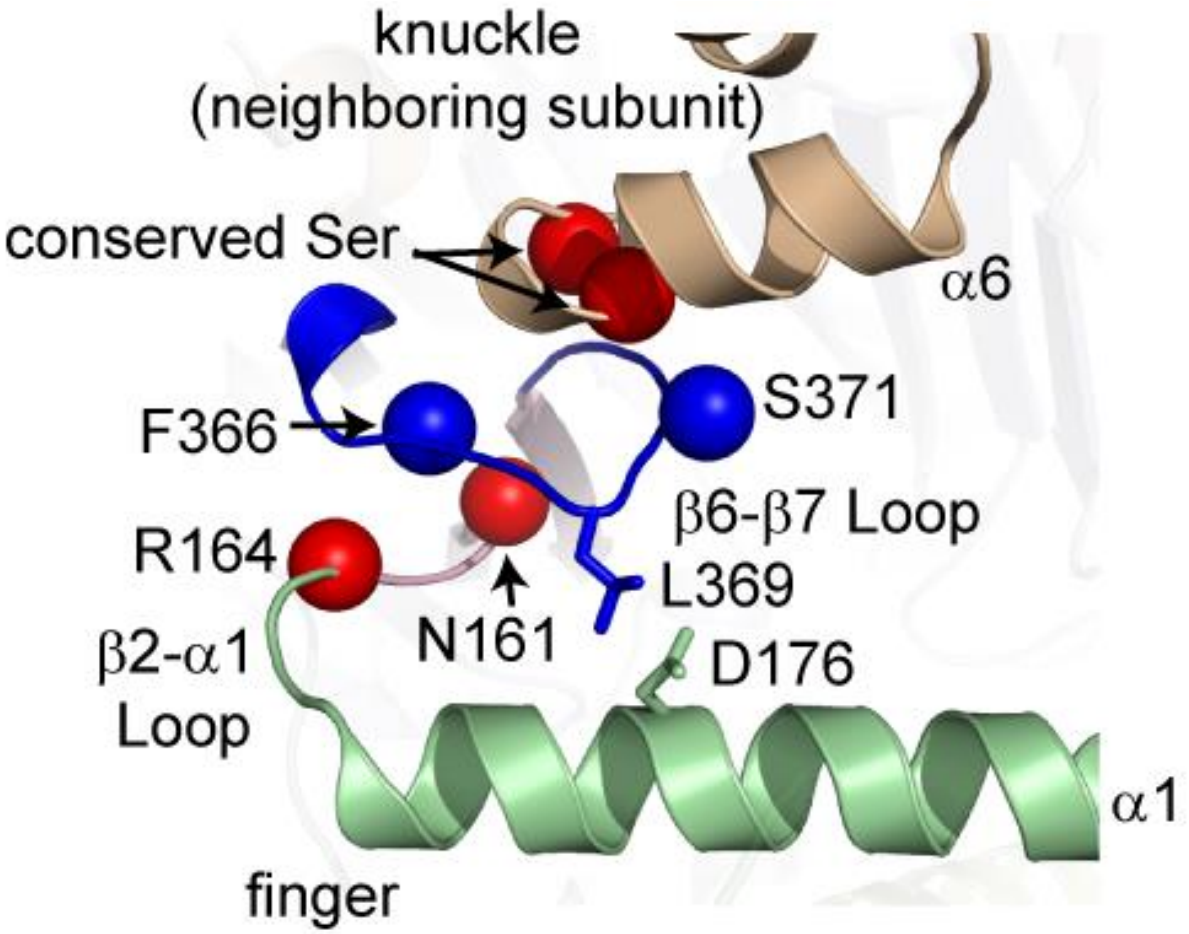


What do we know about Na^+ self-inhibition?

