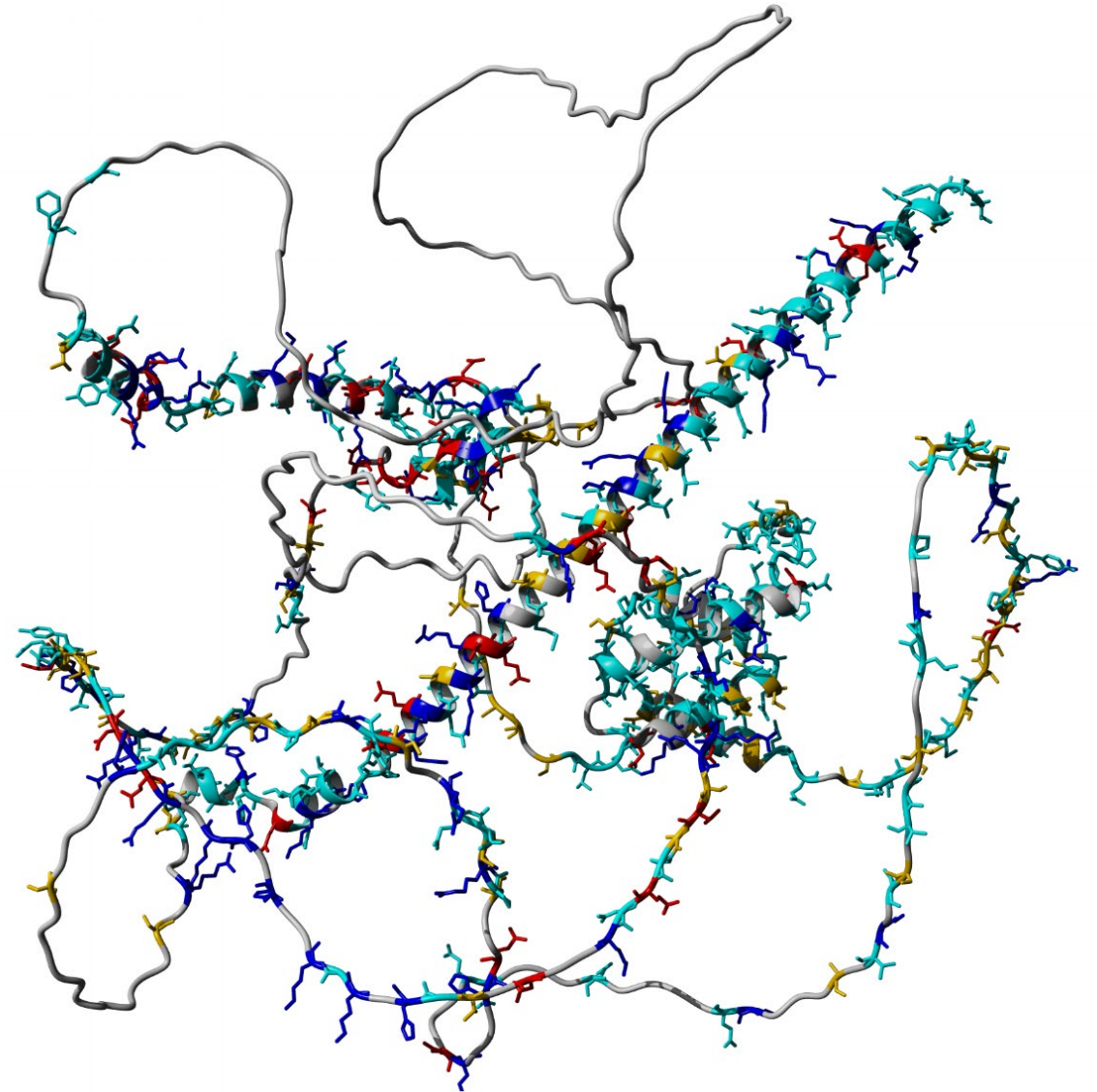


**DESSH Conference**  
**Sept 30<sup>th</sup>, 2023**

**Daniel Vogt**  
**Michigan State University**

**Animal models to  
understand DESH**

**Predicted shape of the WAC protein  
(using alpha fold)**



Rudolph et al., 2023

Most Genetic changes in the *WAC* gene are loss of function/**major recipe change**

Transcription

Translation



**DNA**

**RNA**

**Protein**

Loss of function

Missense/single unit change

# Scientific advances require collaborative teams

## Parent and patient advocates

Caitlin Piccirillo

...and many here today!

Organization, leading, informing and connecting.

## Clinical guidance

Marwan Shinawi

Diagnosing and describing patient concerns and treatments.

## Research validation

Vogt lab

Kim lab

Gabel lab

Mouse model of *WAC*.

Zebrafish model of *WAC*.

Mechanisms by which *WAC* may function.

## Additional experts

Nord lab

Jeong lab

UCI MRI institute

RNA candidates (the copied recipes!)

Craniofacial analyses of *WAC* mouse model.

MRI assessment of *WAC* mouse model.

# What does the WAC recipe look like?

## Can we find some common ingredients?

Very important protein parts will be the same in many species, can use this to our advantage...

Known ingredients include the WW and coiled coil domains, as well as a nuclear targeting domain: these interact with other proteins or direct a protein where to go.

Some regions are highly conserved but do not have a known function.

These domains are conserved between species

Human	MVMYARKQRLSDGCHDRRGSQFPYQALKYSSKSHPSSGDHRHEKMRDAGDSPSPNKMLR
Mouse	MVMYARKQRLSDGCHDRRGSQFPYQALKYSSKSHPSSGDHRHEKMRDAGDSPSPNKMLR
Zebrafish	MVMYARKQRLSDGCHDRRGSQFPYQALKYSSKSHPSSGDHRHEKMRDAGDSPSPNKMLR ***** *.*.*.* *.*.*.* *.*.*.* *.*.*.* *.*.*.* *.*.*.* *.*.*.* *.*.*.*
Human	RSDSPENKYSdstghskaknvHthrVRErdGGTSYSPQENSHNHSALHSSNSHSSNPSNN
Mouse	RSNSPENKYSdstghnkaknvHtQrvRErdGGTSYSPQENSHNHSALHSSNSHSSNPSNN
Zebrafish	RSDSPDNKHMdntGHGRakAIHPhRGREREggTSISpQENSHNHSslHSSNSHS-----N *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *
Human	PSKTSdAPYdSADDWSEHISSSGKkYYncrTEVsqWEKPKewLErEQrQKEAN--KMAV
Mouse	PSKTSdAPYdSADDWSEHISSSGKkYYncrTEVsqWEKPKewLErEQrQKEAN--KLAV
Zebrafish	PNKSSdTFfEPADDWSEHISSSGKkYYncrTEVsqWEKPKewLErEQrQKEATKAAVV *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *
Human	NSFPKDRDYrREVMQATATSGFASGMEDKHSSDASSLLPQNILSQTsrHndrdYrLPRAE
Mouse	NSFPKDRDYrREVMQATATSGFTSGMEDKHSSDASSLLPQNILSQTsrHndrdYrLPRAE
Zebrafish	NSFPKDRDYrREAMQATPAS----- ***** *.*.*.* *.*.*.* *
Human	THSSSTPVQHPiKpVvHPTATpStVpSSpFTLQSDHQPKKsFDANGASTLSKLPtPSSV
Mouse	THSSSTPVQHPiKpVvHPTATpStVpSSpFTLQSDHQPKKsFDANGASTLSKLPtPAsL
Zebrafish	-----
Human	PAQKTERKESTSGDKPVSHSCTTPSTSSASGLNPTsAPpTSASAVpVSPVPQSPiPP-LL
Mouse	PAQKTERKESAPGDKSISHSCTTPSTSSASGLNPTpAPpTSASAVpVSPVPQSTiPP-LL
Zebrafish	---YSSTKSSiATEKpSSLTpSSSSAAVSGLNVPNSASASGStVpVSPVmqSPAPpTLL *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.*
Human	QDPNLLRQLLPALQATLQLNNSNVDISKINEVLTAAVTQASLQSIiHKFLTAGPSAFNIT
Mouse	QDPNLFRLQLLPALQATLQLNNSNVDISKINEVLTAAVTQASLQSIiHKFLTAGPSAFNIT
Zebrafish	QDPsLLRQLLPALQATALQLNNSVdMAKINEVLTAAVTQASLQSIiLHKILTAGPSAFNIT *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.*
Human	SLISQAAQLS-TQAQPSNQSPMSLTSDASSPRSYVSPRISTPQNTNTVPIKPLISTPPVSS
Mouse	SLISQAAQLS-TQAQPSNQSPMSLTSDASSPRSYVSPRISTPQNTNTVPMKPLISTPPVSS
Zebrafish	TLLSQATQLSNQVAQQSSQSPMSLTSDASSPRSYVSPRISTPQNTNTASLKPPLSTTPVSS *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.*
Human	QPKVSTPVVKQGPVQSATQQPVTADKQGHepVSPRSrLQRSSQRSPSPGPNHTSNSSN
Mouse	QPKVSTPVVKQGPVSHSATQQPVTADKQGHepVSPRSrLQRSSQRSPSPGPNHTCSSN-
Zebrafish	QTKINAMTVKSSSLPPSSQQPLSTEKHHDNG-NSPRTLQRQSSQRSPSPGPNHMGSSNS *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.*
Human	ASNAT-----VVPQNSARSTCSLTPALAAHFSENLIKHVQGWPA
Mouse	ASTAT-----VVPQNASARPACSLTPTLAAHFNDNLIKHVQGWPA
Zebrafish	SSSNNGGGGGQGPVVGAMPpGsvPPGTAPGRATCSFTPTLAAHFNENLIKHVQGWPA *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.*
Human	DHAEKQASRLREEAHNMGTiHMSEiCTELKnlRSLVrVCEiQATLrEQrILFLRQqIKEL
Mouse	DHAEKQASRLREEAHNMGSVHMSEiCTELKnlRSLVrVCEiQATLrEQrILFLRQqIKEL
Zebrafish	EhVEKQASRLREEAHtmGsiYmSenCTELKnlRSLVrVCEiQATLrEQrILFLRQqIKEL *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.* *.*.*.*.*
Human	EKLKNQNSFM- ★ Fully conserved
Mouse	EKLKNQNSFMV : Strong conservation
Zebrafish	EKLKNQNSFMV . Weak conservation
	*****
	bpNLS
	WW
	Unknown
	CC



