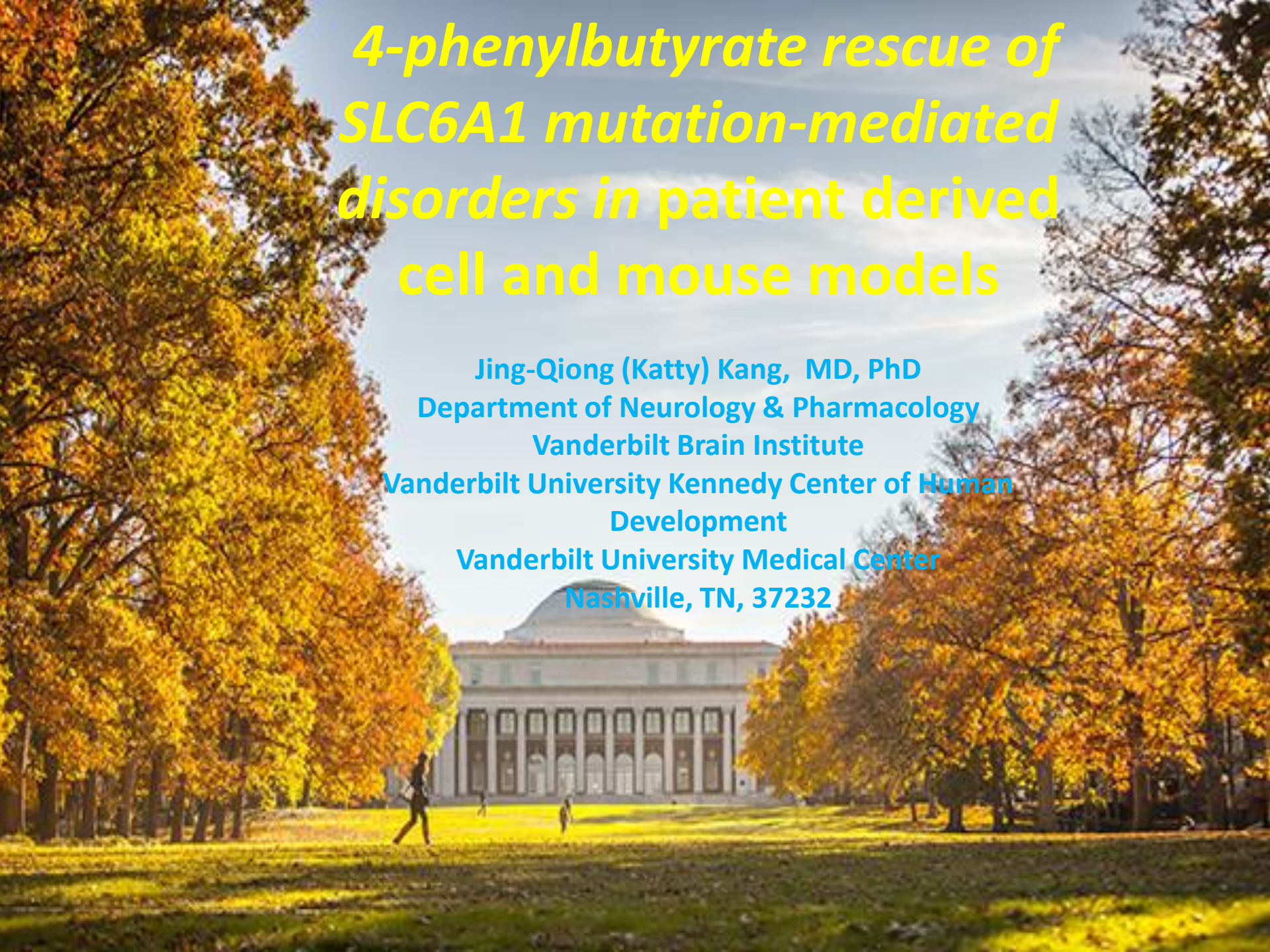


4-phenylbutyrate rescue of SLC6A1 mutation-mediated disorders in patient derived cell and mouse models

Jing-Qiong (Katty) Kang, MD, PhD
Department of Neurology & Pharmacology
Vanderbilt Brain Institute
Vanderbilt University Kennedy Center of Human Development
Vanderbilt University Medical Center
Nashville, TN, 37232

2011

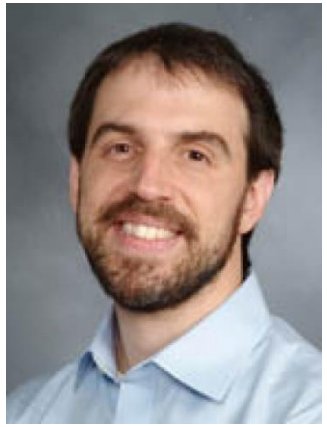
My VICTR award “ 4-phenylbutyrate as a novel treatment option for epilepsy”

Gene Liau

Terry Jo Bichell

Tom Davis

Amber Freed



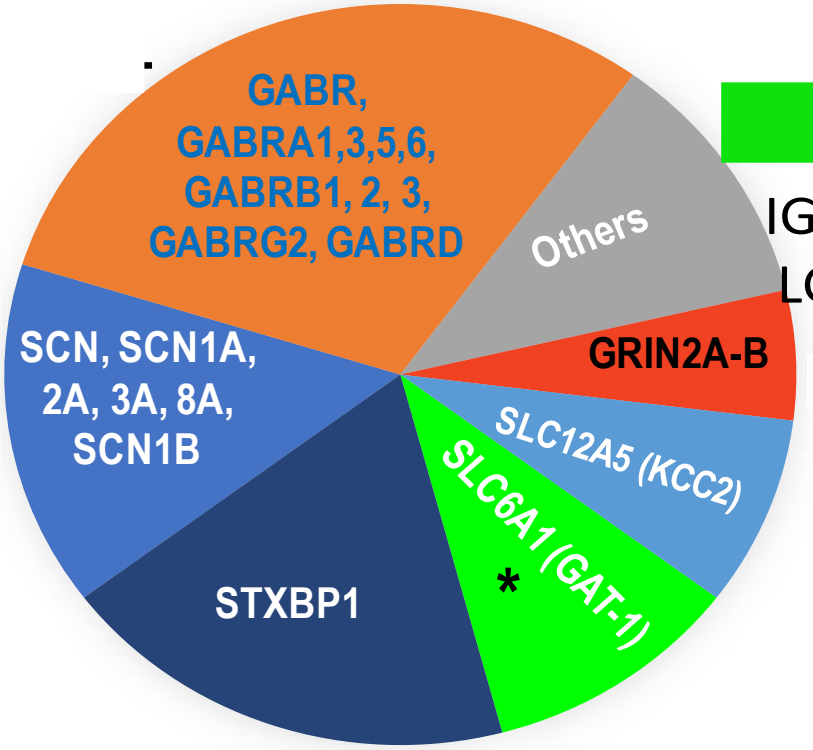
Zachery Grinspan, then Scott, Kim….

The story about Maxwell

I got SLC6A1(S295L) mutation, let's fight!!!

~80 established epilepsy genes and ~1000 epilepsy associated genes

Mutations in genes directly or indirectly affecting GABAergic pathway and the associated epilepsy syndromes, autism and intellectual disability



SLC6A1

IGE, CAE, JME, IAE, MAE, LGS, ID, autism

CAE=Childhood absence
IAE=Idiopathic absence epilepsy

ID=intellectual disability
LGS=Lennox-Gastaut syndrome

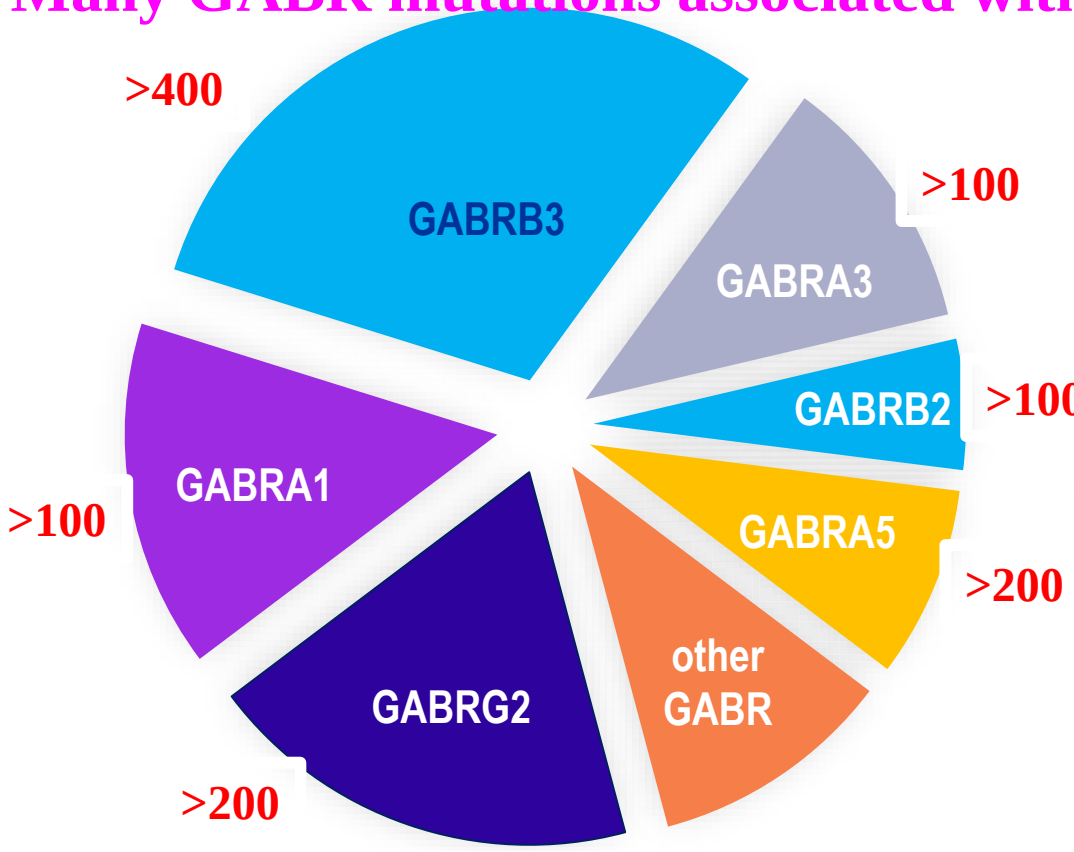
JME=Juvenile myoclonic epilepsy

LGS=Lennox-Gastaut syndrome

MAE=Myoclonic astatic epilepsy

* Gene will be studied

Many GABR mutations associated with epilepsy



Many mutations in multiple GABR have been associated with epilepsy, autism and ID

GABR mutations
GABRA1,2,3,5,6,
GABRB1-3, GABRG2,
GABRD
GABRG1

CAE=Childhood absence

DS=Dravet syndrome

EE=Epileptic encephalopathy

EIEE=Early infantile epileptic encephalopathy

EIMFS=Epilepsy of infancy with migrating focal seizures

EOME=Early onset myoclonic encephalopathy

FS=Febrile seizures

GEFS+=Generalized epilepsy with febrile seizures

IAE=Idiopathicabsence epilepsy

ID=Intellectual disability

IGE=Idiopathic generalized epilepsy

IS= Infantile spasms

JAE=Juvenile absence epilepsy

JME=Juvenile myoclonic epilepsy

Lennox-Gastaut syndrome

LGS=Lennox-Gastaut syndrome

MAE=Myoclonic astatic epilepsy

OS=Ohtohara syndrome

SUDC=Sudden unexplained death in childhood

What does it mean if a child carries a mutation in *SLC6A1*?

Loss of function?

Gain of function?

Altered function?

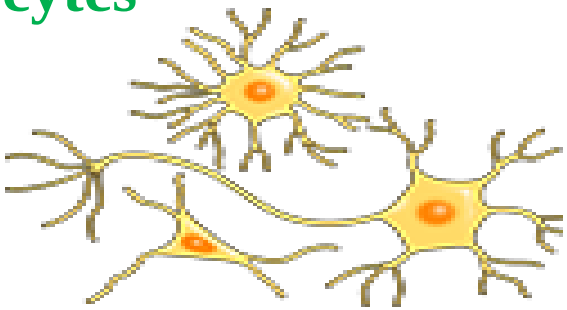
How do we study the impact of a mutation?

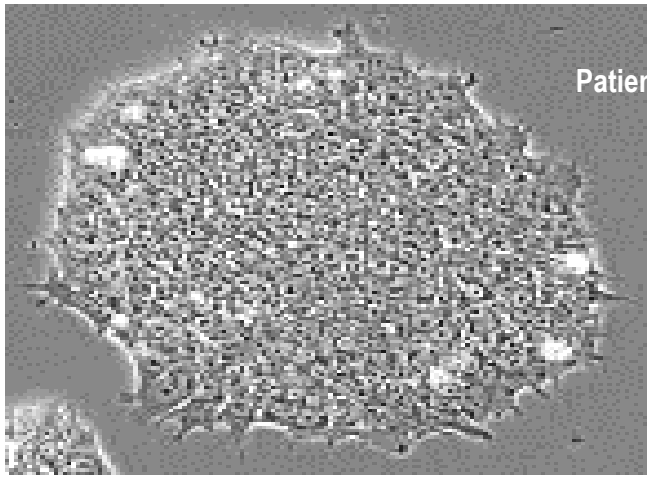
Insights, tools and protocols from GABAA receptors

We have studied the trafficking and function of mutant GAT-1 in HEK293T, iPSCs, mouse neurons and astrocytes

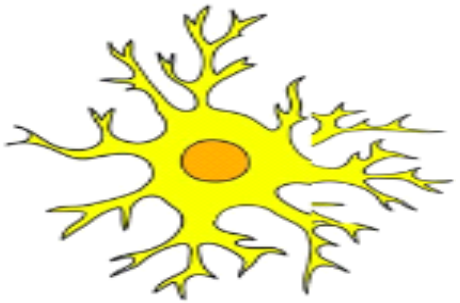
HEK293

Neurons



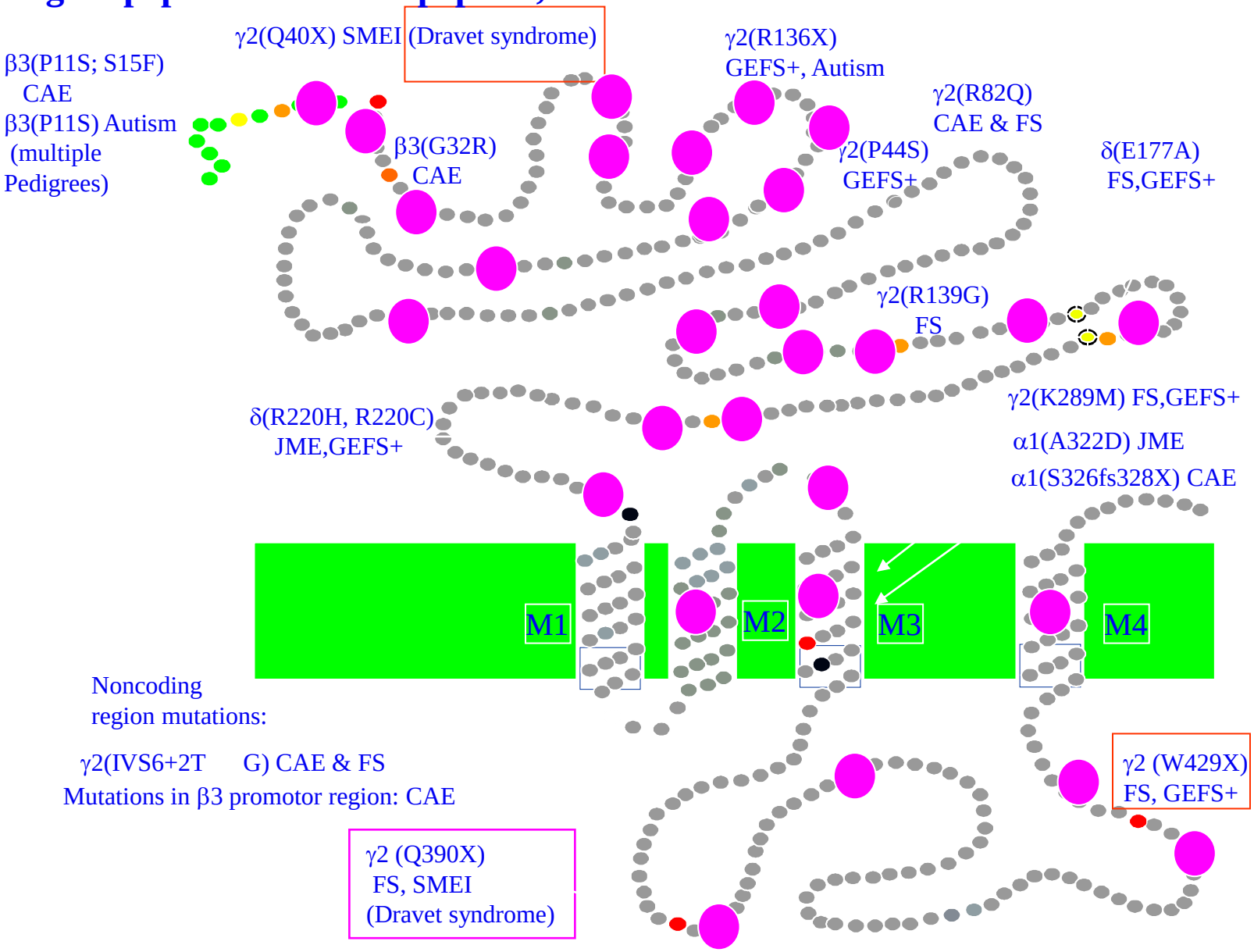


iPSCs (induced pluripotent stem cells)



astrocytes

We have extensively studied $GABA_A$ receptor subunits (~100 mutations)
Signal peptide to mature peptide, from transmembrane domain to intracellular loop