- weak, Na^+ and Li^+ specific
- extracellular
- allosteric decrease in P_o

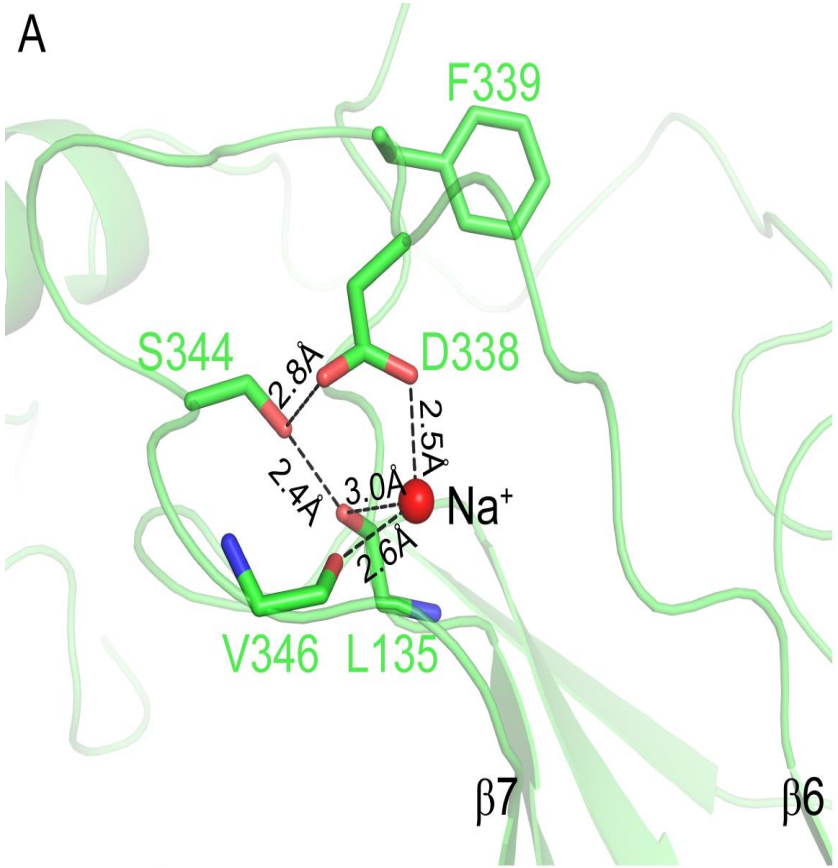
Numerous (>90) ENaC mutants alter the Na^+ self-inhibition response

Allosteric effector binding site for Na^+ in the α subunit



Kashlan et al. JBC 2015

Cation binding site in the resolved structure of the α subunit

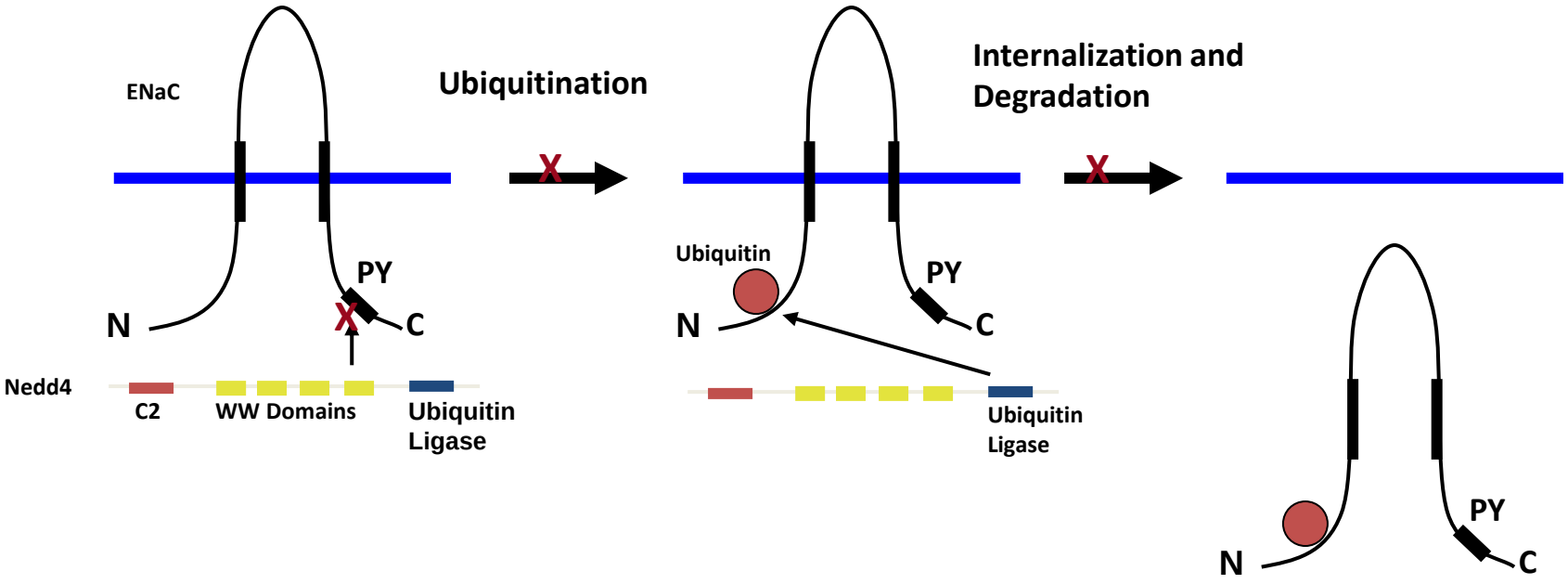


Noreng et al eLife 2020

Are there human ENaC variants that affect channel function?