# Predictions and findings

## Clinical reports

## Mouse model

## Zebrafish model

**Craniofacial**

**Yes**

**Yes**

**Behaviors**

**Yes**

**Yes**

**Hypotonia**

**NT**

**NT**

**Seizures**

**Yes**

**NT**

**Gastrointestinal**

**NT**

**NT**

**Blood glucose**

**Yes**

**NT**

**NT not tested**



# What do these changes look like?

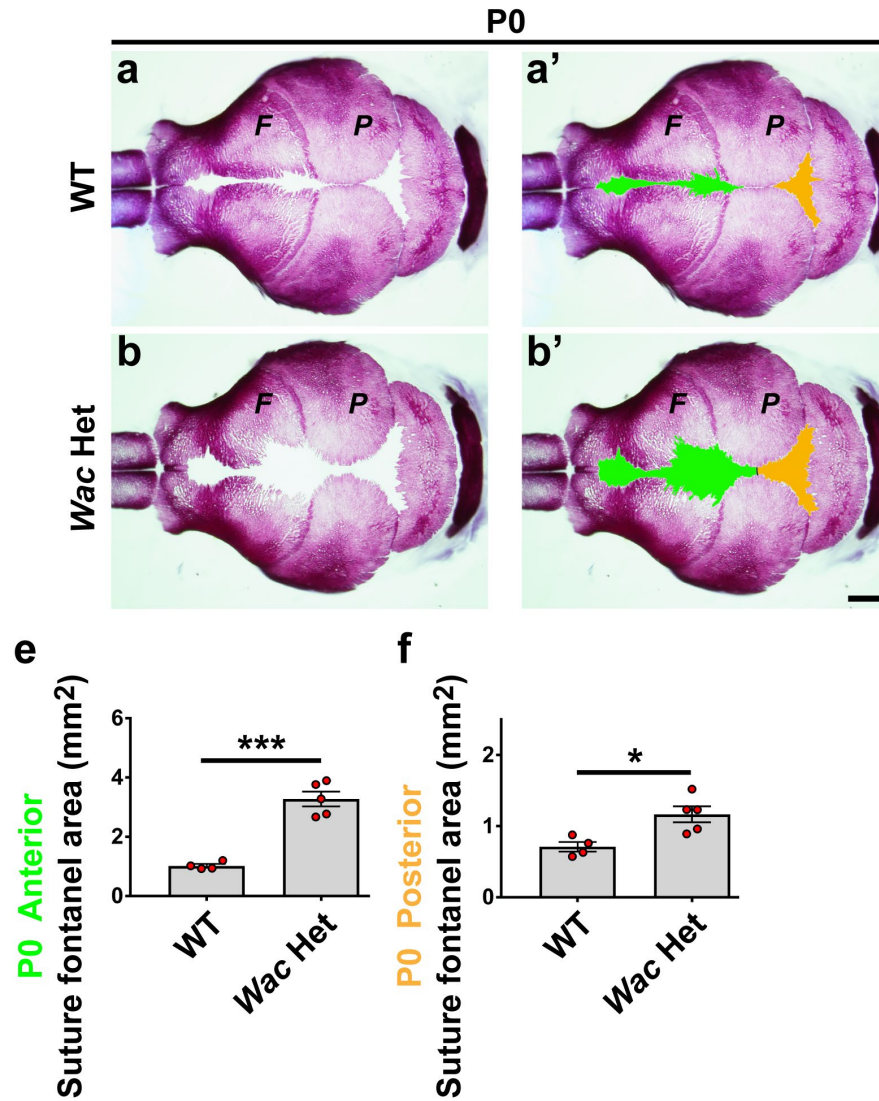
Craniofacial changes were observed early (Neonatal)

Work performed by:

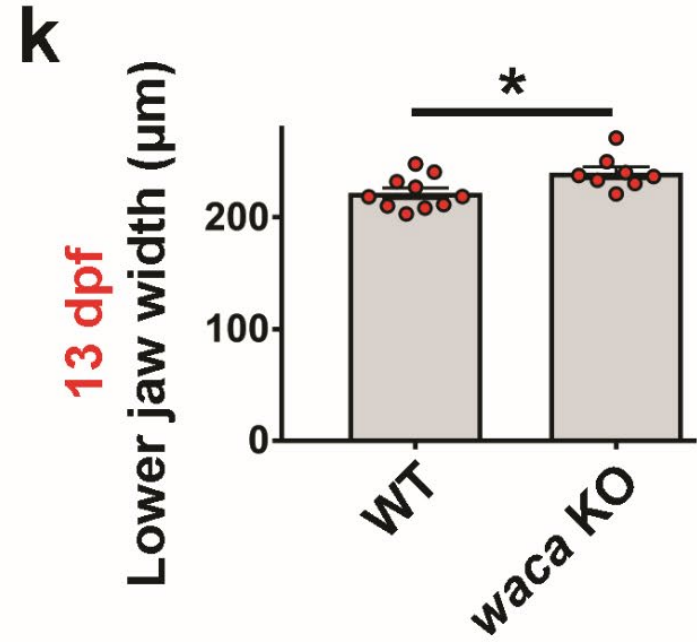
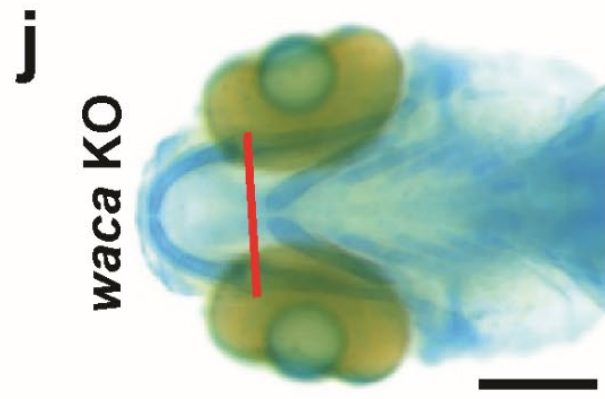
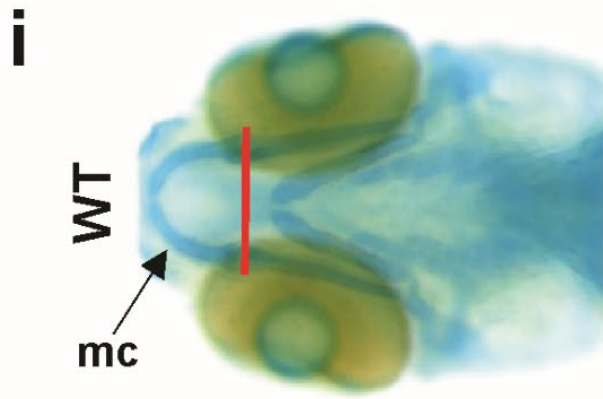
Maria Pacheco and Juhee Jeong