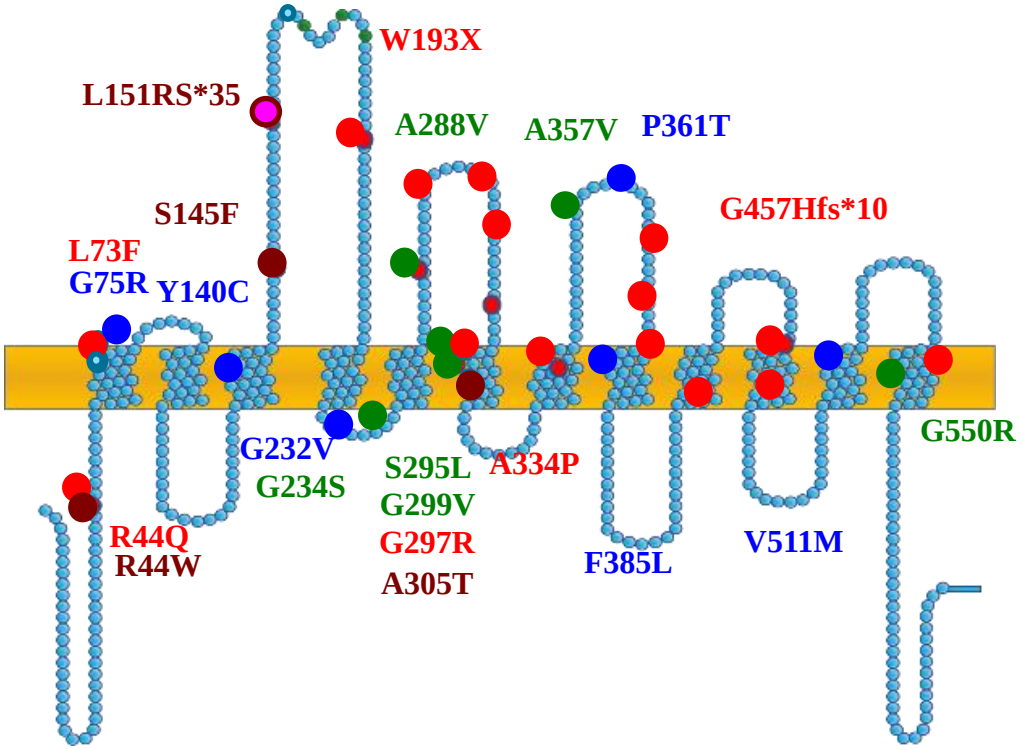


$GABA_A$ receptor subunit gene mutations associated with epilepsies

In collaboration with
Dr. Macdonald

GABA Transporter 1

>60 mutations



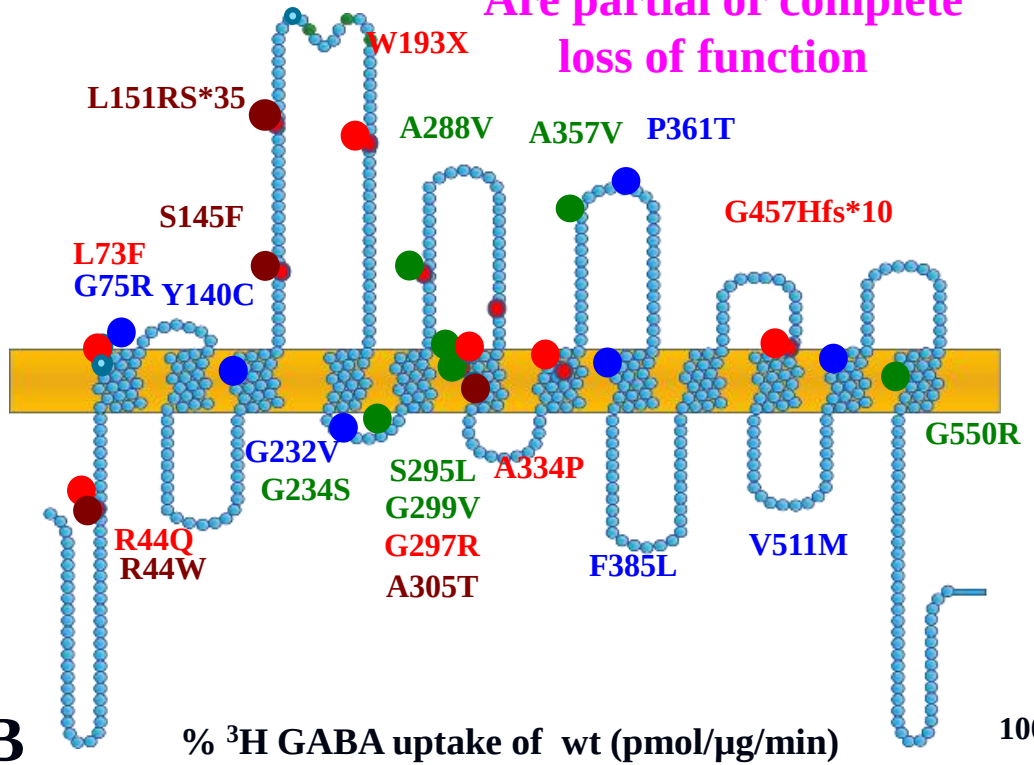
R44Q[#]: epilepsy with myoclonic-atonic seizures
R44W: developmental disorder
L73F: epilepsy
G75R[#]: generalized epilepsy, intellectual disability
Y140C[#]: epilepsy with myoclonic-atonic seizures, mild to moderate intellectual disability
S145F[#]: mild intellectual disability
L151RS*35[#]: intellectual disability
W193X[#]: epilepsy with myoclonic-atonic seizures
G232V[#]: epilepsy with myoclonic-atonic seizures, mild to moderate intellectual disability
G234S: Lennox-Gastaut Syndrome
A288V[#]: autism spectrum disorder
S295L: SLC6A1-related disorder, hypotonia
G297R[#]: epilepsy with myoclonic-atonic seizures
G299V: autism spectrum disorder
A305T: developmental disorder
A305V: myoclonic atonic epilepsy
A334P[#]: epilepsy with myoclonic-atonic seizures
A357V[#]: Rett-like syndrome
P361T: autism spectrum disorder, epilepsy with absence and atonic seizures
F385L[#]: epilepsy with myoclonic-atonic seizures, mild to moderate intellectual disability
G457Hfs*10[#]: epilepsy with myoclonic-atonic seizures
V511M[#]: Generalized epilepsy, mild intellectual disability
G550R[#]: autism spectrum disorder

Cai .. Kang Exp Neuro 2019
Wang .. Kang Mol Brain 2020
Poliquin .. Kang Exp Neuro 2021
Mermer .. Kang Brain 2021
Nwosu.. Kang Brain Comm 2022
Mermer .. Kang Neurobiology of Disease 2022

Mermer et al., Brain, 2021

22 mutations

Most of the SLC6A1 mutations, if not all, Are partial or complete loss of function



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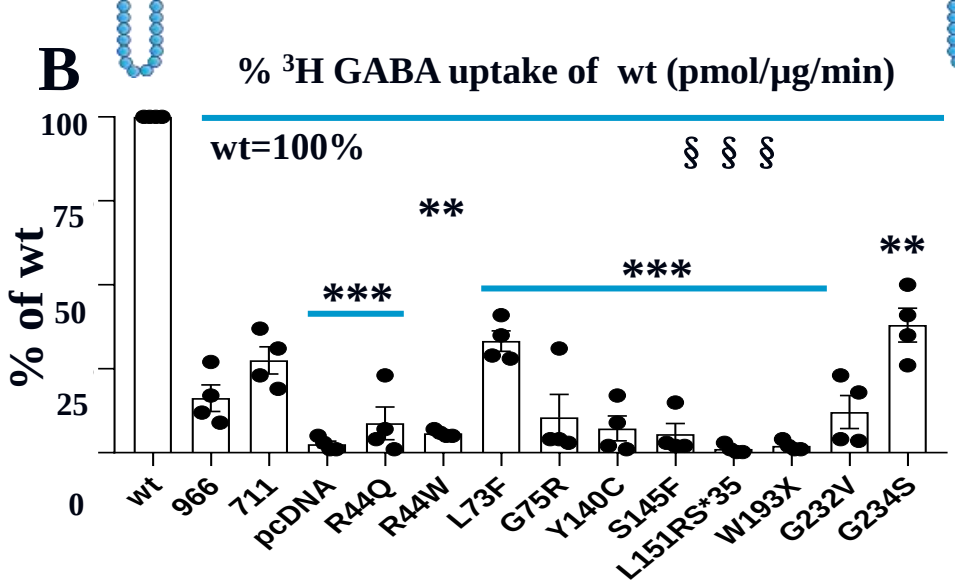
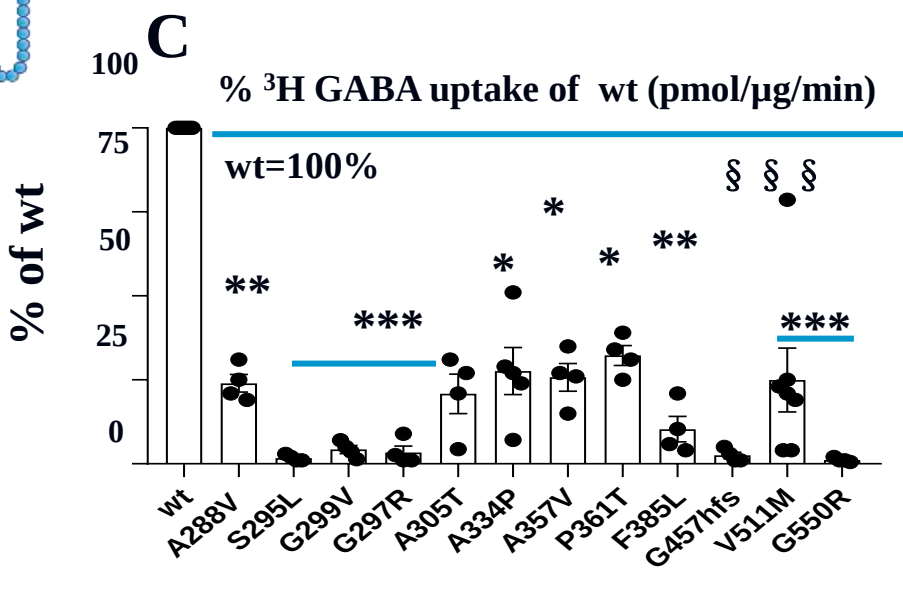


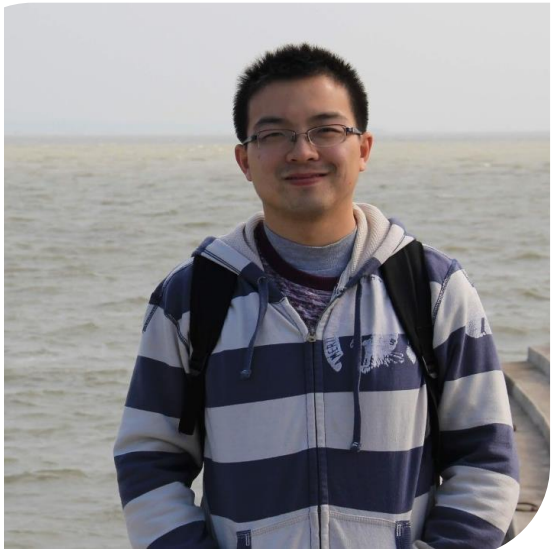


What can cause the mutant transporters to lose function?

insights gained from collaboration with two experts from deep learning

Prof. Dong Xu

Chair/Distinguished Professor
Univ of Missouri



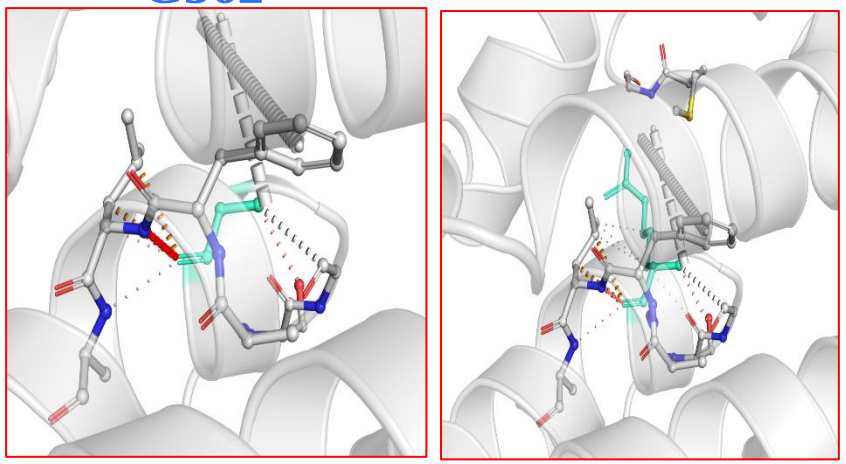
Dr. Juexin Wang

Assistant professor
Indiana Univ-Purdue Univ

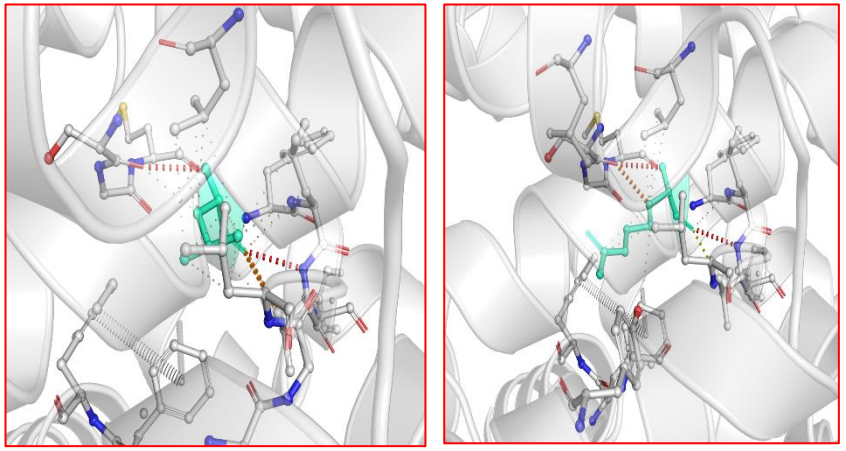
Expert in Computer Science/
Bioinformatics/protein structural biology/Single cell RNAseq/AlphaFold

AI tools predict the mutations (two MAE patients in a Chinese cohort) to have reduced protein stability