Liddle syndrome mutations disrupt the interaction between Nedd4 and ENaC

Liddle syndrome mutations disrupt the interaction between Nedd4 and ENaC



ENaC non-synonymous SNPs

There are hundreds of non-synonymous SNPs within the three ENaC genes reported databases

ENaC non-synonymous SNPs

72 ENaC variants analyzed to date
47% significantly altered channel activity
25% of the variants exhibited a gain-of-function

Many ENaC mutations result in a gain-of-function

While it is common for mutations to result in no change of function or in a loss of function, it is unusual to find proteins with many activating mutations.

Why is this observed with ENaC?

Many ENaC mutations result in a gain-of-function

While it is common for mutations to result in no change of function or in a loss of function, it is unusual to find proteins with many activating mutations.

Why is this observed with ENaC?

It is “easy” to lose the inhibitory effect of external Na^+

Do ENaC variants that alter the Na^+ self inhibition response affect blood pressure?

αC479R found in 2 siblings with a mild Liddle syndrome phenotype

Salih et al. JASN 2017

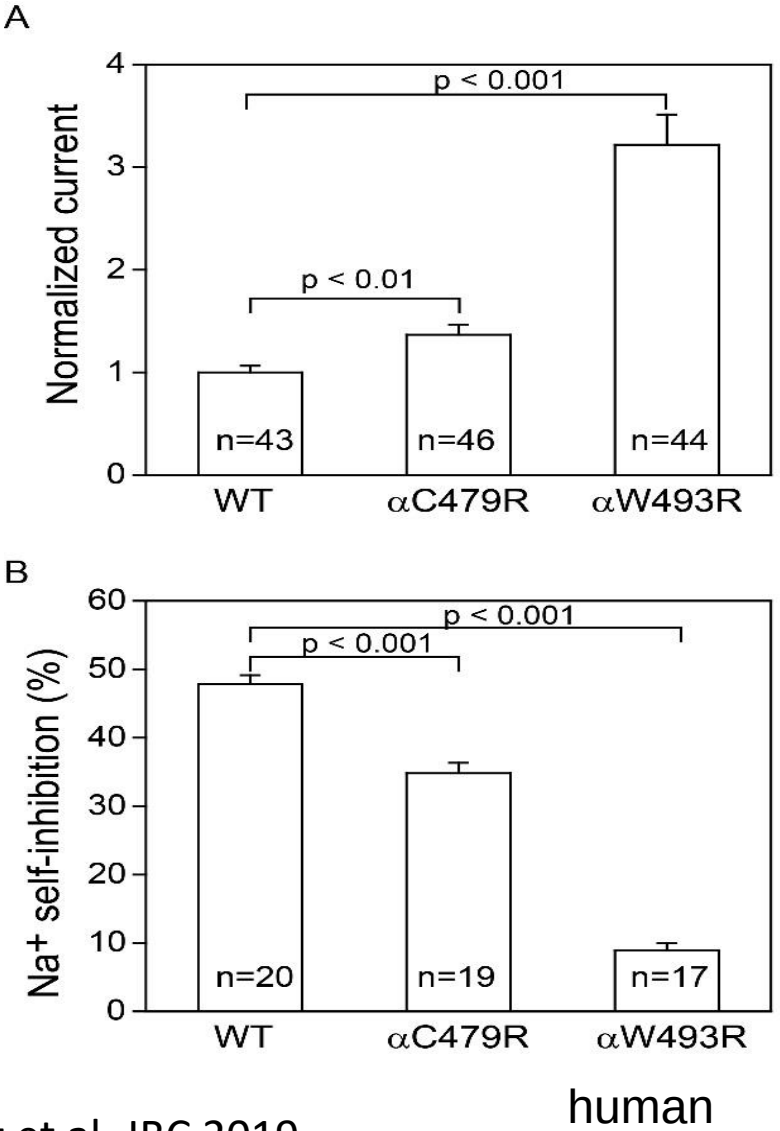
Equivalent to mouse αC506

We reported that αC506A is a gain of function mutant, due to a loss of Na^+ self-inhibition)