New York University

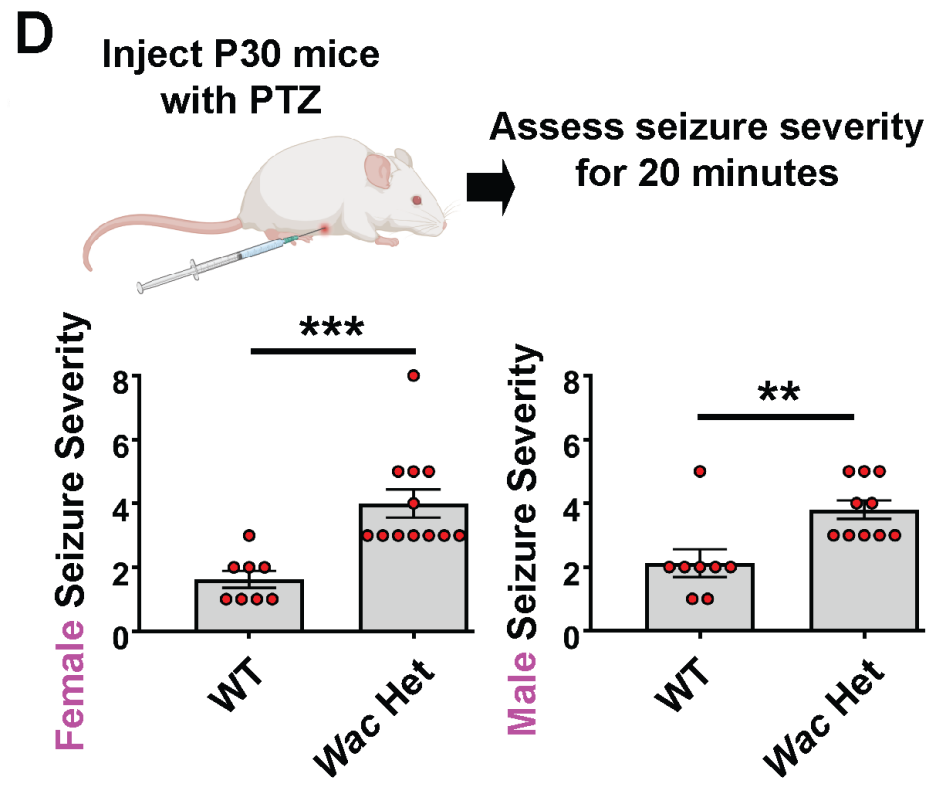


# Zebrafish jaws are wider when there is a decrease in *wac*



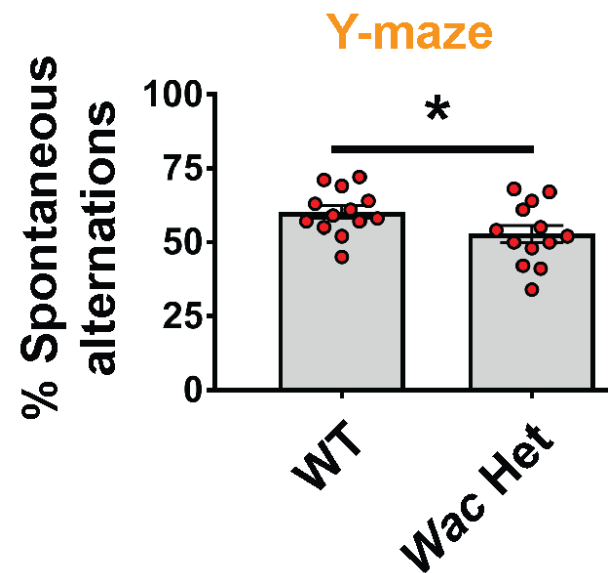


Challenging *Wac* Hets with a seizure causing drug leads to greater response

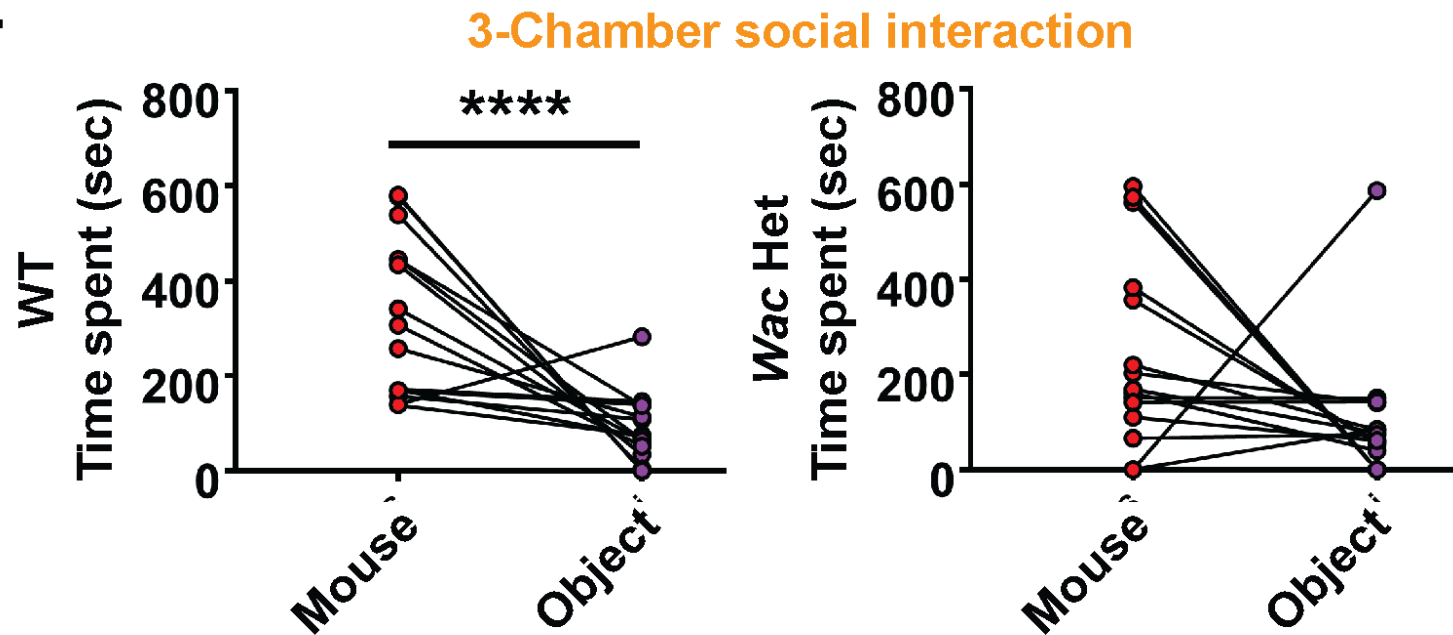


Some behaviors relevant to social interactions and short-term memory were altered in *Wac* Hets