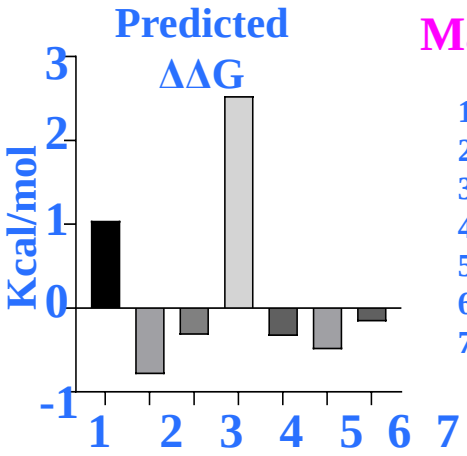
A G362 R362



B L460 R460



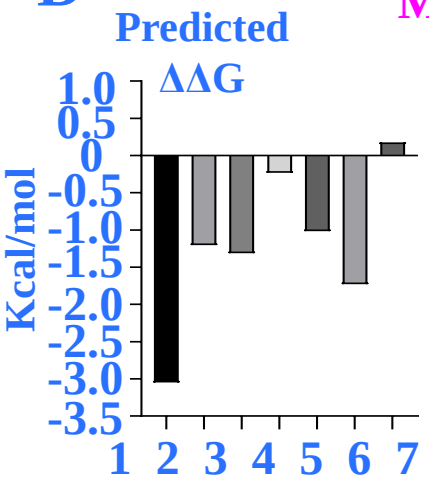
C



Machine learning tools

1. SDM
2. mCSM
3. DUET
4. Dynamut
5. INPS-MD(sequence only)
6. INPS-MD(with structure)
7. MAESTROweb

D



Machine learning tools

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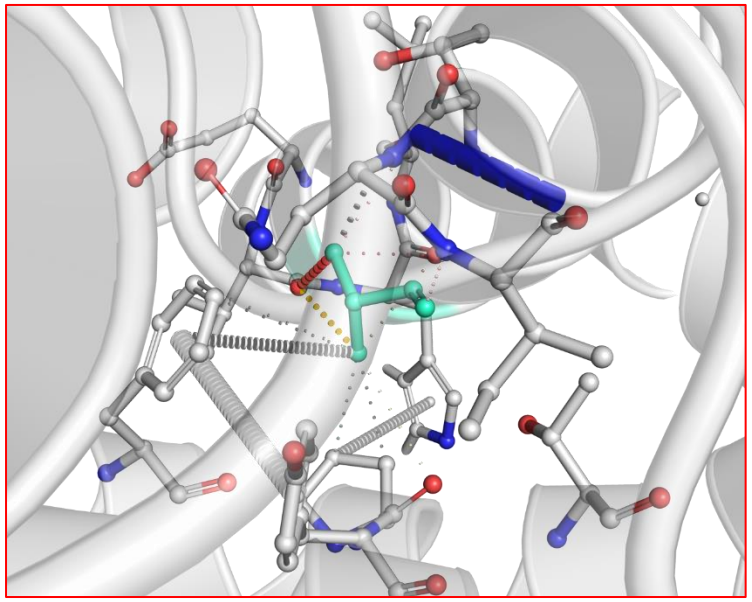
6. INPS-MD(with structure)
7. MAESTROweb

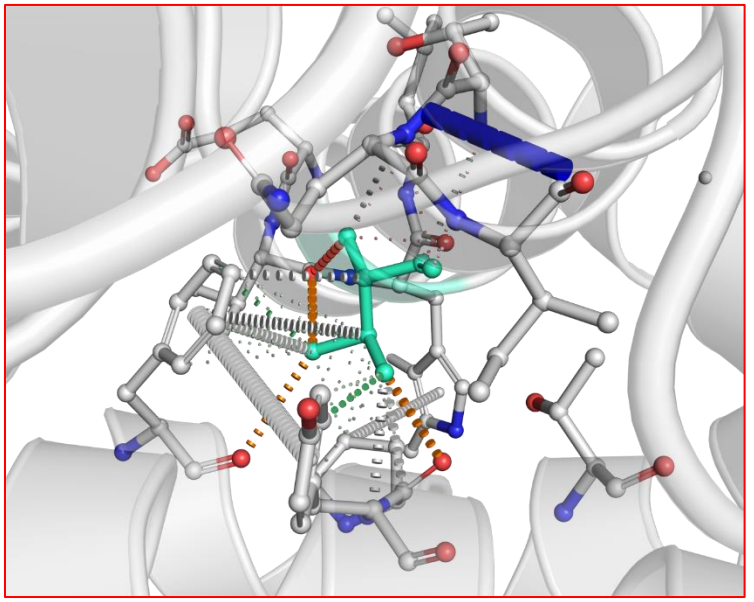
Mermer et al., 2022
Neurobiology of Disease

Machine learning and structure modeling suggest the GAT-1(V288) protein is more hydrophobic but less stable compared with the wildtype.

Wildtype GAT-1(A288)



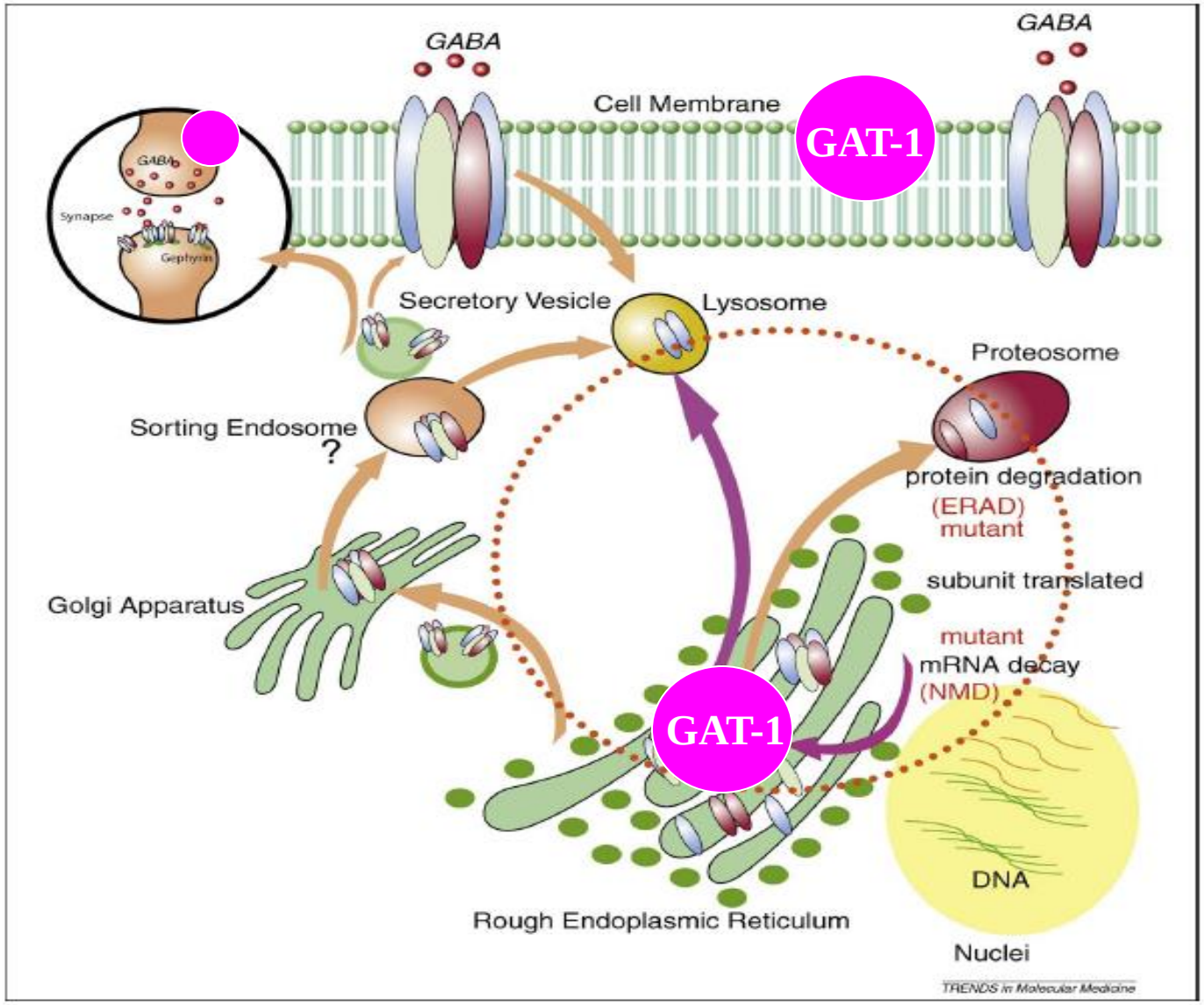
mutant GAT-1(V288)



Mutation from Alanine to Valine at residue 288 adds a side chain isopropyl group, making the residue much more hydrophobic.

Kang & Macdonald, JAMA Neurology, 2016

where are the mutant GAT-1 transporters?

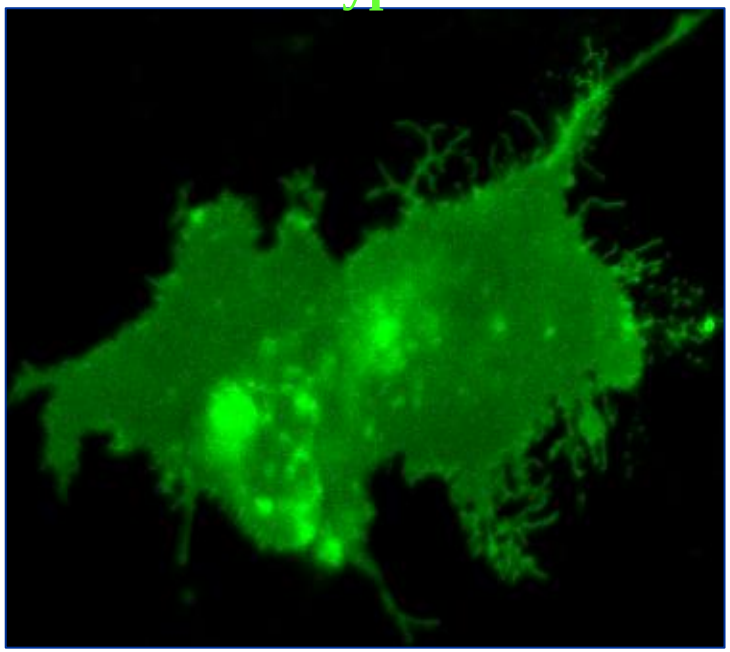


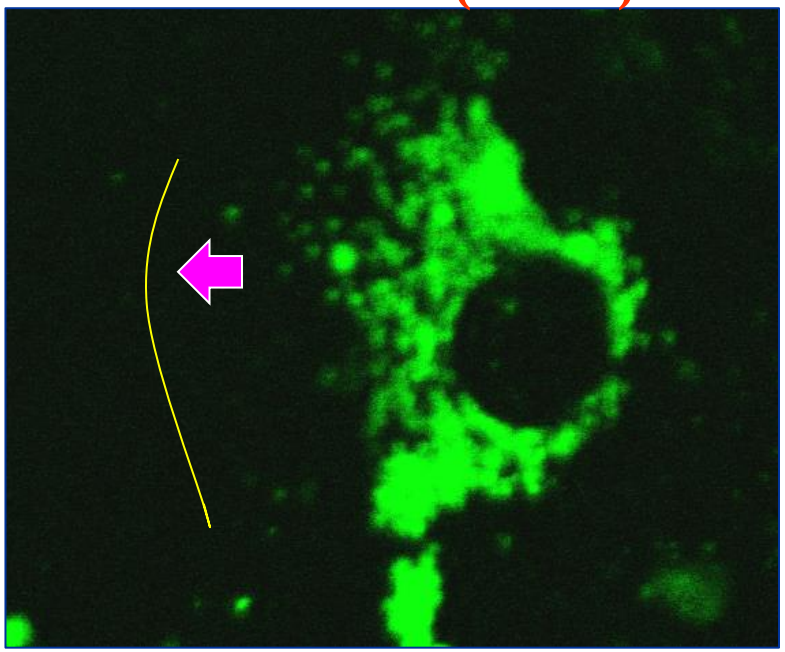
Maxwell's mutant protein is retained inside ER

wildtype

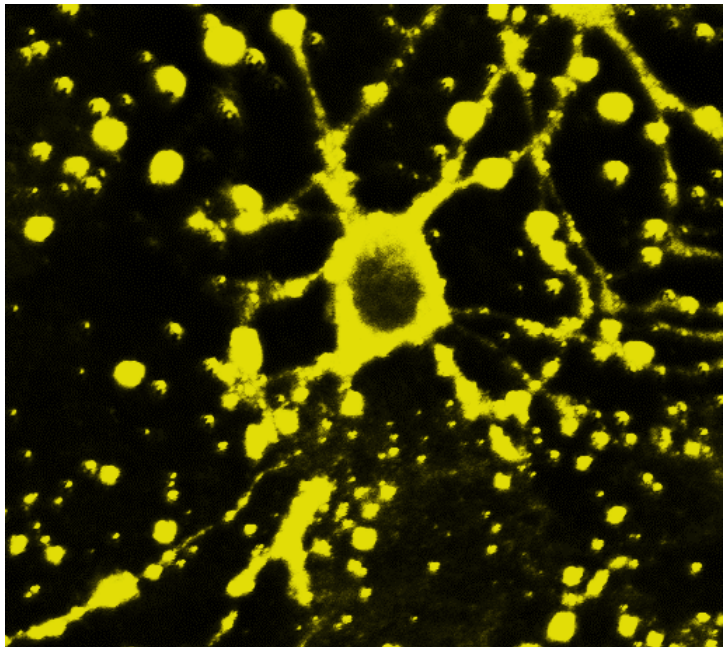
mutant (S295L)