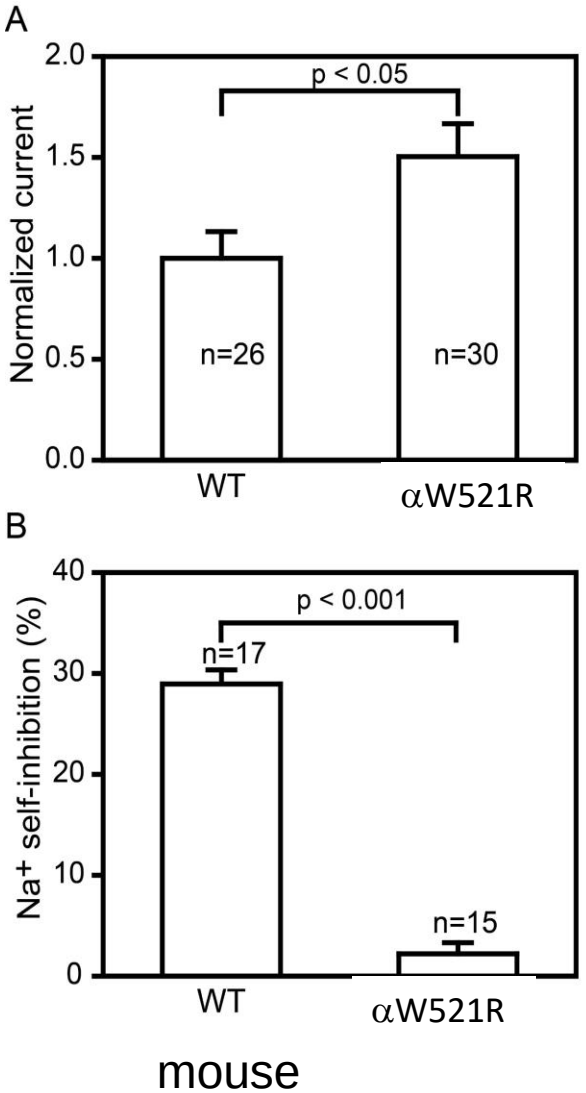
Sheng et al. JBC 2007

αC506R is also a gain of function mutant

Comparing human and mouse ENaC variants



human



mouse

Wang et al. JBC 2019

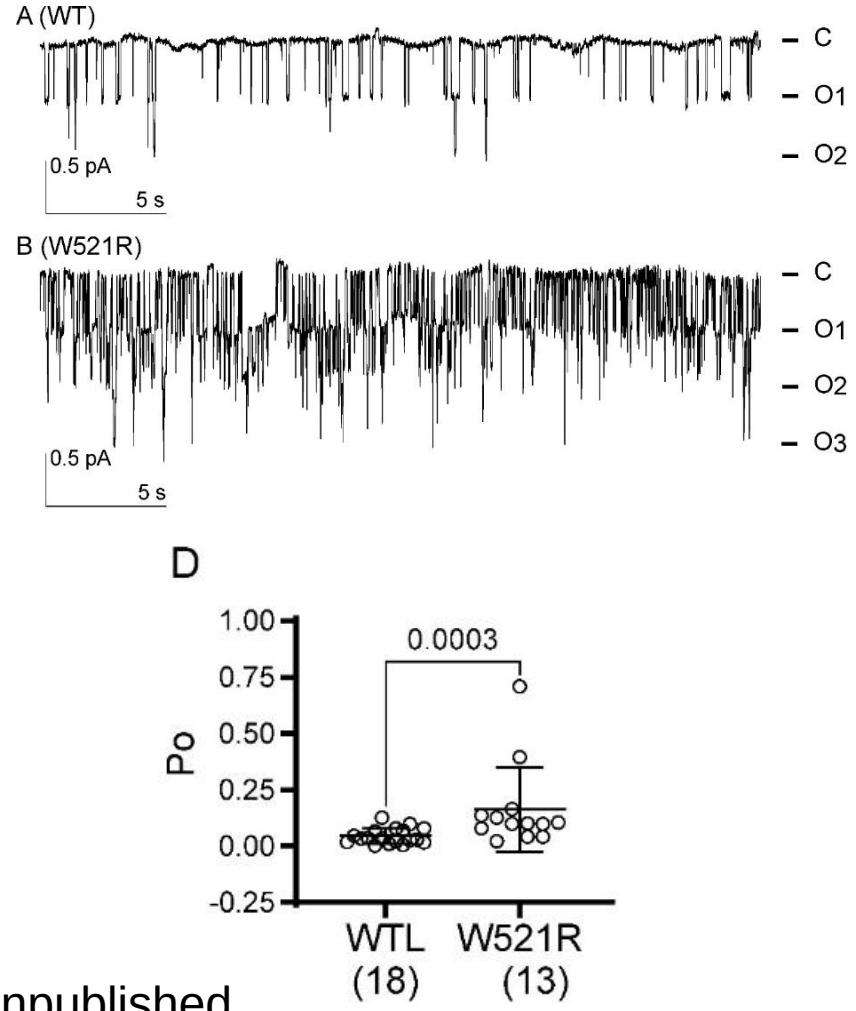
Questions

Are “non-traditional Liddle” ENaC gain-of-function variants associated with an increase in blood pressure?