**E**



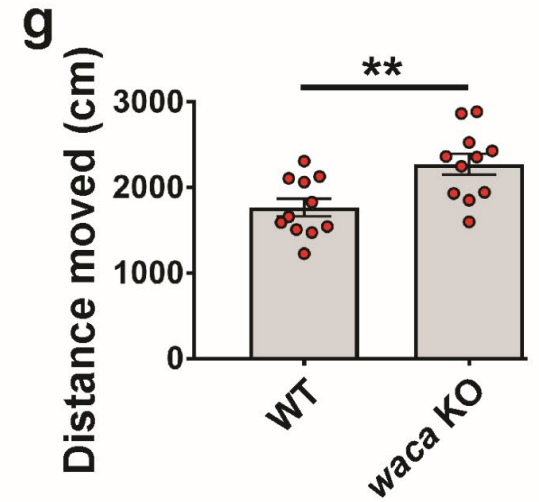
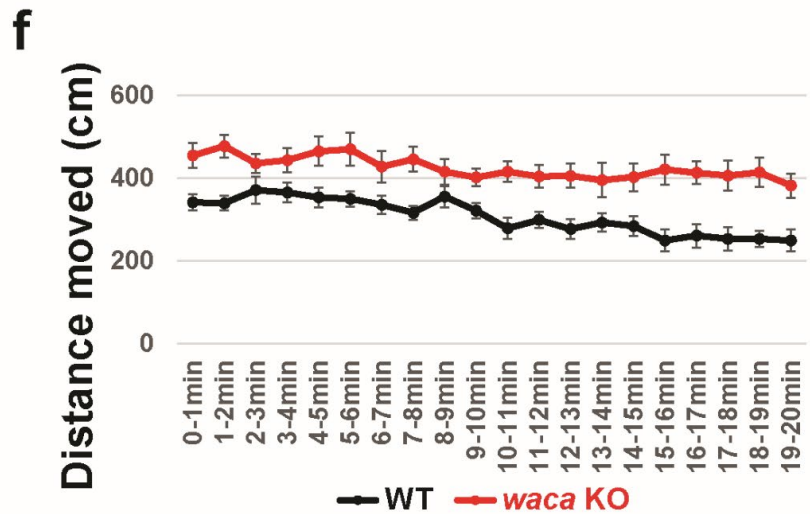
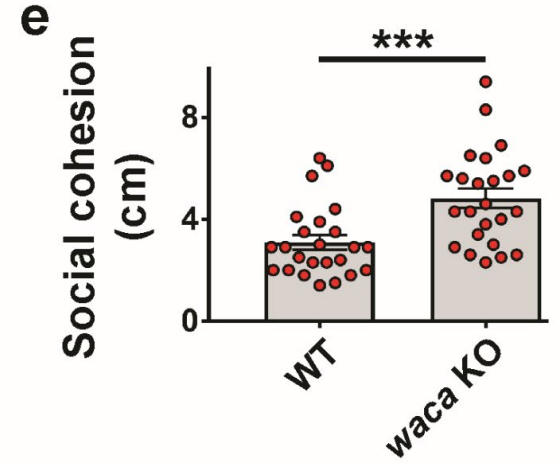
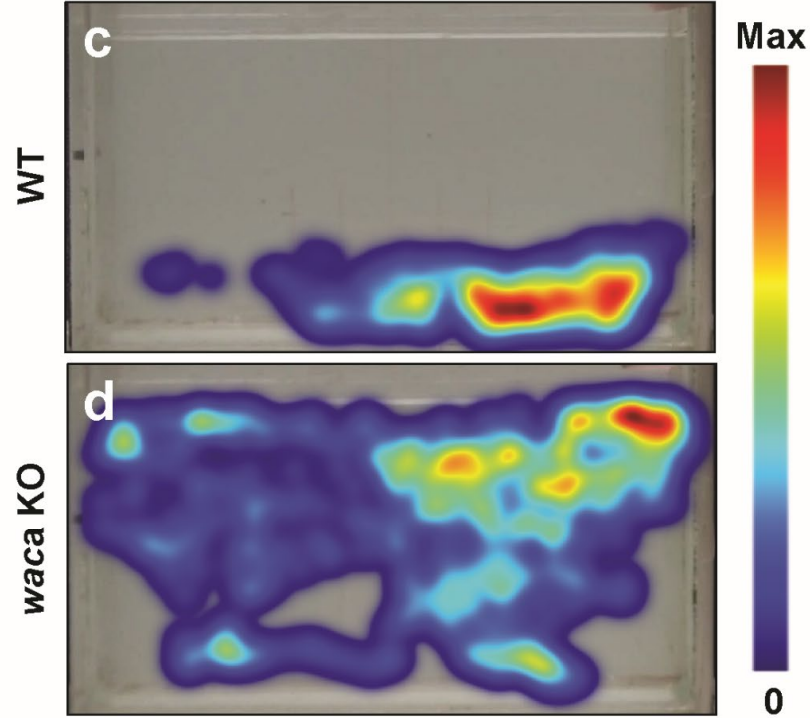
**F**



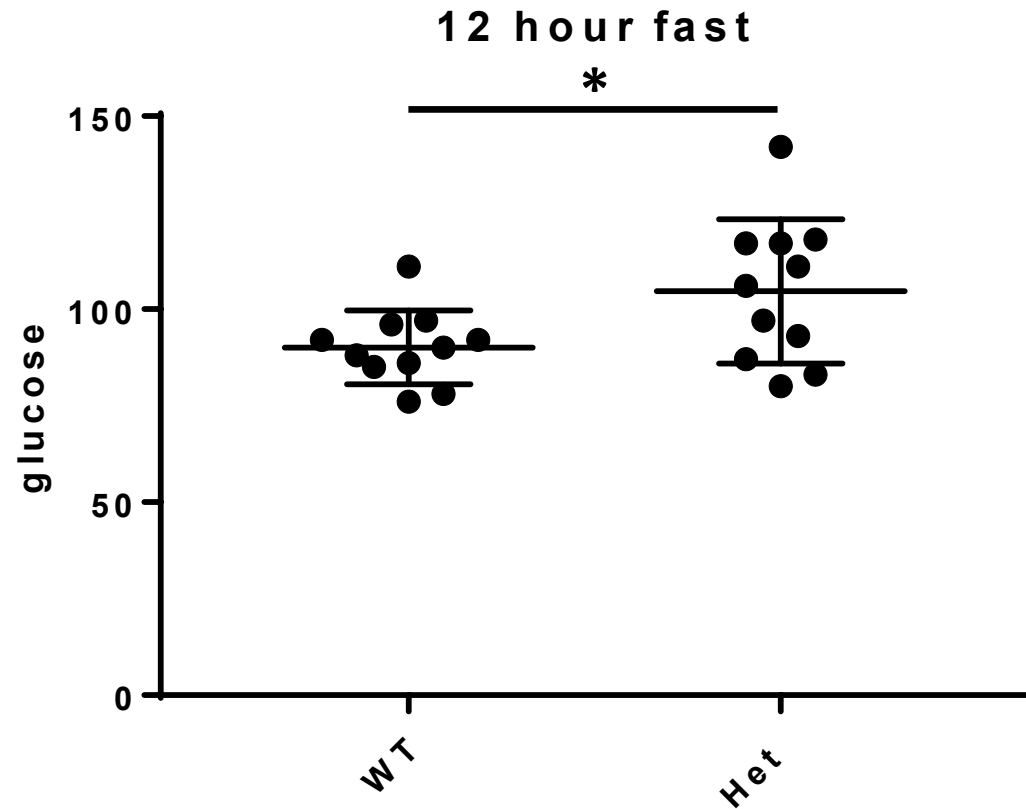
# Decreased *wac* in Zebrafish leads to less social behavior and hyperactivity

Zebrafish

## Social cohesion



# At young adult ages, *Wac* hets exhibit elevated blood glucose levels



However, low blood glucose observed in young pups (still collecting data)

## Part one conclusions

Both mouse and zebrafish *WAC* models exhibit relevant craniofacial and behavior outcomes; mice also have a susceptibility to seizures, and potential glucose alterations.

Still many DESSH symptoms to test

- This is beyond our abilities, please ask your doctors if they know researchers that would be interested in helping?
- We have the mice and are happy to provide to any lab that wants to investigate.

What are some mechanisms/cell types that we could be targeting to treat DESSH symptoms?