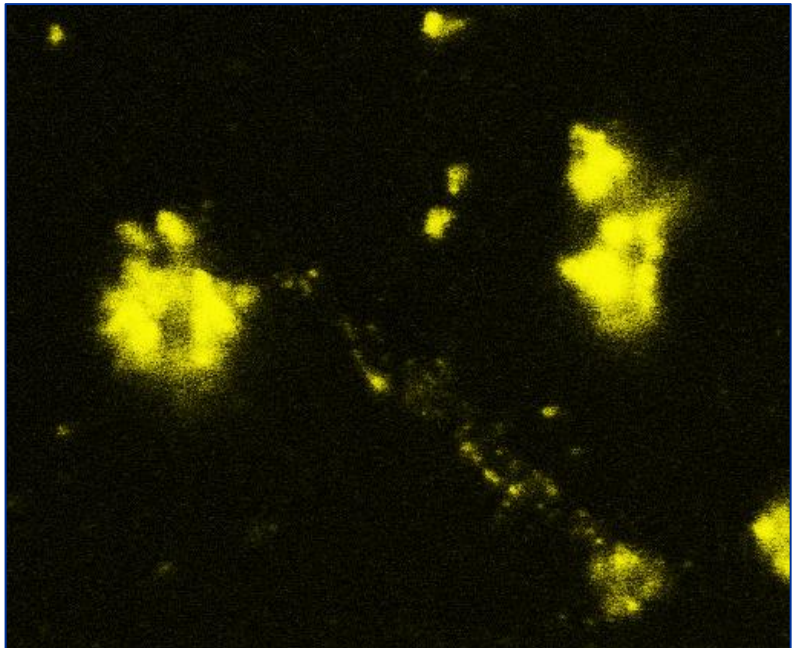
Live Astrocyte





Live neuron



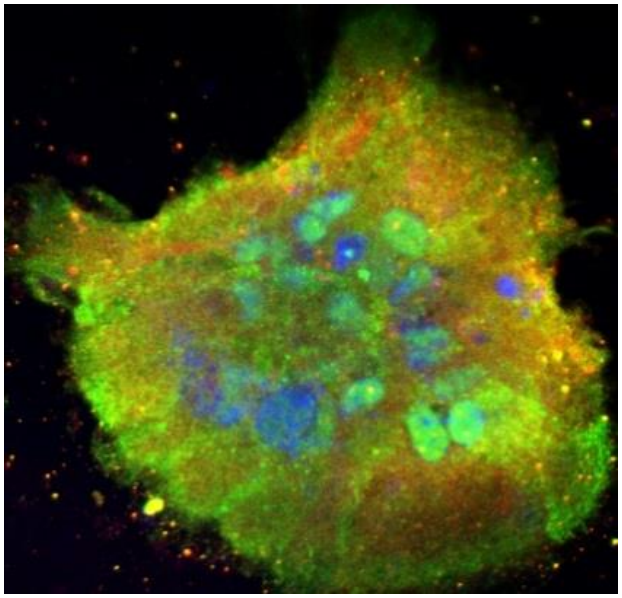


Impaired trafficking: the mutant transporters may interfere with the wildtype

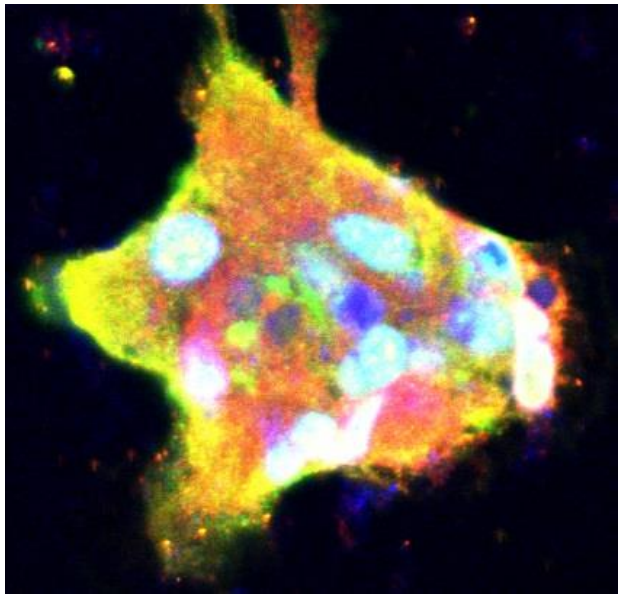
Red=wildtype endogenous GAT-1

Green=recombinant GAT-1

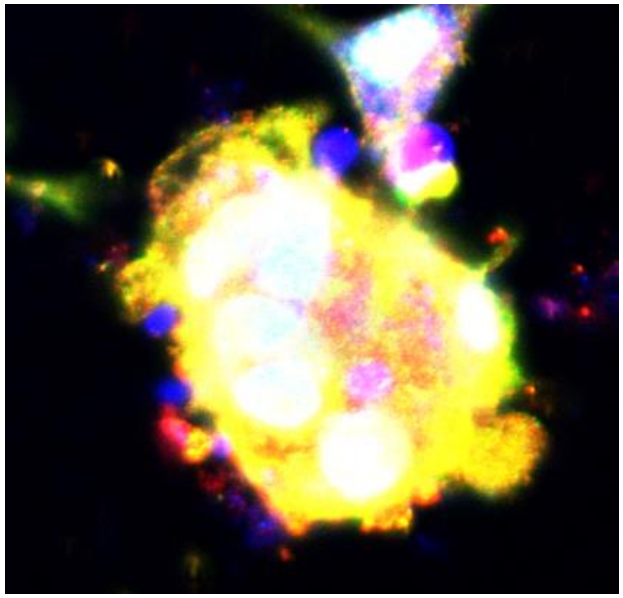
wildtype



mutant



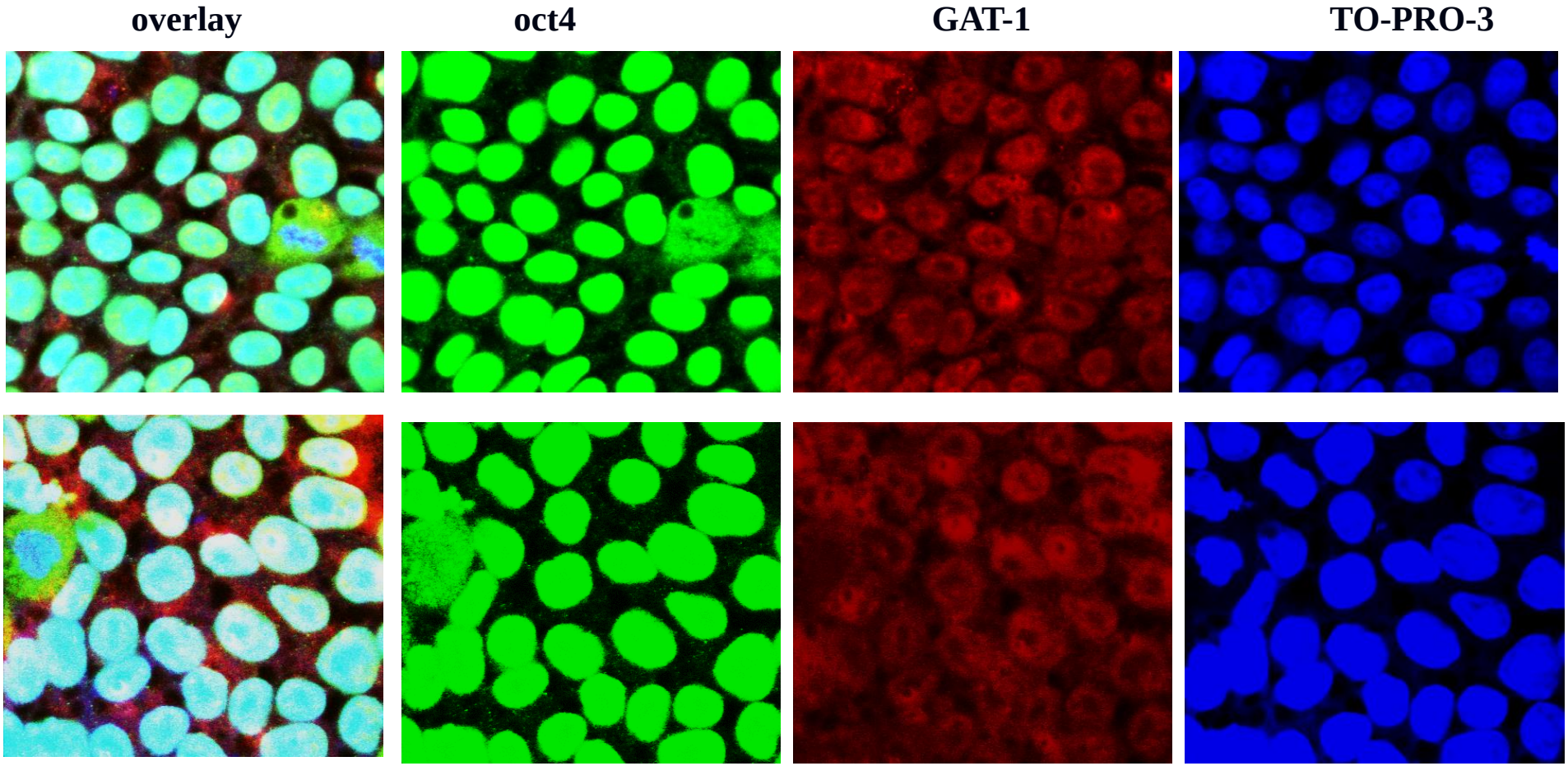
mutant



astrocytes

When will the cells start to generate the mutant protein?

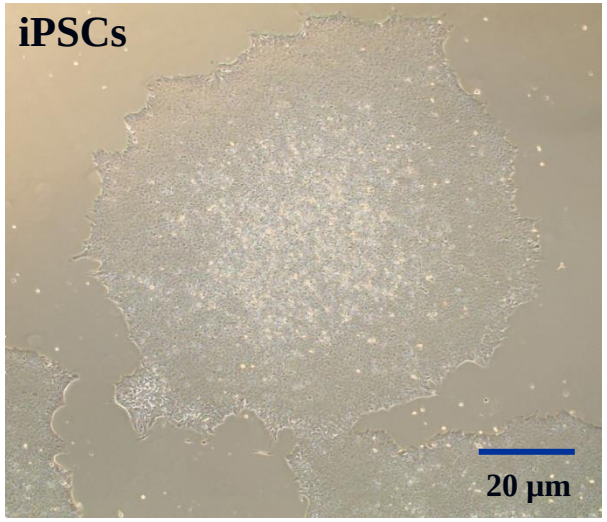
human iPSC cells From conception on?

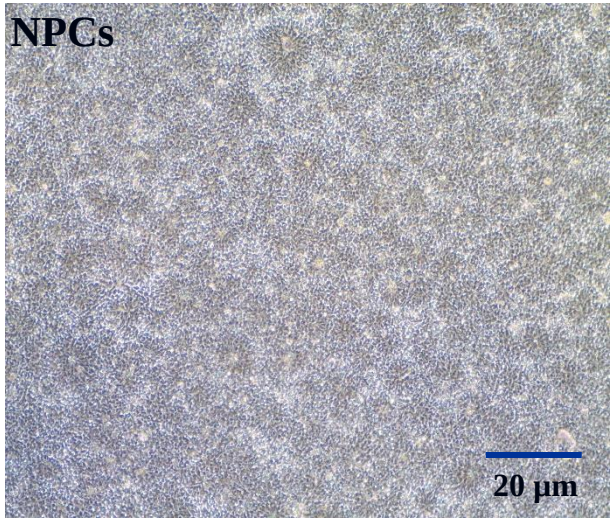


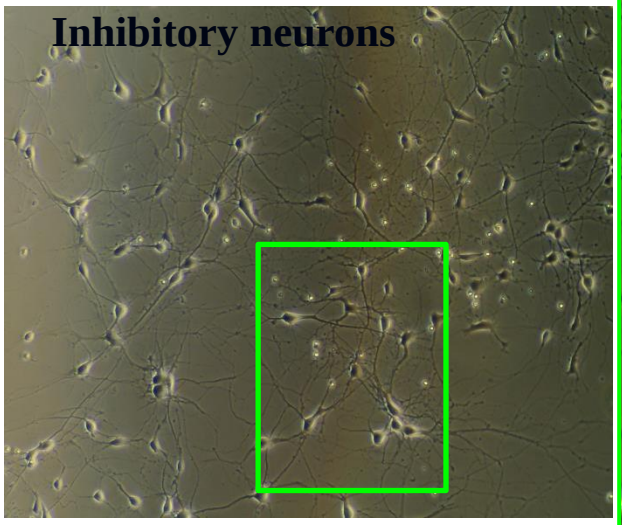
A low level of GAT-1 was detected in human iPSCs

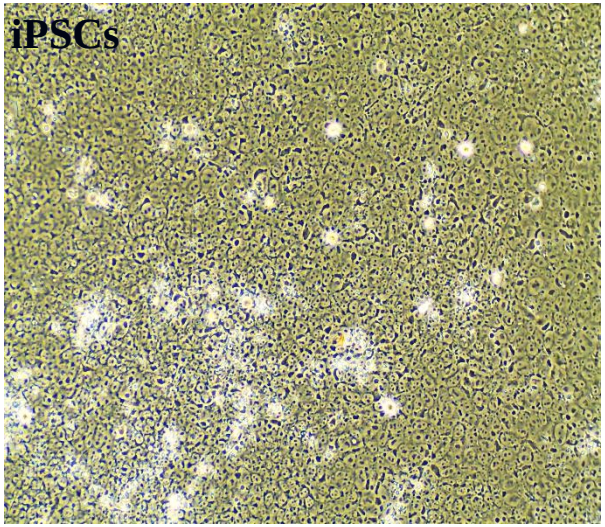
We then tested PBA effect in various cell models: Human iPSC derived NPC, neurons and astrocytes