Should individuals with hypertension and an ENaC gain of function variant be treated with an ENaC inhibitor?

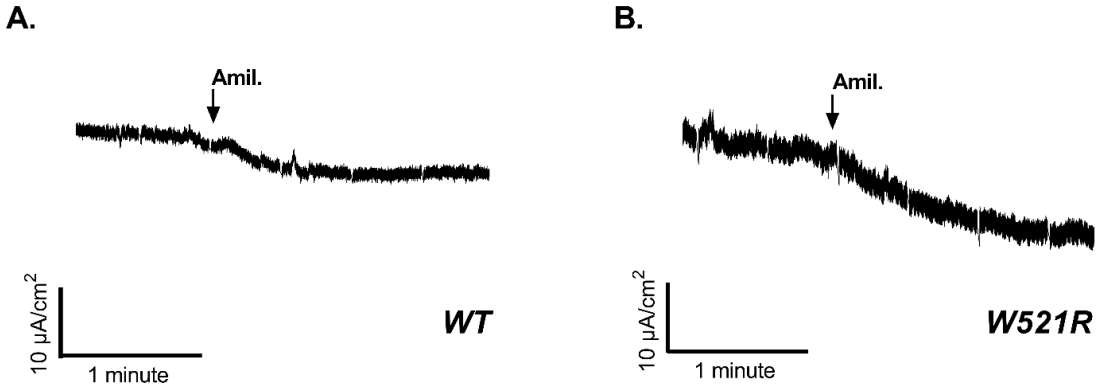
Generated a mouse expressing the αW521R mutant using CRISPR-cas9.

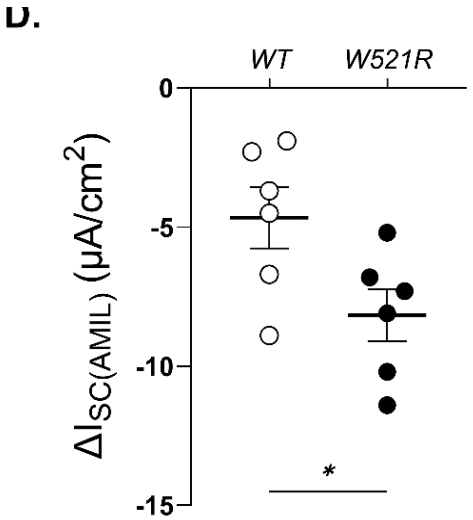
CCD/ENaC ENaC has a higher open probability in αW521R mice (control diet)