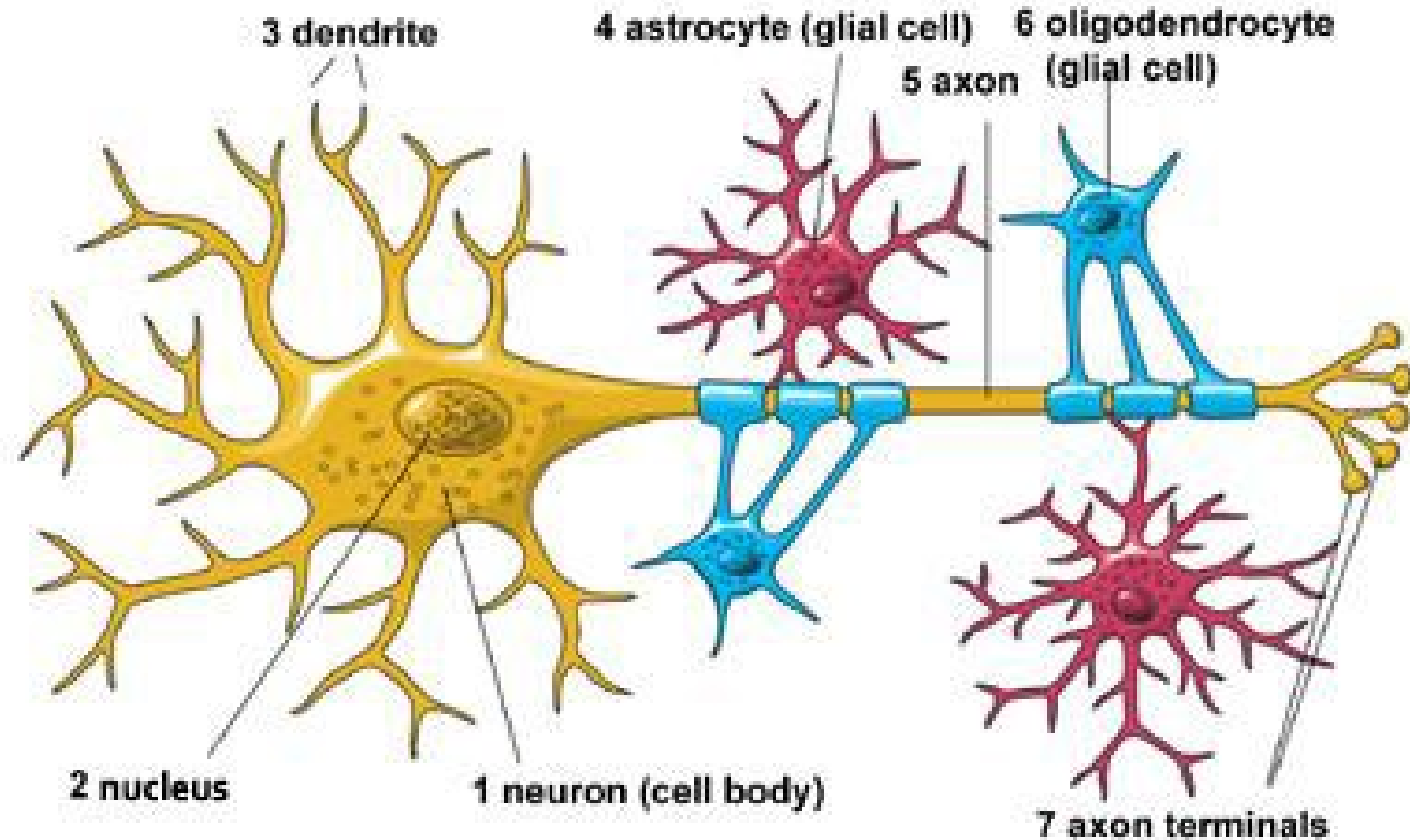
# Our brains are balanced to transmit information using neurons

Each part of the brain forms a circuit made up of different cells

Dysfunction of any cell type can often alter the function of a circuit

Which brain regions are impacted by loss of WAC?

How are different cell types effected?





**Most major brain cell types are present**

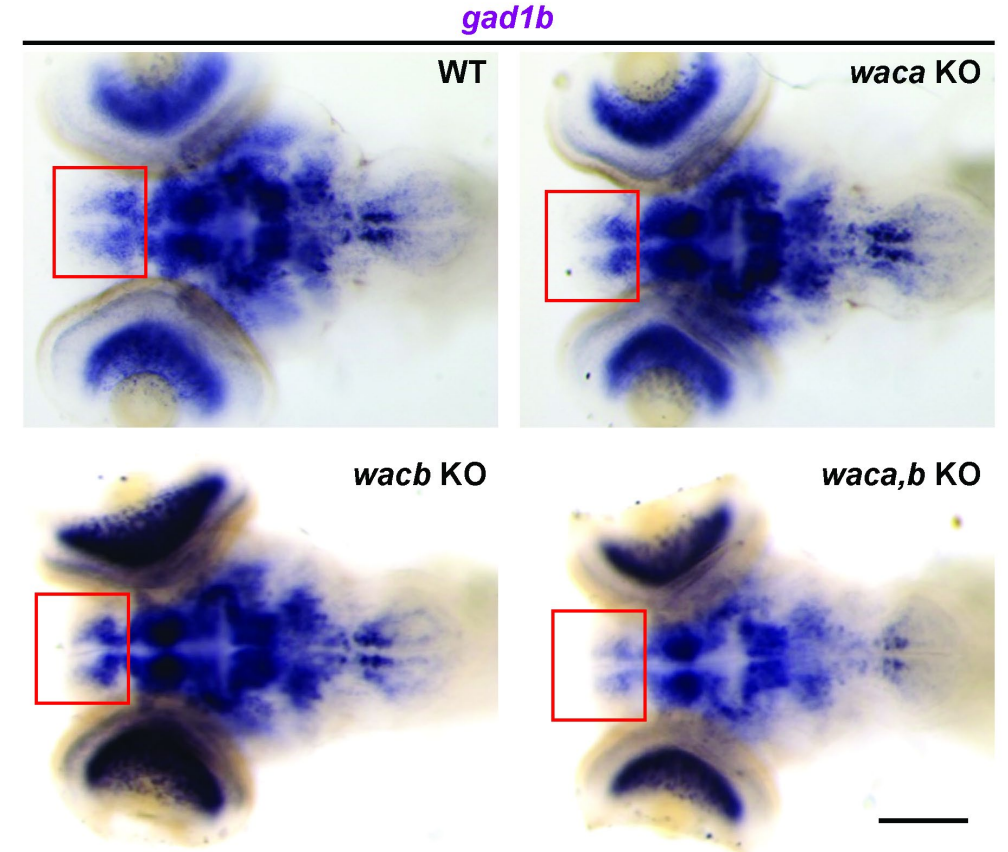
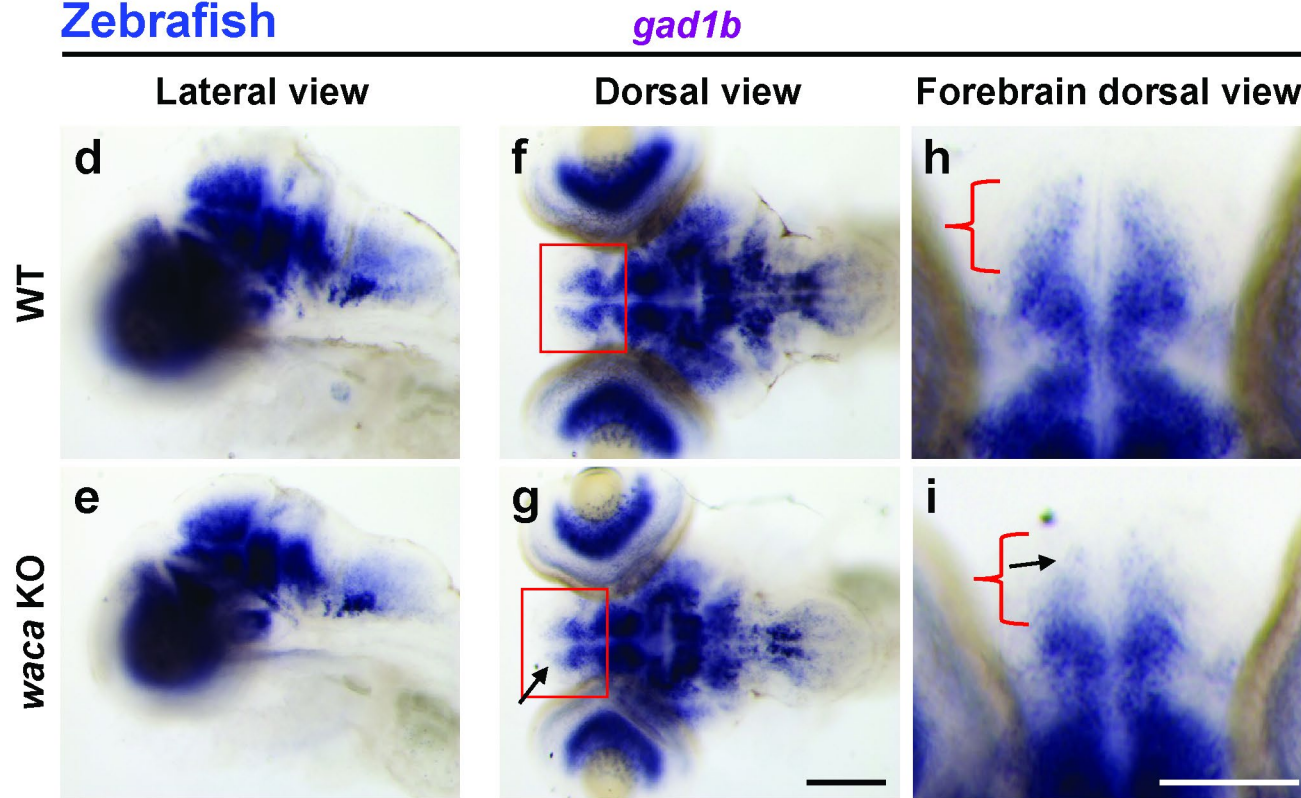
a