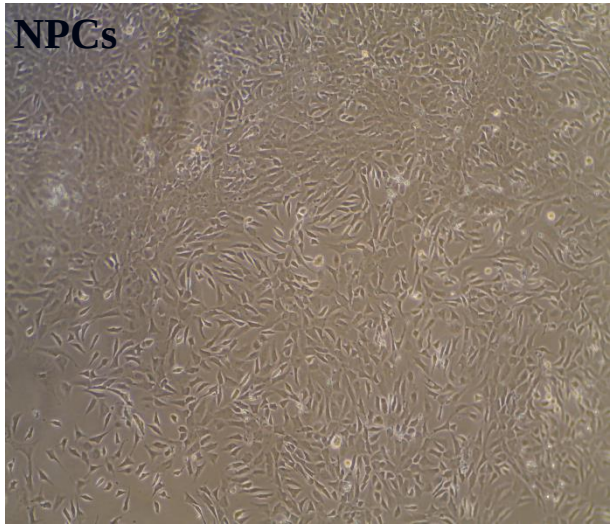
A

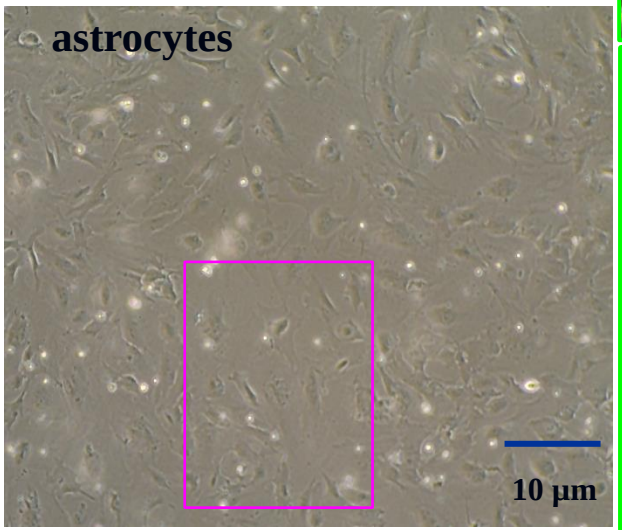












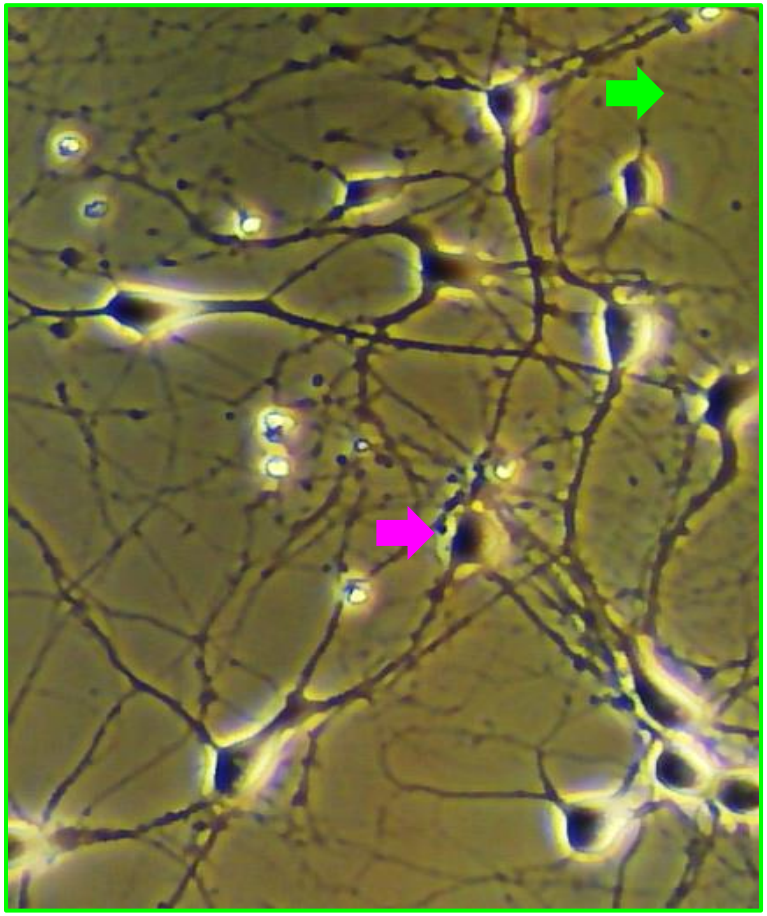
B

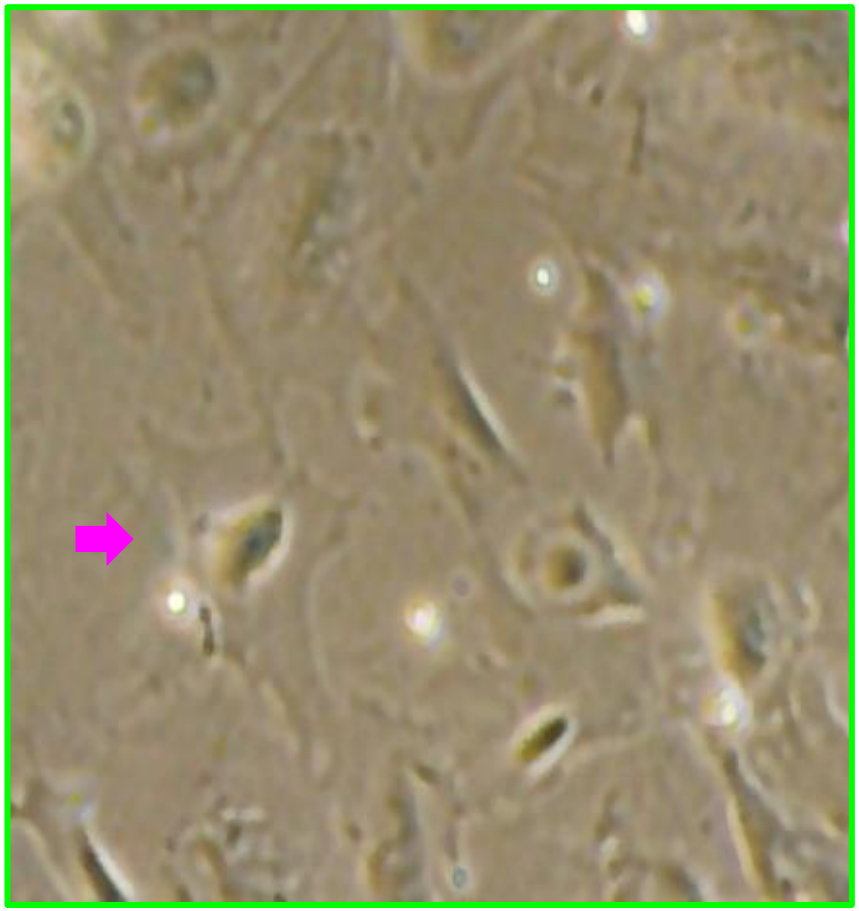
iPSC derived

Enlarged

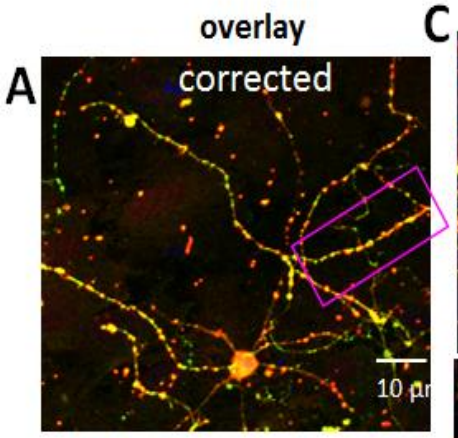
Neurons

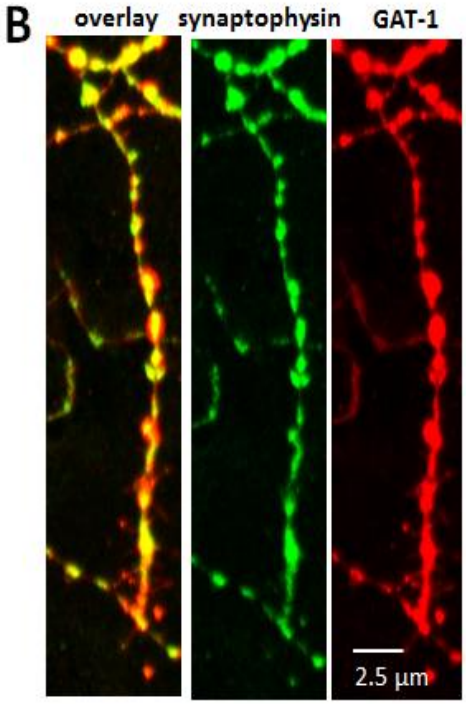
astrocytes

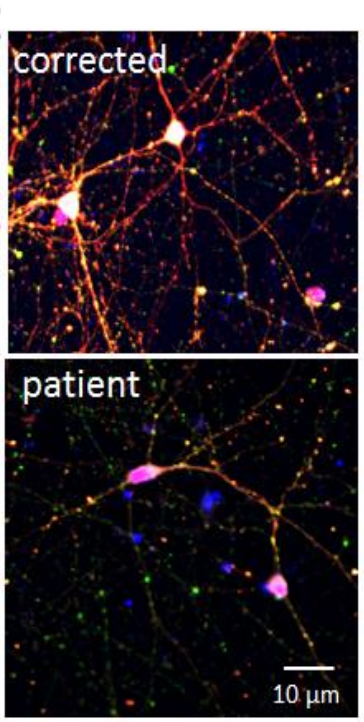


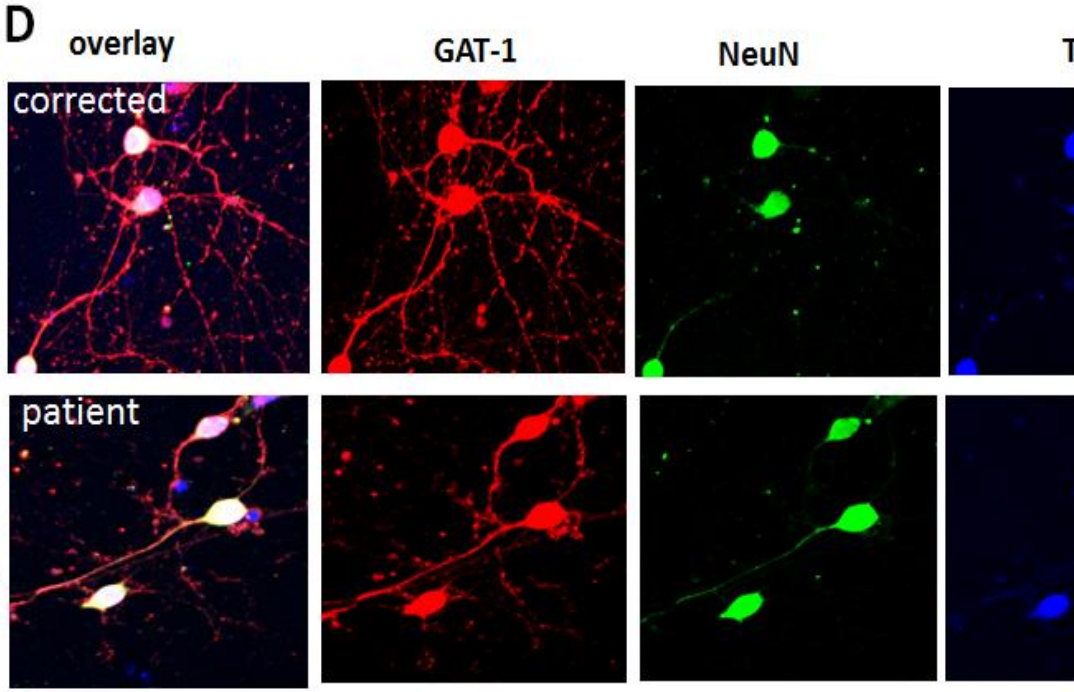


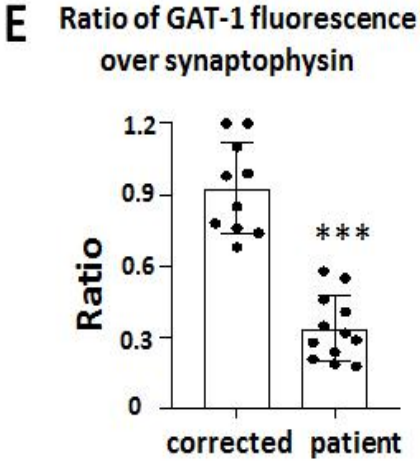
Maxwell's neurons show reduced GAT-1 at synapse

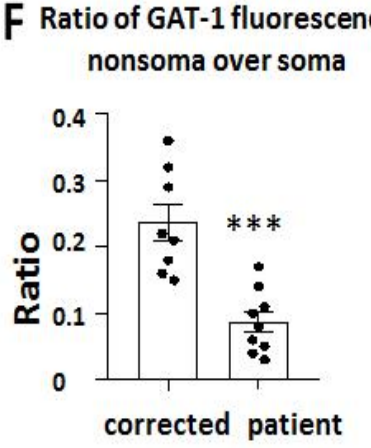


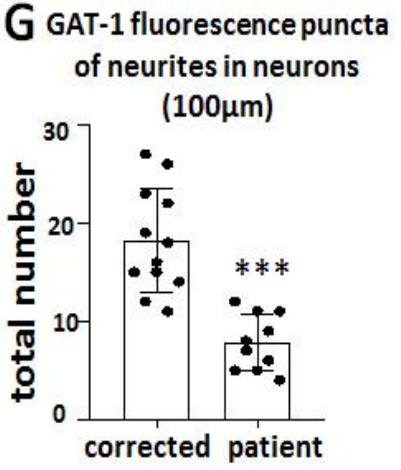




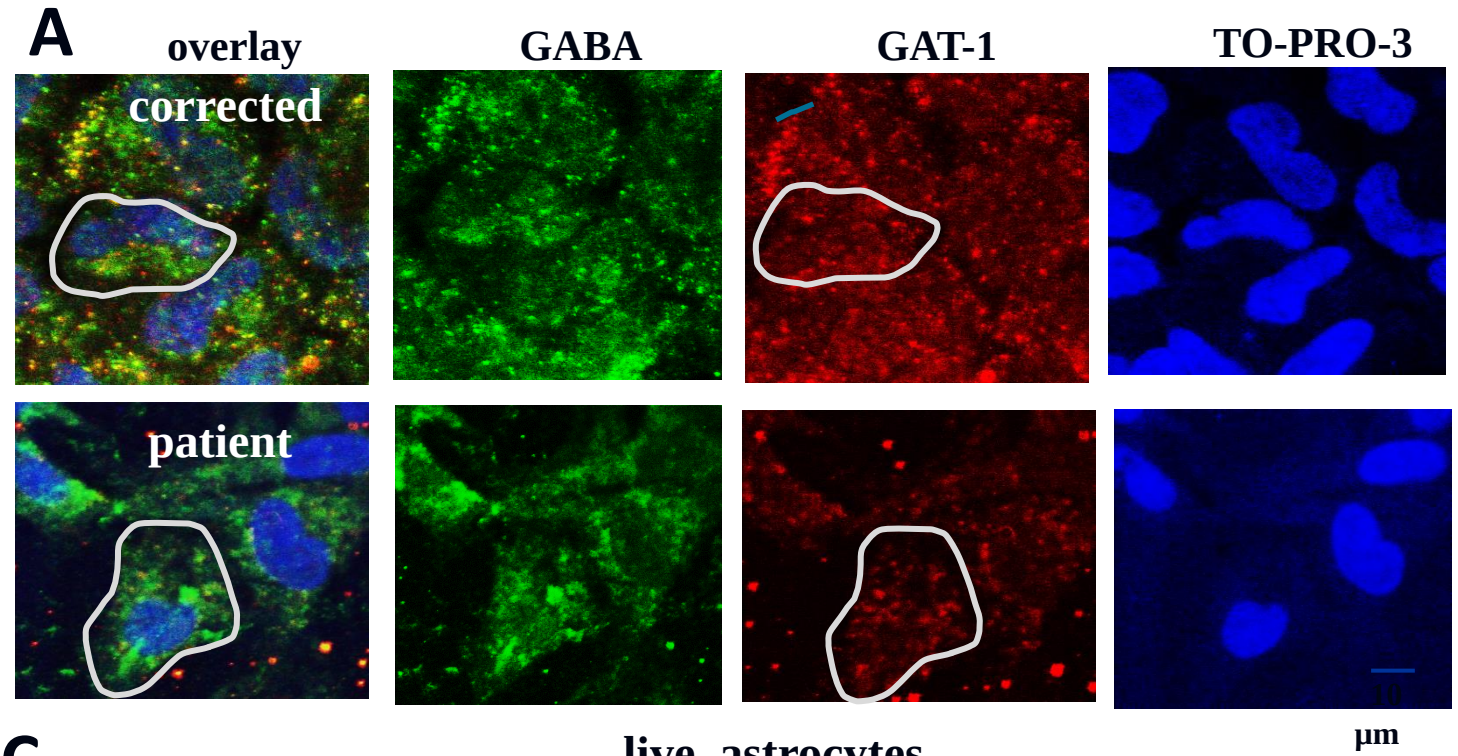


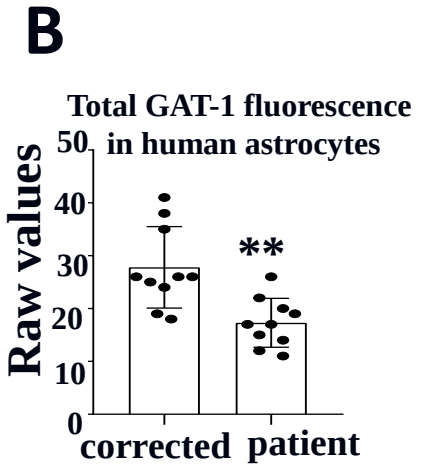


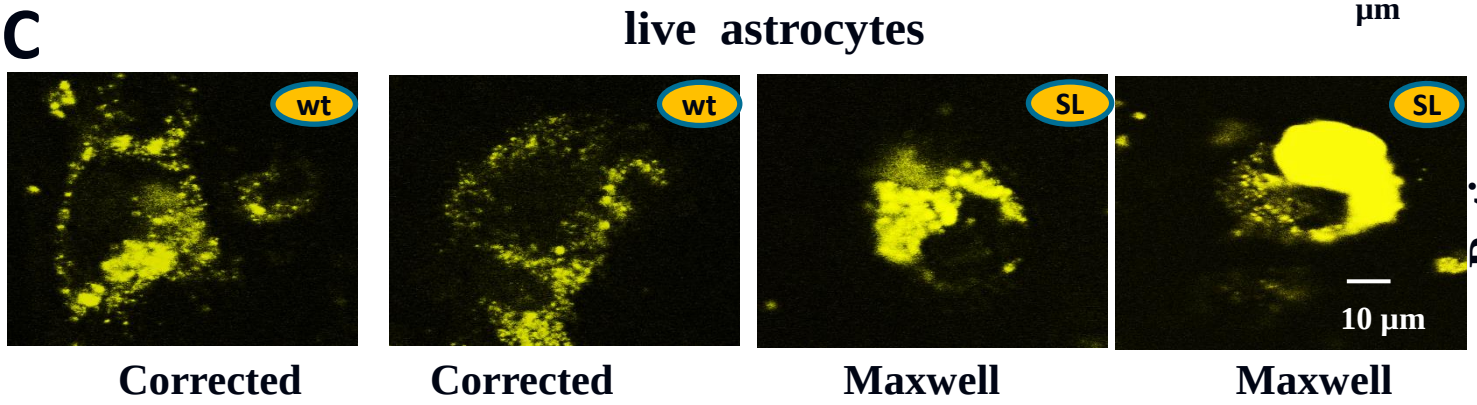


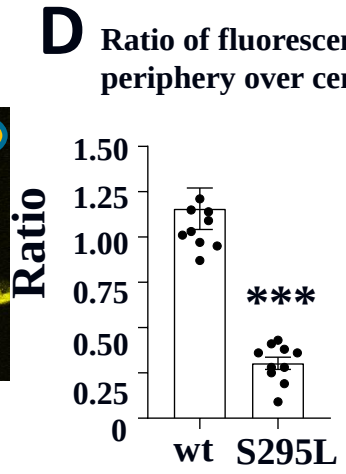


Maxwell's astrocytes show reduced and ER-retained GAT-1

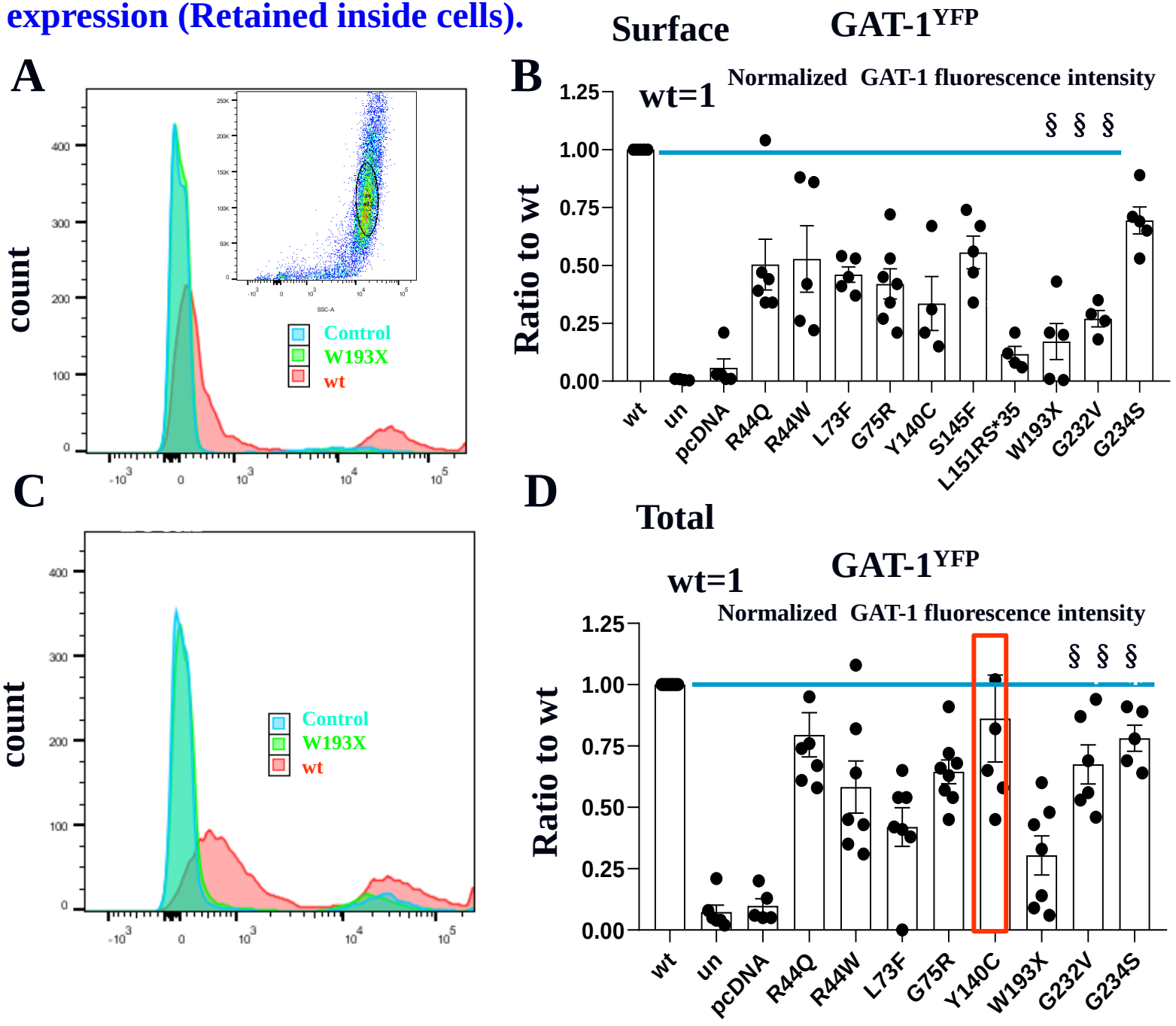






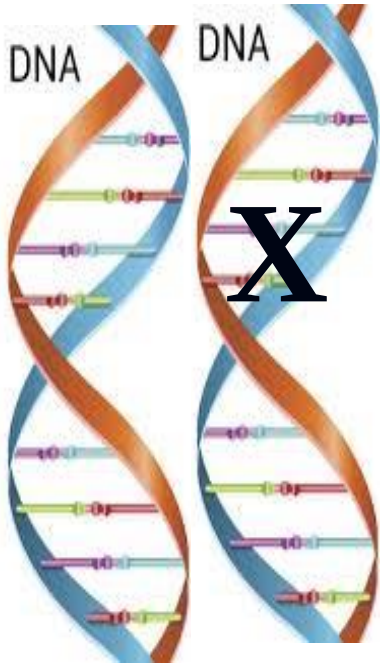


High throughput flow cytometry identified that the mutant GAT-1 had reduced cell surface expression (Retained inside cells).