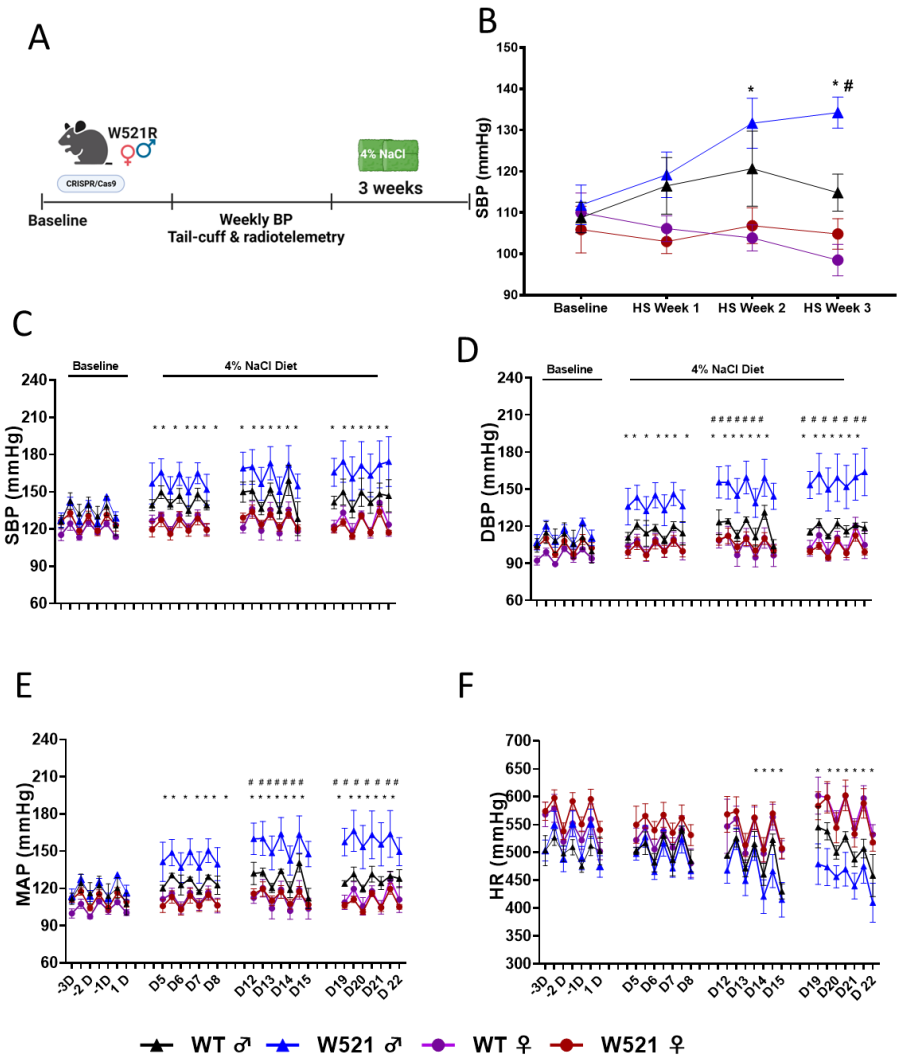
Ishimwe, Ray et al, unpublished

Distal colon ENaC activity is higher in αW521R mice (control diet)

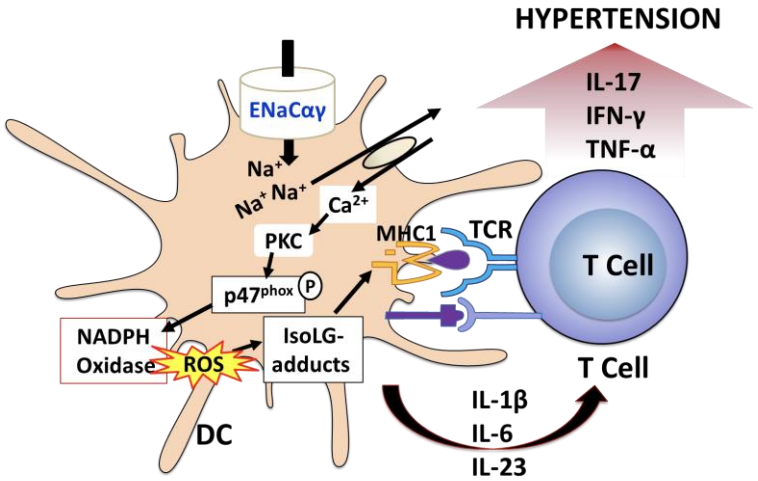




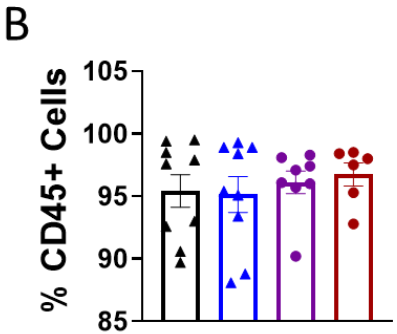
Male αW521R mice have an increase in BP on a high salt diet

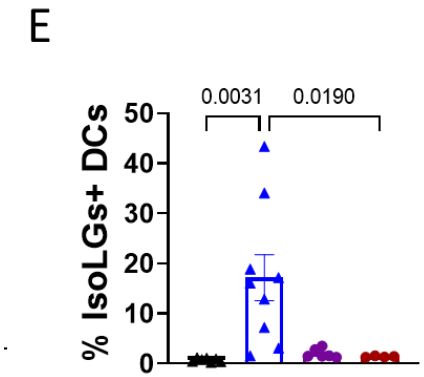


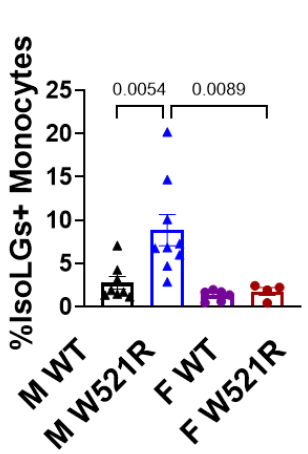
αW521R on a high salt diet have markers of dendritic + monocyte cell activation