**P30 Somatosensory Cortex Cell Counts**

Cell classification	Marker	WT Cells/mm <sup>2</sup> (SEM)	Het Cells/mm <sup>2</sup> (SEM)
Neuron (General)	NeuN	3324 (±179)	3380 (±48)
Oligodendrocyte	OLIG2	725 (±9)	752 (±7)
Astrocyte	S100beta	606 (±8)	599 (±8)
Microglia	IBA1	431 (±15)	440 (±30)
Interneuron	PROX1	185 (±10)	186 (±11)
Interneuron	Somatostatin	129 (±9)	121 (±8)
Interneuron	Parvalbumin	171 (±6)	141 (±6) *
Interneuron	LHX6	326 (±15)	323 (±15)

# GABAergic cell types are altered in Zebrafish as well

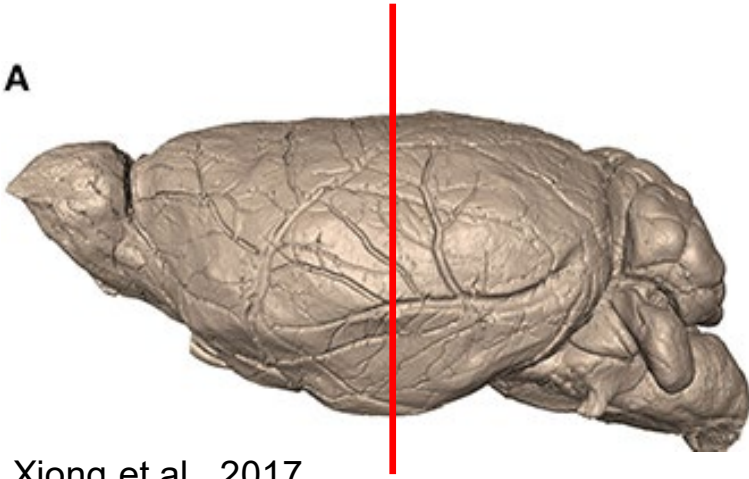
## Zebrafish



# Our previous data were very targeted, can we be unbiased?

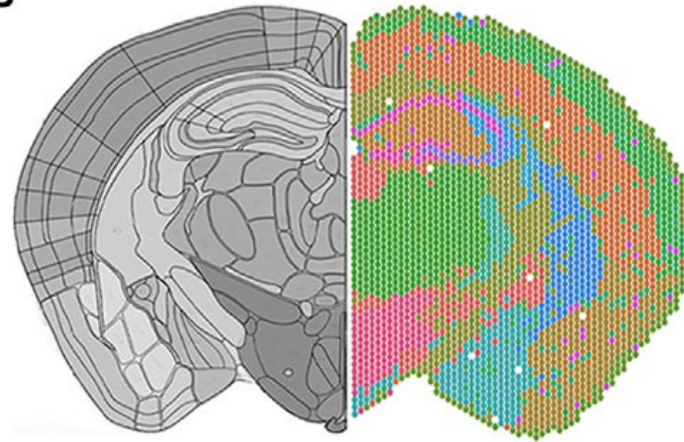
New techniques allow us to view the whole menu (recipes being used by a cell)

A

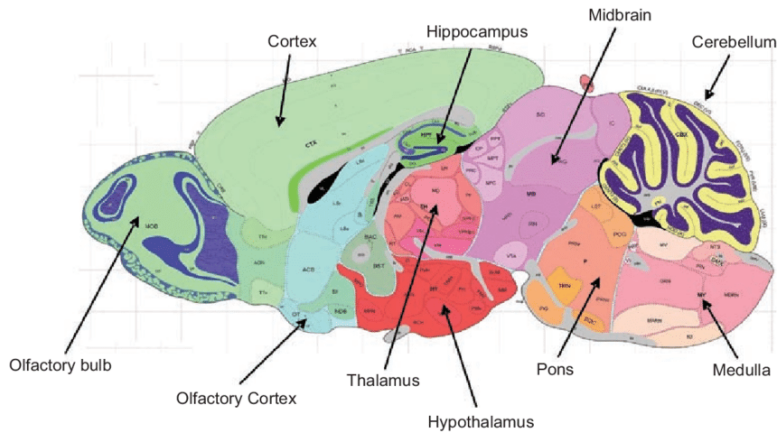


Xiong et al., 2017

B



- 0 Subcortex
- 1 Isocortex
- 2 Molecular layers
- 3 Olfactory cortex
- 4 Fiber tracts
- 5 Meninges
- 6 Thalamus nuclei
- 7 Isocortex
- 8 Cortex Sst+
- 9 Subcortex
- 10 HT, Mb, and cerebral nuclei
- 11 Ventricular surface
- 12 Ventricle neighborhood
- 13
- 14
- 15
- 16 Cortex Tyrobp+
- 17
- 18 HP somatic layers
- 19