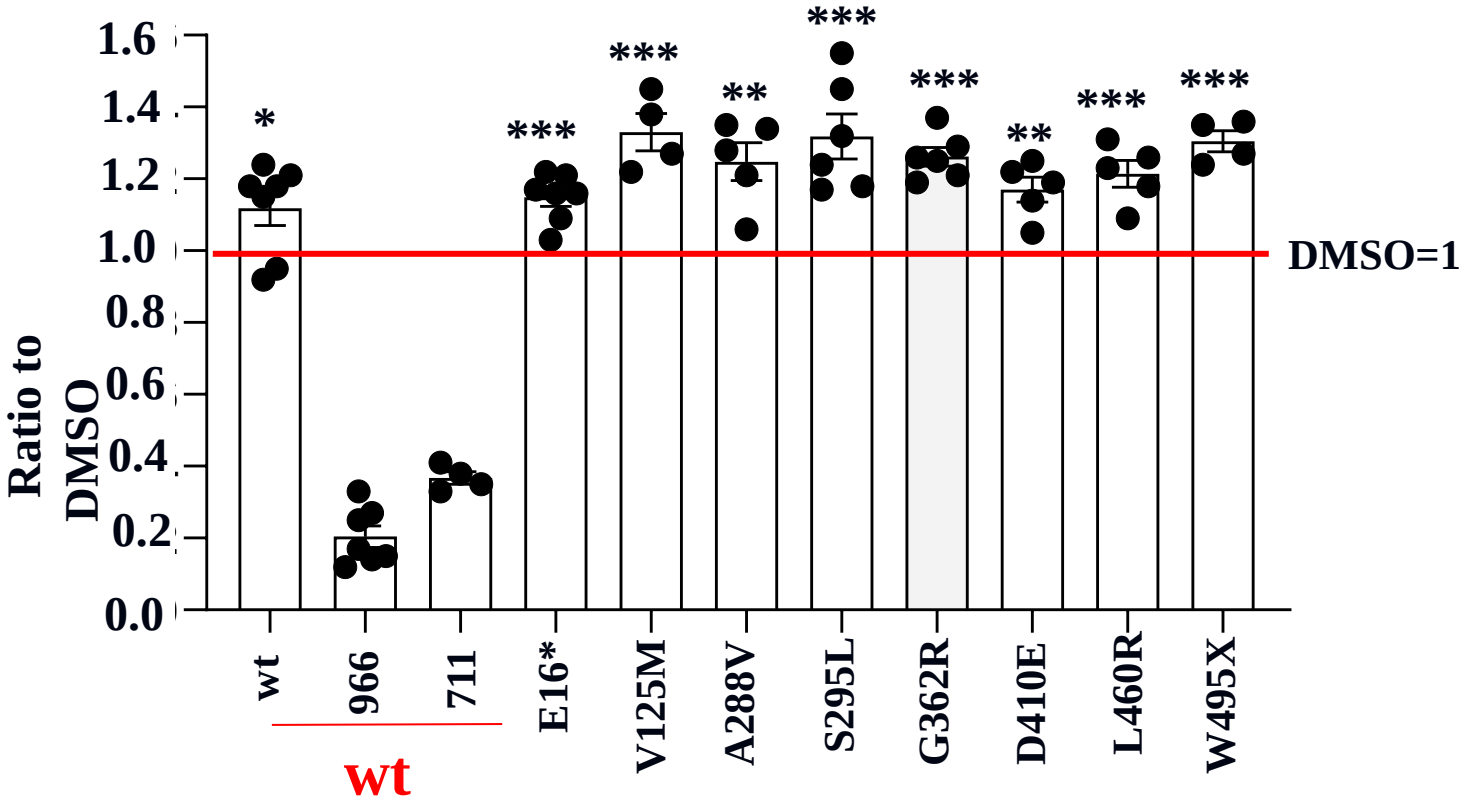
PBA restored GABA uptake in all 8 tested mutations
In “heterozygous” condition

mixed cDNAs
Patient condition
wt mut



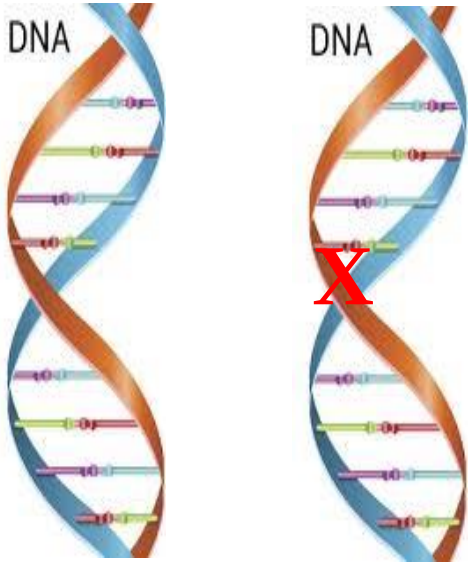
Ratio of ^{3}H GABA uptake PBA vs DMSO
“heterozygous”

PBA 2mM
24 hrs.

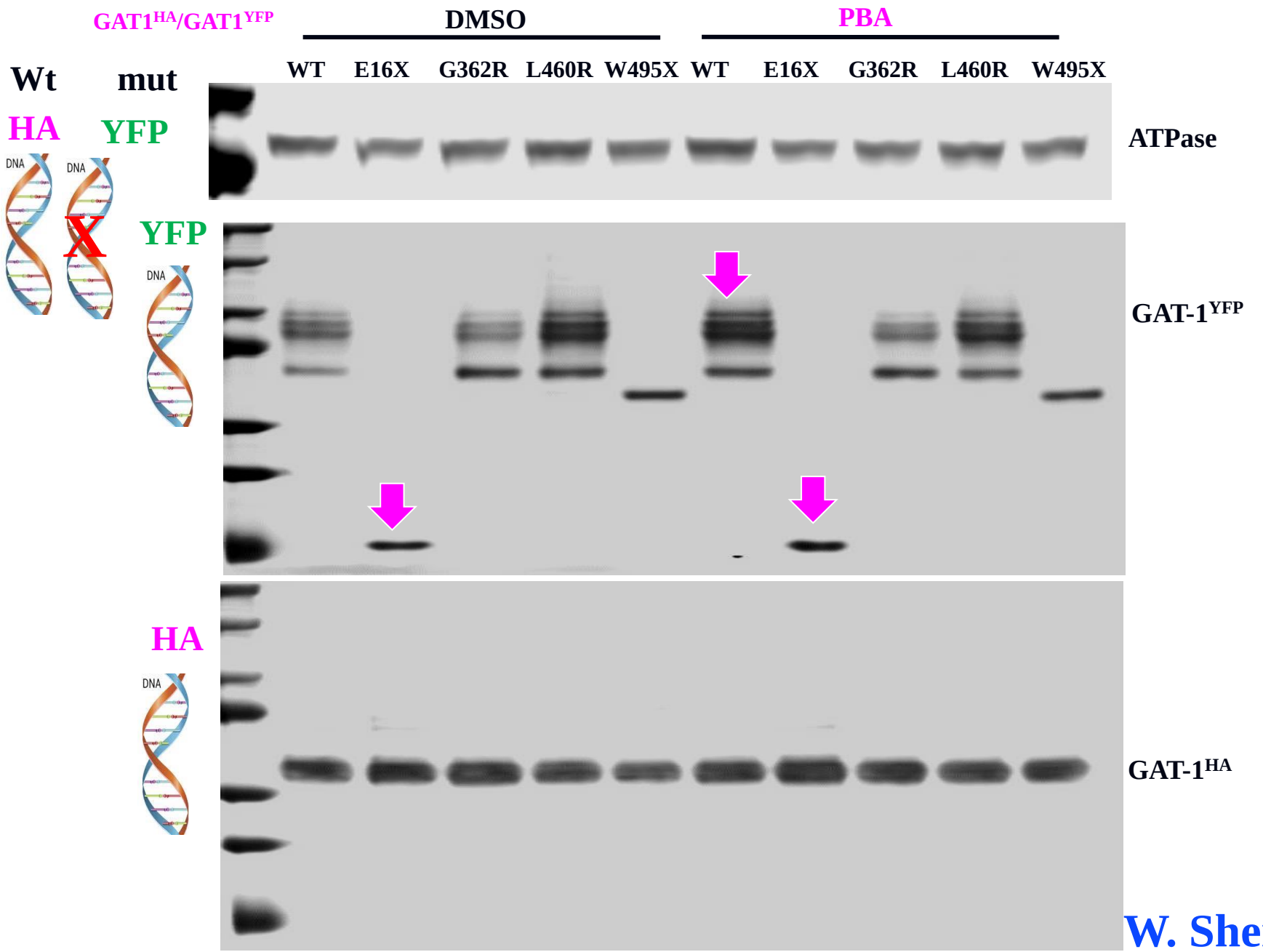


In heterozygous patients, which allele is rescued by PBA?

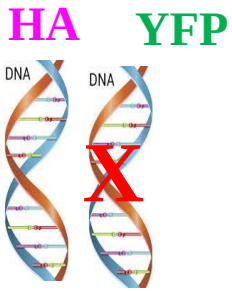
Wt mut



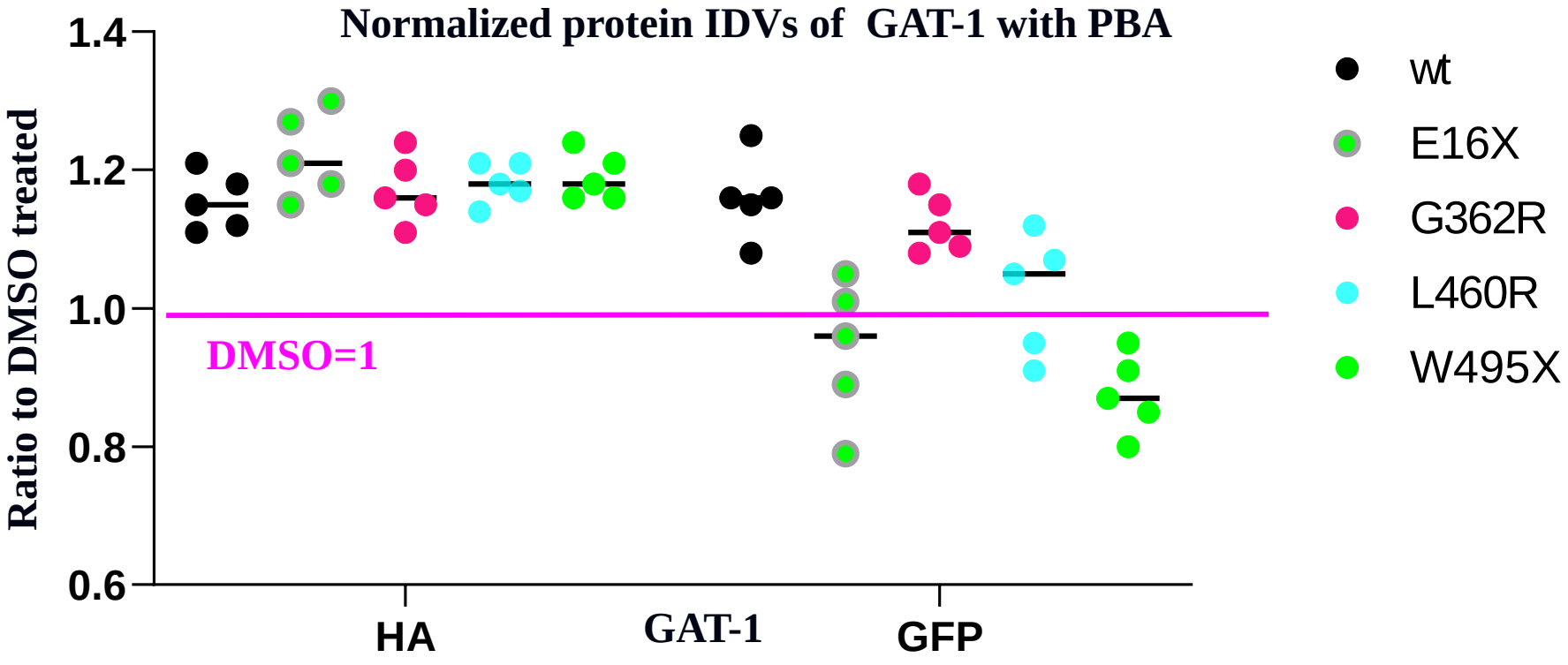
The wildtype GAT-1 protein expression is upregulated across mutations.



W. Shen

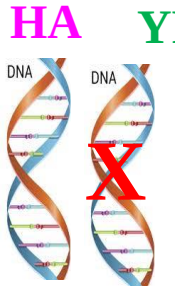


PBA effect on mutant GAT-1 is mutation-dependent



HA=wildtype allele

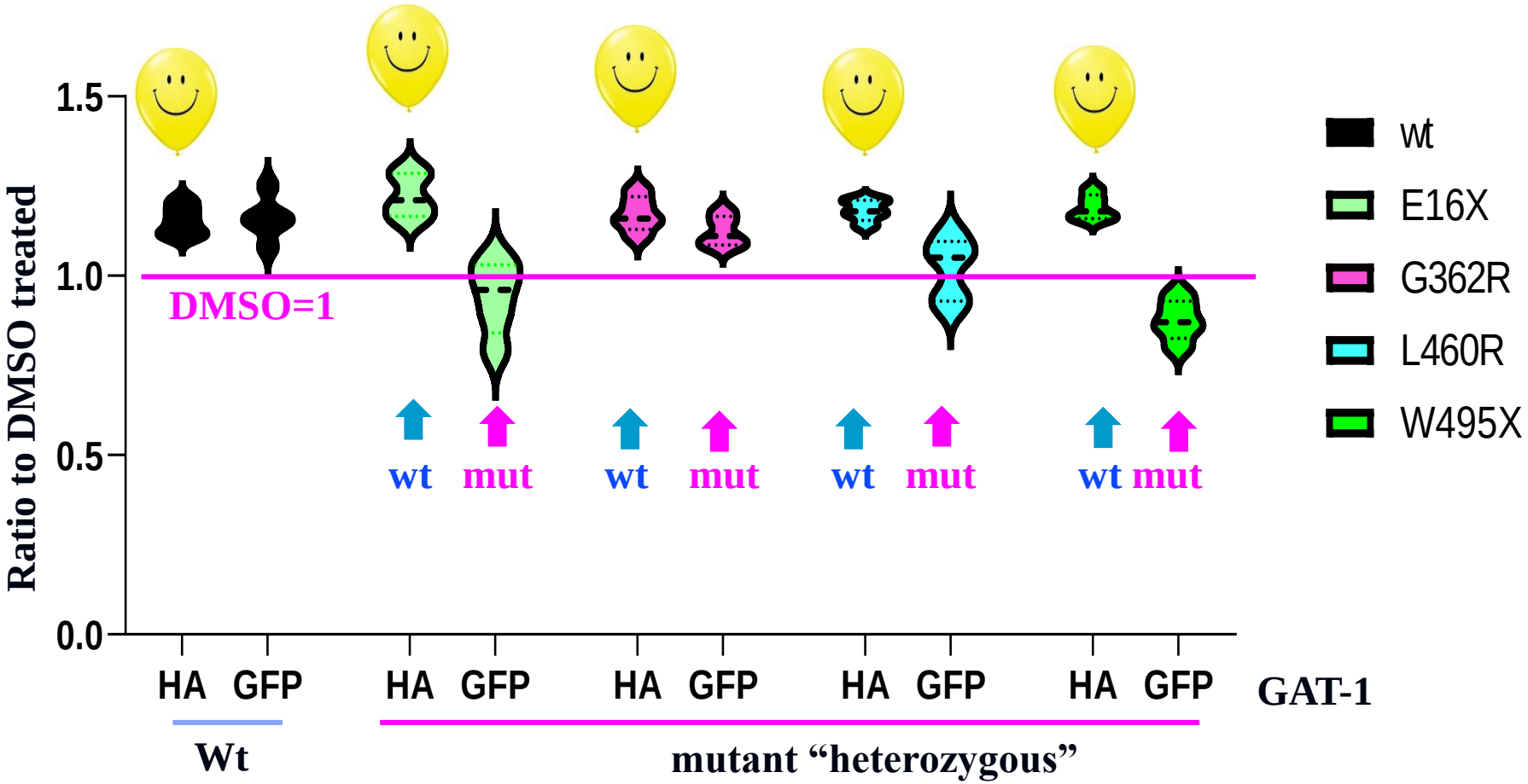
YFP(GFP)=mutant allele



PBA effect is allele-dependent wildtype vs mutant allele

Good news for heterozygous patients that wildtype in all mutations is upregulated!!