Barbaro et al Cell Rep 2017

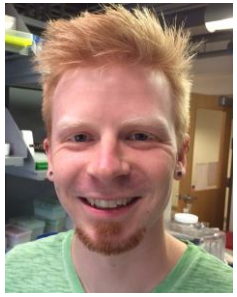






Are rare and low frequency ENaC variants associated with changes in blood pressure

- Trans-Omics for Precision Medicine (TOPMed) Program
- Whole-Genome Sequence Data
- Sequence kernel association tests



Brandon Blobner

SKAT: gene level test for association between a set of rare (or common) variants and quantitative phenotypes

Studies included in BP analyses

Study	N	Abbreviation
Genetics of Cardiometabolic Health in the Amish	732	Amish_FC13
	269	Amish_FC2
Atherosclerosis Risk in Communities Study VTE cohort	3039	ARIC
Cleveland Family Study	744	CFS
Framingham Heart Study	3270	FHS
Genetic Studies of Atherosclerosis Risk	1626	GeneSTAR
Genetic Epidemiology Network of Arteriopathy	1138	GENOA
Genetic Epidemiology Network of Salt Sensitivity	1616	GenSalt
Hypertension Genetic Epidemiology Network	1635	HyperGen
Jackson Heart Study	3124	JHS
Samoan	1167	Samoan
Women's Health Initiative	4692	WHI_ctr
	4403	WHI_stroke_case
	944	WHI_VTE_case

Blobner et al. Hypertension, 2022

Single variant association tests of common ENaC variants

Variant	rs ID	SBP *p*-value	DBP *p*-value	MAP *p*-value	PP *p*-value
αA334T	rs11542844	0.9	0.9	0.9	0.7
αT663A	rs2228576	0.1	0.6	0.9	0.06
βG165R	rs2303157	0.3	0.9	0.6	0.07

Sequence kernel association tests of low frequency and rare ENaC variants with blood pressure measures

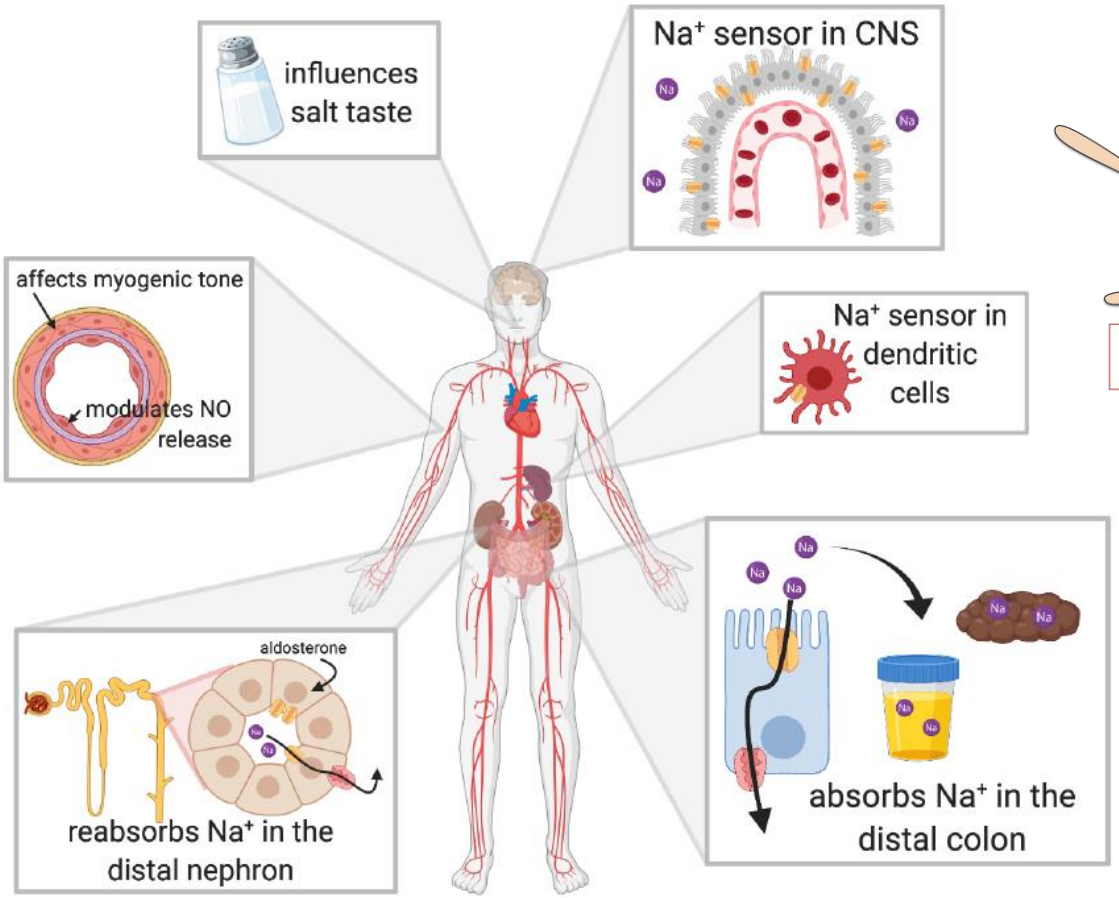
		Variants with MAF < 0.05	
Gene. Chromosome:Start–End	***Measure***	***n***	***p* value**
SCNN1A. 12:6346824–6379751	SBP	3,972	0.02
	DBP	3,972	**0.00004**
	PP	3,972	0.7
	MAP	3,972	**0.0002**
SCNN1B. 16:2329812–23381320	SBP	12,334	0.02
	DBP	12,334	**0.002**
	PP	12,334	0.3
	MAP	12,334	**0.003**
SCNN1D. 1:1280417–1292050	SBP	2,405	**0.0008**
	DBP	2,405	**0.002**
	PP	2,405	**0.003**
	MAP	2,405	**0.0008**
SCNN1G. 16:23184696–23216904	SBP	4,093	0.5
	DBP	4,093	0.2
	PP	4,093	0.06
	MAP	4,093	0.5