Heydel et al., 2010

# Can investigate changes in all brain cell types at the menu/RNA level

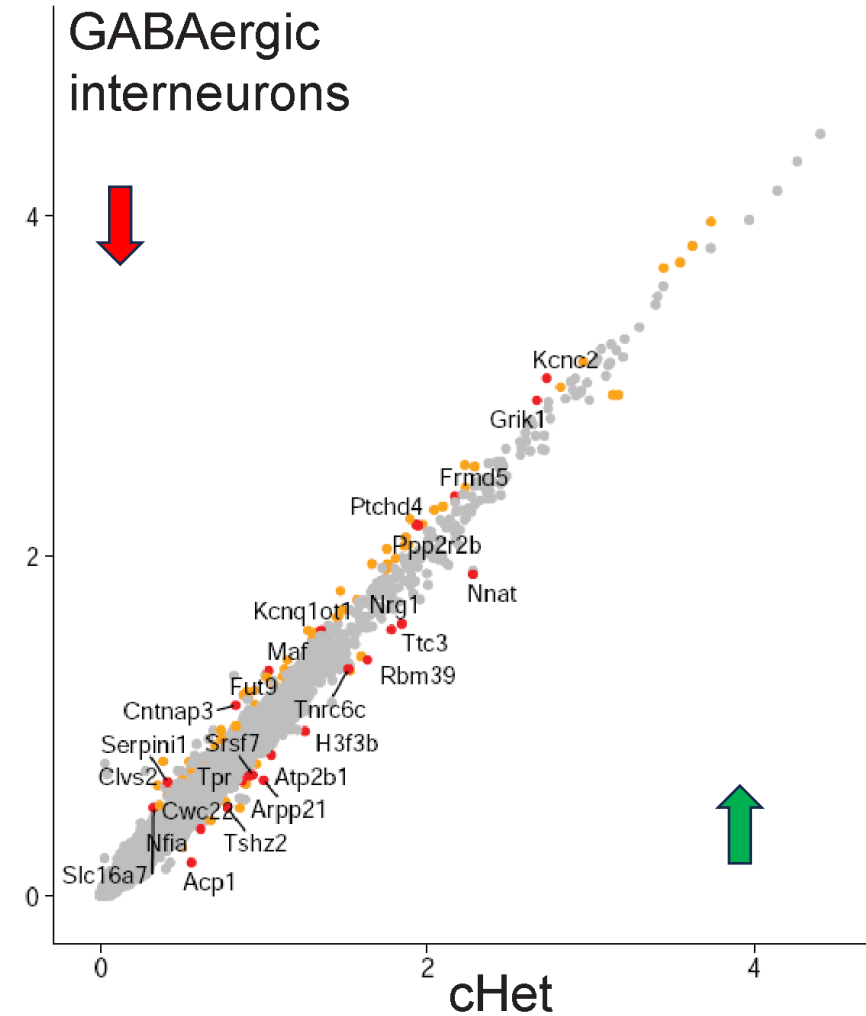
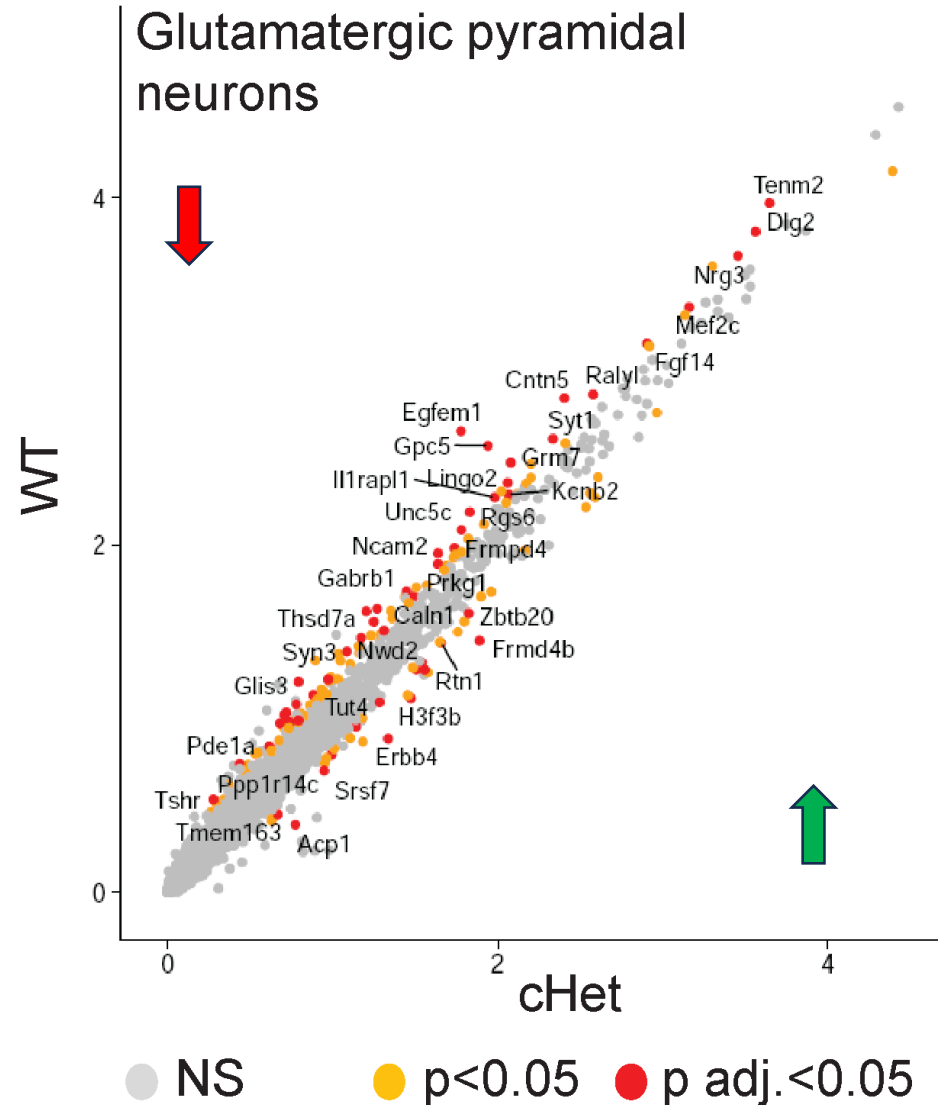
Excitatory (Glut) and inhibitory (GABA) neurons were the most impacted cells



Gene is reduced



Gene is increased

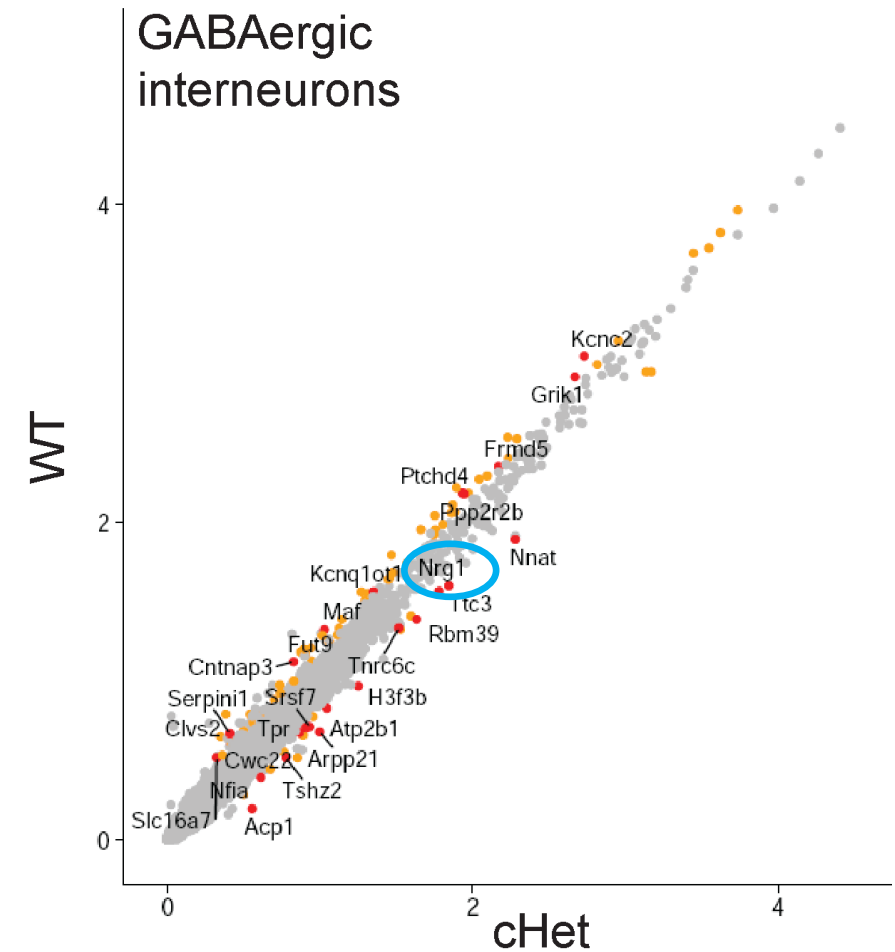
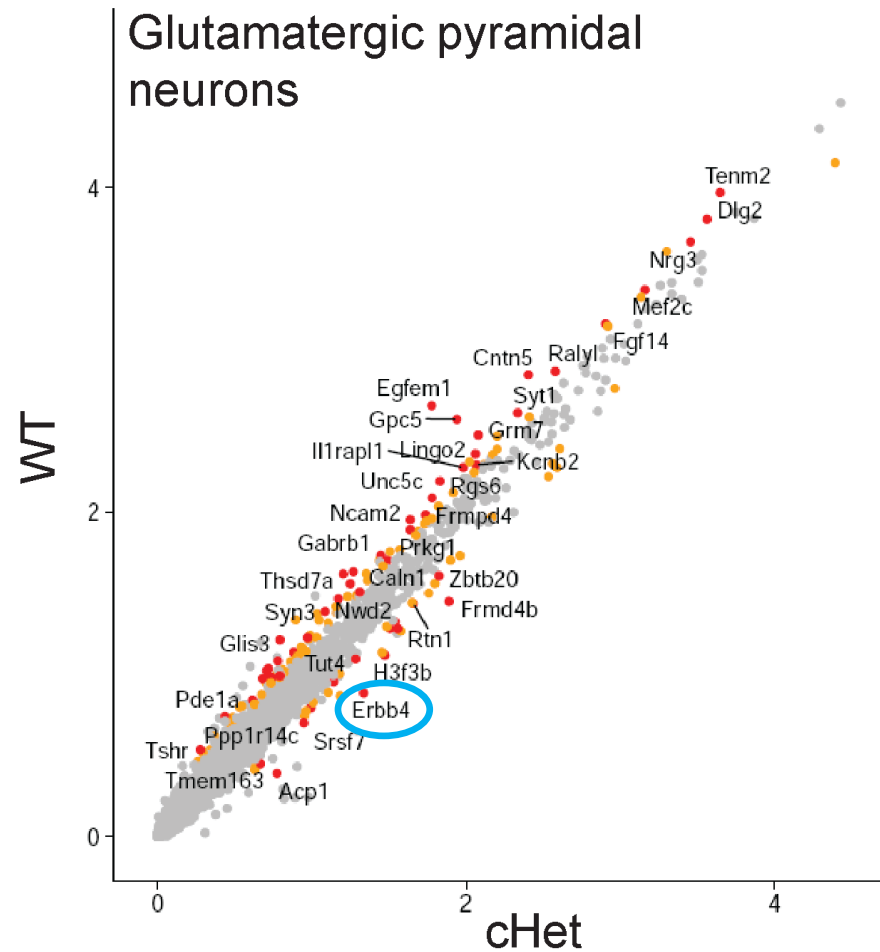