Normalized protein IDVs of GAT-1 with PBA



Kang unpublished

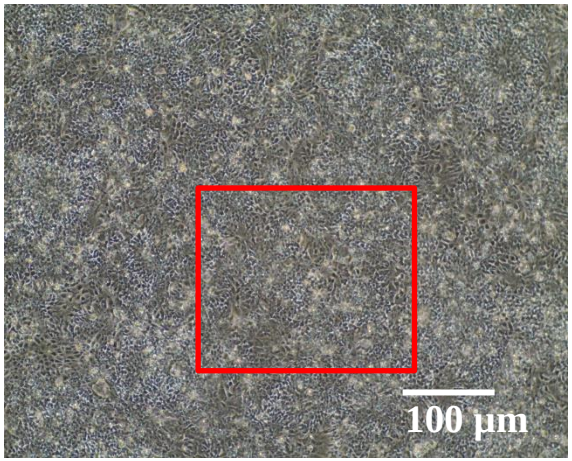
Ongoing work
is testing
PBA for 30 mutations

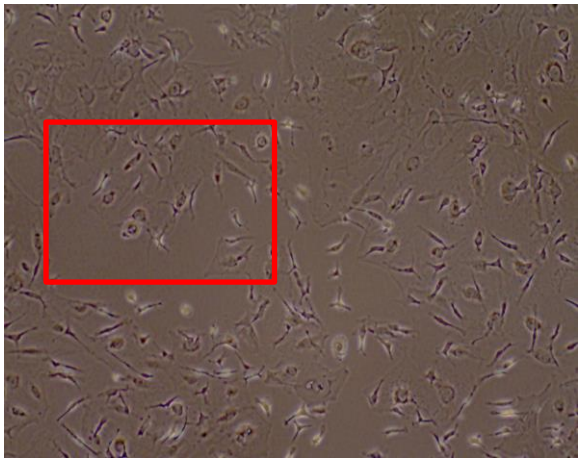
We differentiate iPSCs to NPCs to Astrocytes or to inhibitory neurons

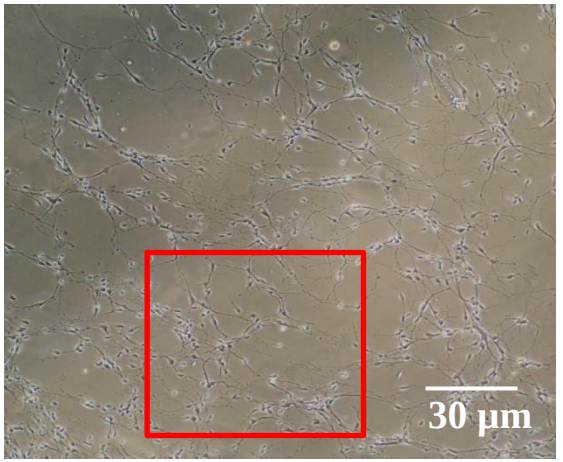
NPCs

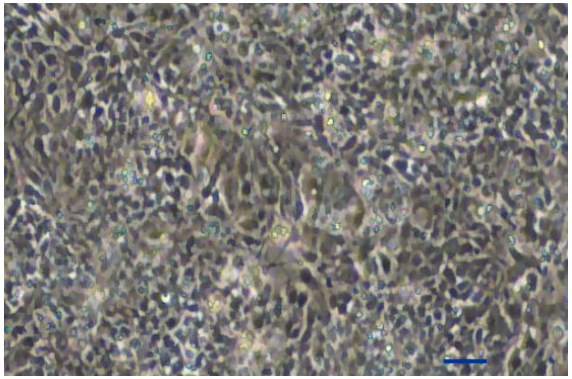
astrocytes

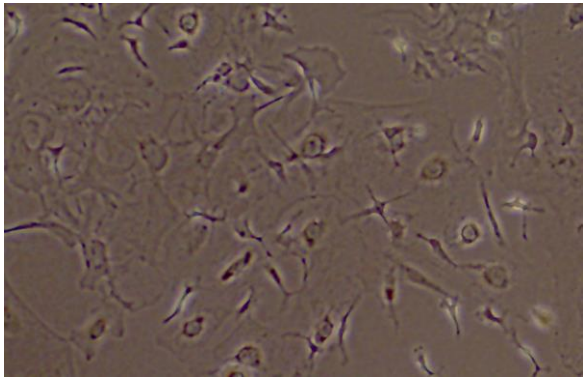
inhibitory neurons

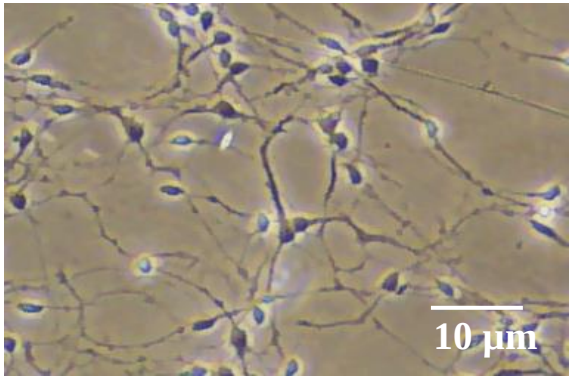












Two knockin mouse models

Focused on two representative mutations A288V and S295L

***Slc6a1*$^{+/A288V}$**
Charlie

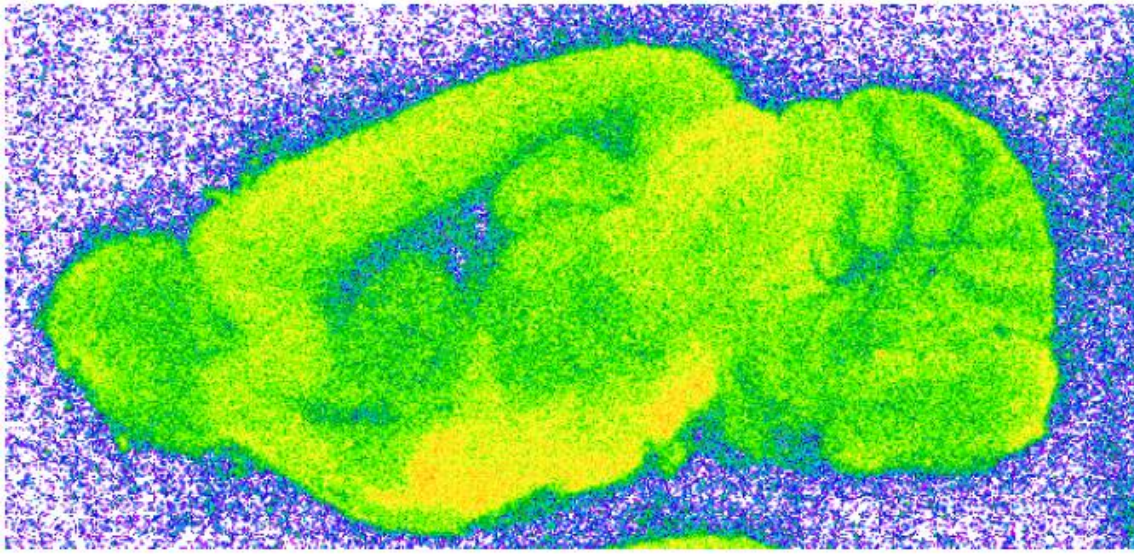
Partial loss of function

***Slc6a1*$^{+/S295L}$**
Maxwell

Complete loss of function

Robust GAT-1 expression in all major brain regions shown by [^{3}H]Tiagabine (5.2 nM) binding

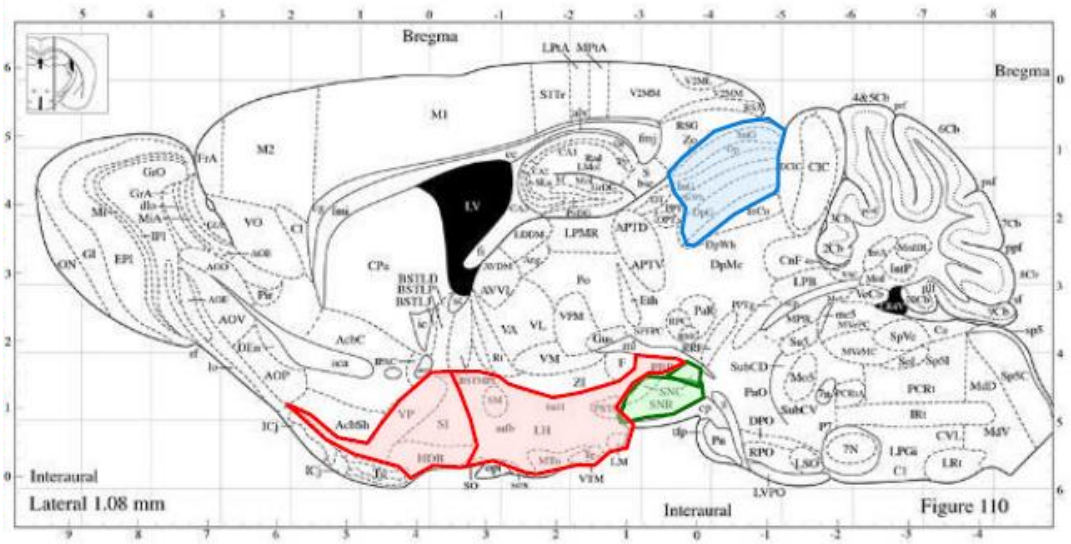
wildtype



High binding:

Superior colliculus
Substantia nigra
Lateral hypothalamic area
VP: ventral pallidum

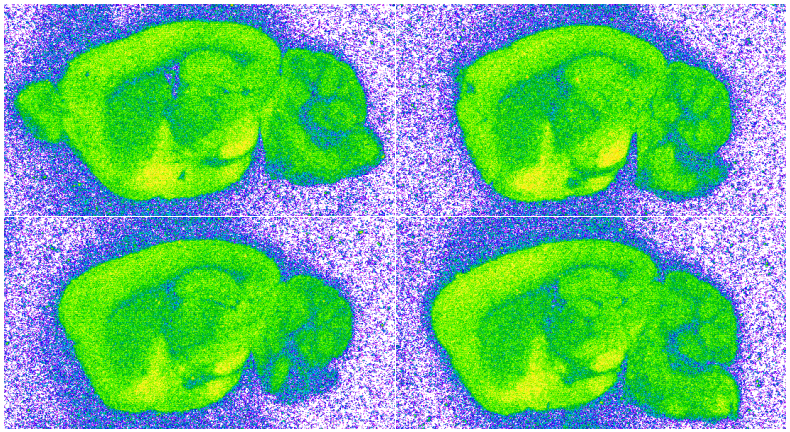
Low binding: Striatum (caudate, putamen ventral striatum)



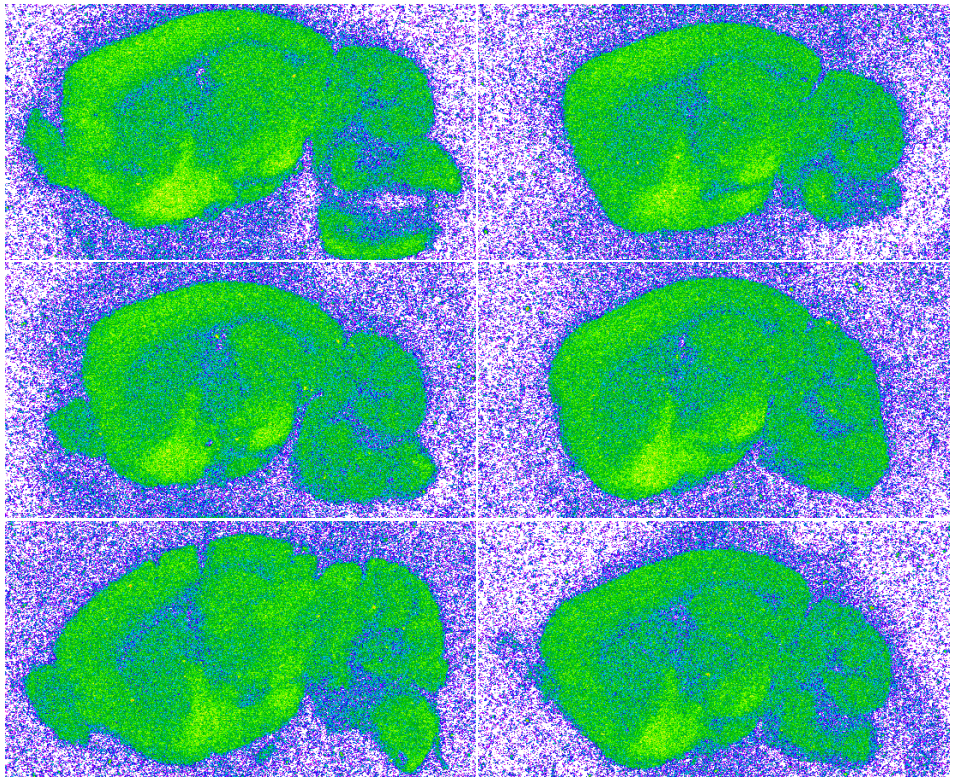
~50% lower [3H]Tiagabine binding (GAT-1 expression) in *Slc6a1*$^{+/A288V}$ Het vs. WT littermates

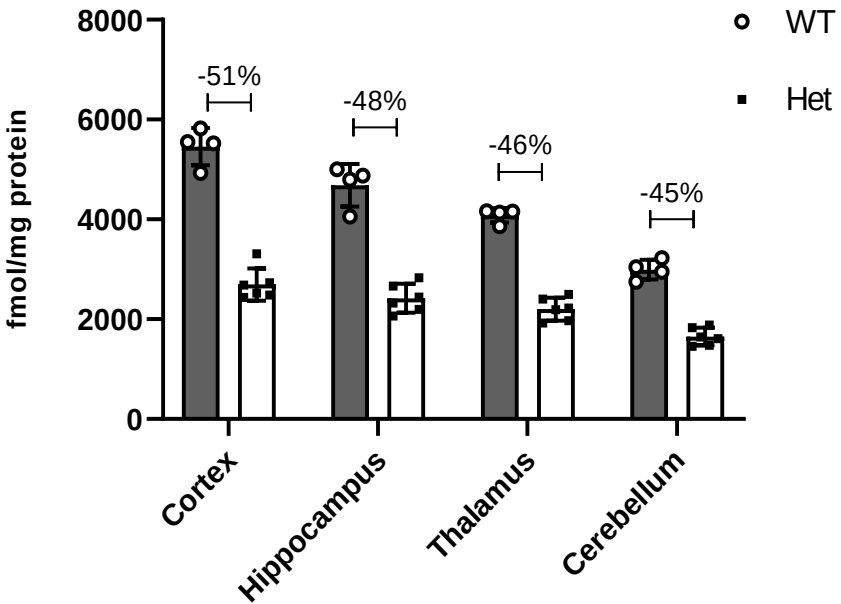
A

WT



A288V **heterozygous**





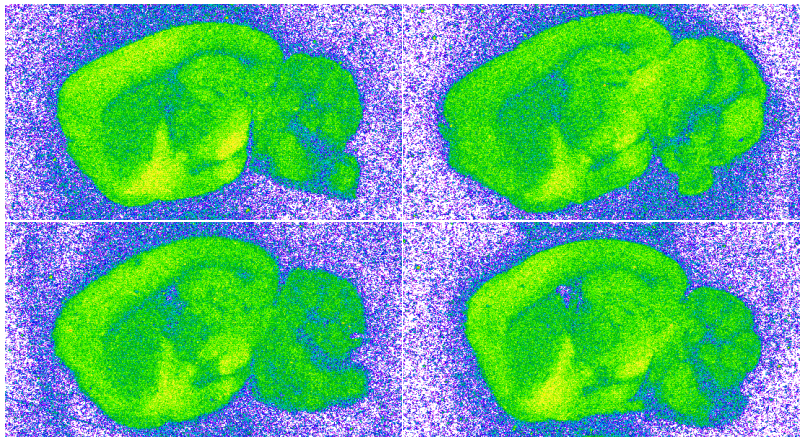
Data generated in collaboration with Roche by Michael Honer's lab-Neuroscience and Rare Diseases, Roche Pharma Research and Early Development