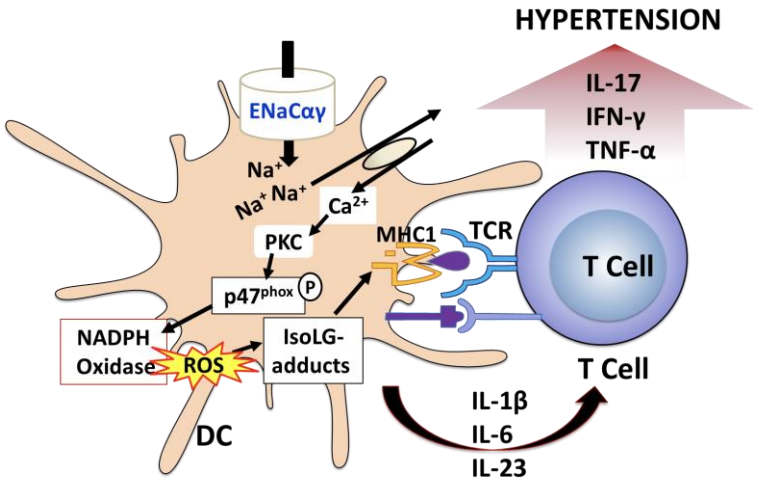
* *p*-values < 0.00625 are significant

α, β, and δ subunit variants (MAF <0.05) are associated with changes in blood pressure

- ENaCs – αβγ or δβγ
- Human kidney - ASDN
  - Primarily αβγ channels
    - Not δ
- δβγ channels have roles in the control of blood pressure
  - Dendritic cells/monocytes?
  - Endothelium?
  - Other sites?

ENaCs are expressed in tissues that affect total body Na^+ stores, extracellular fluid volume, vascular tone and blood pressure





Barbaro et al Cell Rep 2017

Mutchler and Kleyman, Hypertension 2021

Summary

- Specific rare and low frequency ENaC variants are associated with changes in blood pressure
- Should individuals with hypertension and have an ENaC mutation that results in a loss of the Na^+ self-inhibition response be treated with an ENaC inhibitor (amiloride)?
- What is the role of the δ subunit in the control of BP?

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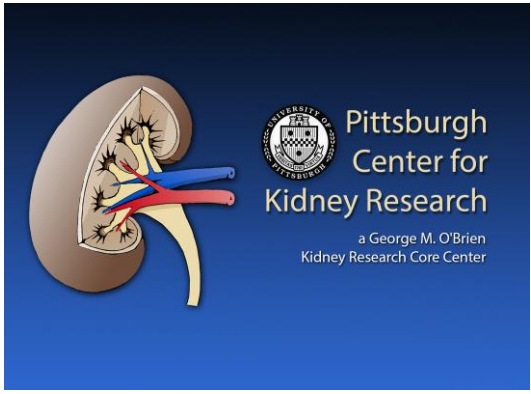

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