# Now validating candidates to see which RNA-menu is used by each cell



*Dariangelly Pacheco-Cruz,  
(Graduate Student)*



● NS      ● p < 0.05      ● p adj. < 0.05

Some candidates have  
FDA-approved drugs to test  
-- Now being investigated

## Ongoing and future work

Collaboration with Kim and Gabel labs

Cell specific loss of function; using engineered mice to study

- Inhibitory neurons
- Excitatory neurons and glia

Unbiased screen to profile RNA abundance in single brain cells  
(Alex Nord lab, UC Davis)

Any labs interested in trying out ideas/therapeutics?

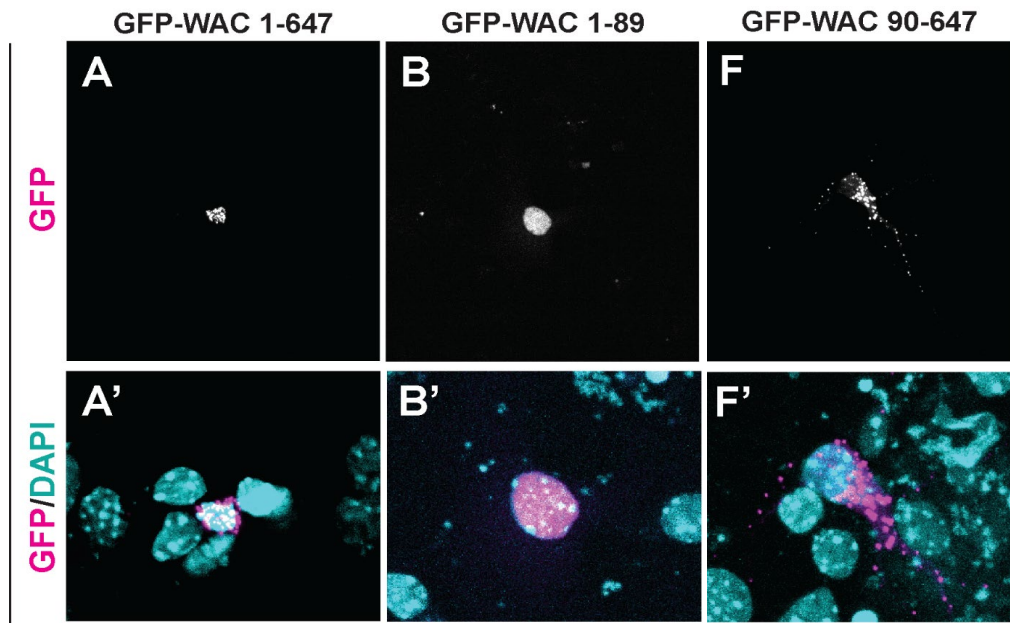
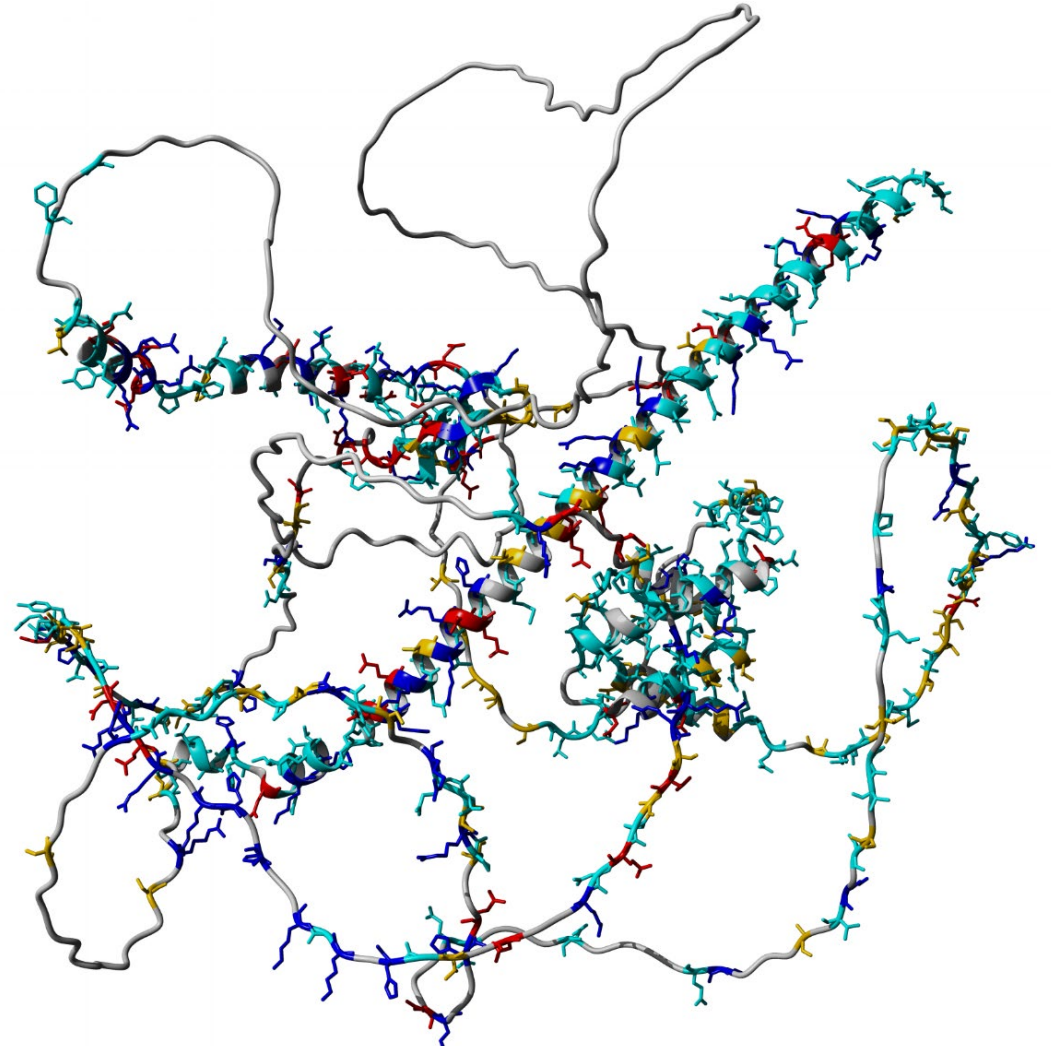


# Funding and thanks

Spectrum Health – Michigan State University Alliance

Michigan State University IPSTP NIH training grant  
To Dariangelly Pacheco-Cruz

Lab manager, April Stafford (generated the mouse  
WAC mice)



# Questions?

1. Can DESSH be diagnosed in utero?
  - This is possible, but may need good indication to attempt
2. Are researchers looking at the gene therapy treatments in utero?
  - Our lab is attempting to introduce a full-length human WAC gene to rescue symptoms
3. Is there hope for treatment in the future?
  - Yes, but depends on finding druggable targets and/or therapeutics
4. Will cell manipulation work for DESSH?
  - Let's discuss
5. Have you researched targeted cell therapy? Have you researched gene therapy or protein replacement?
  - Let's discuss
6. Can WAC protein be synthesized? Has anyone tried?
  - This is possible, but I am not aware of anyone who has synthesized the WAC protein