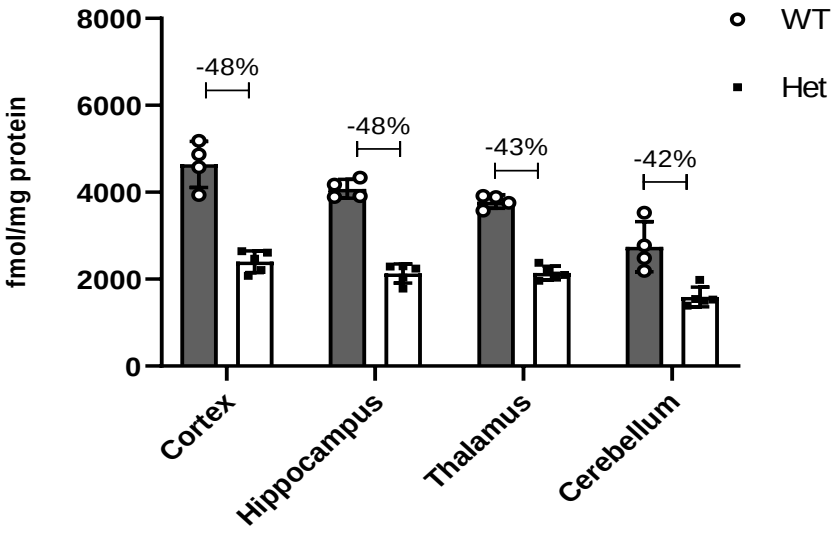
50% lower [3H]Tiagabine binding (GAT-1 expression) in *Slc6a1*$^{+/S295L}$ Het vs. WT littermates

A

wildtype

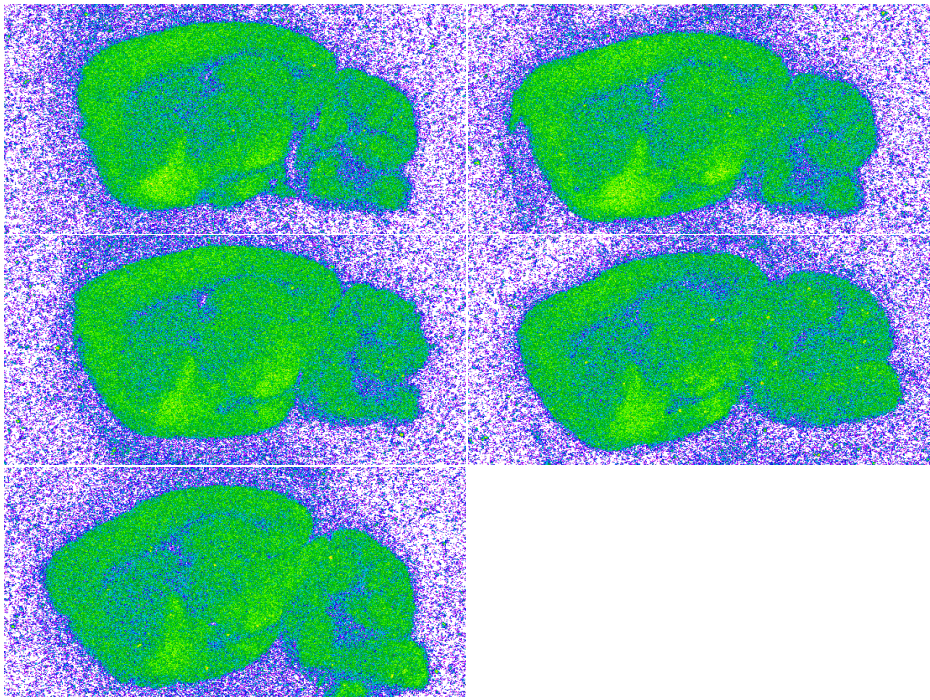
S295L





B

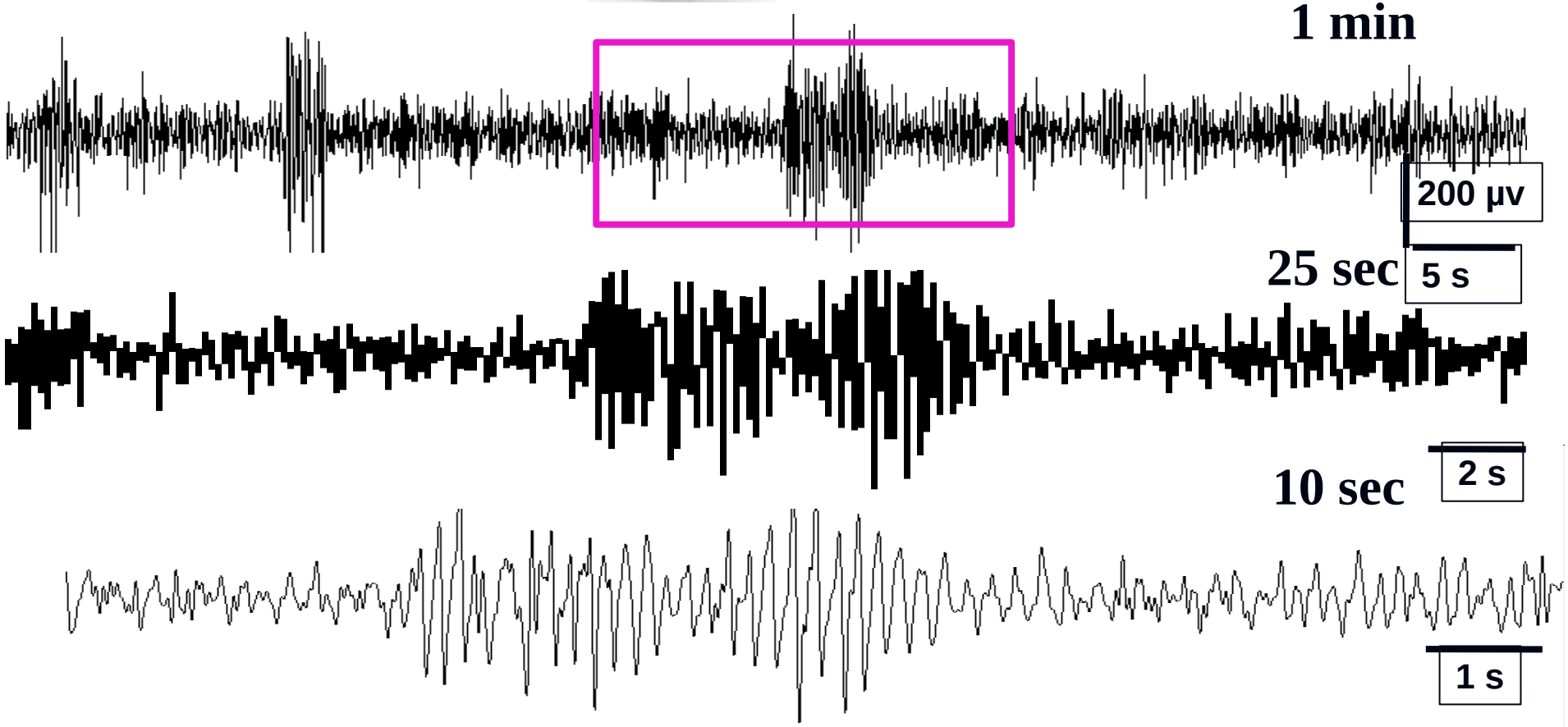
heterozygous



Data generated in collaboration with Roche by Michael Honer's lab-Neuroscience and Rare Diseases, Roche Pharma Research and Early Development

Both mouse models display absence seizures

Slc6a1$^{+/A288V}$



Baseline

Slc6a1$^{+/S295L}$

1 min

A

500µV

5 sec

10 sec

500µV

1 sec

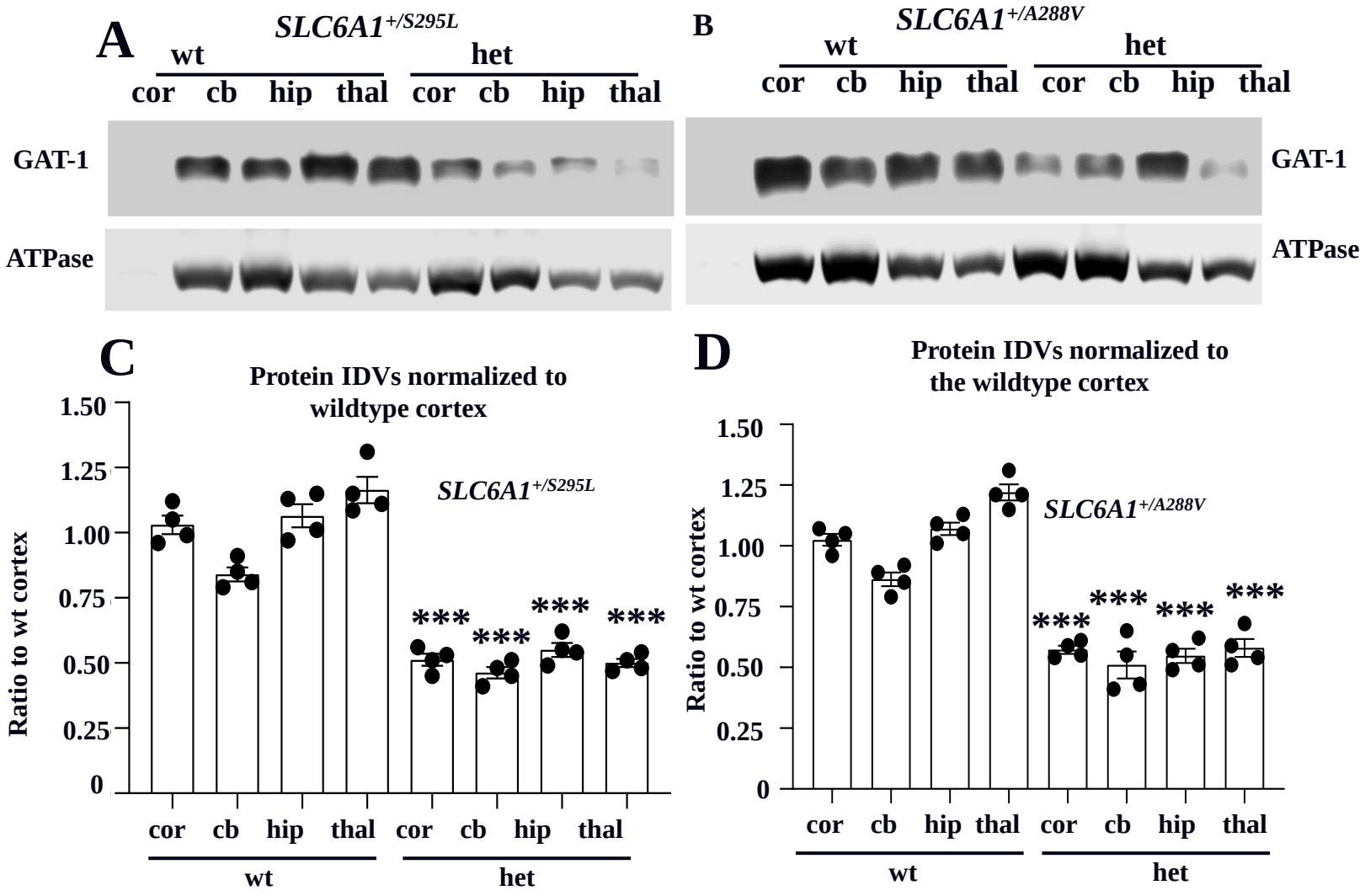
Slc6a1$^{+/S295l}$

3 min

B

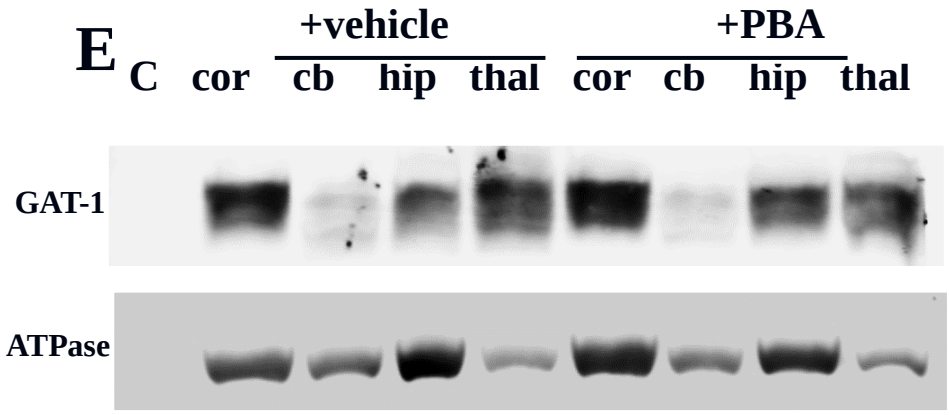
500µV

GAT-1 is abundantly expressed in all major brain regions and is reduced globally in both mouse models

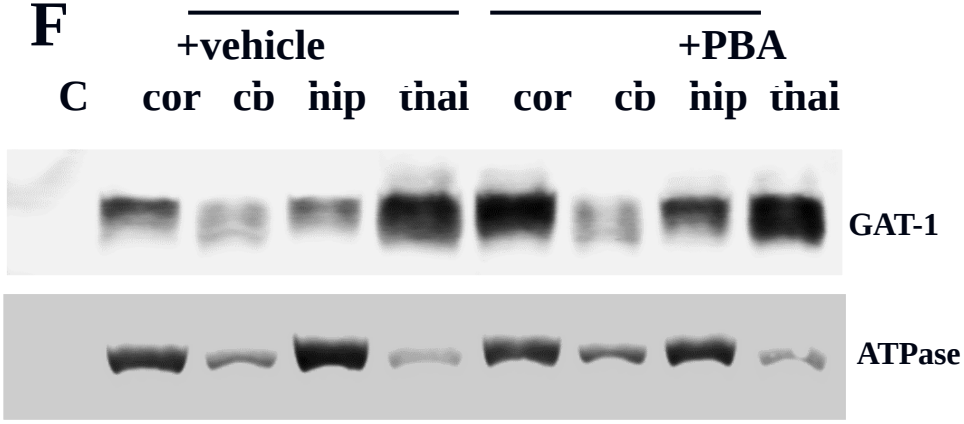


PBA increased GAT-1 expression in the cortex, hippocampus and thalamus in both mouse models

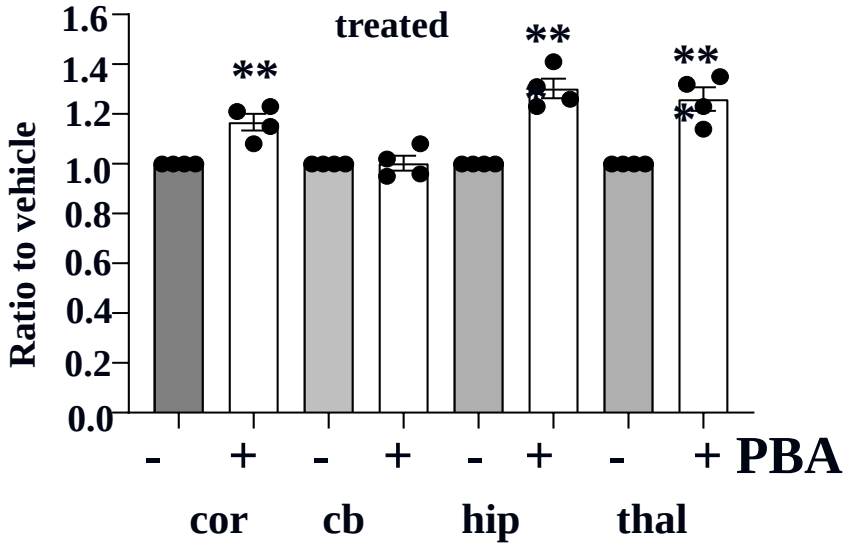
E *Slc6a1*$^{+/S295L}$



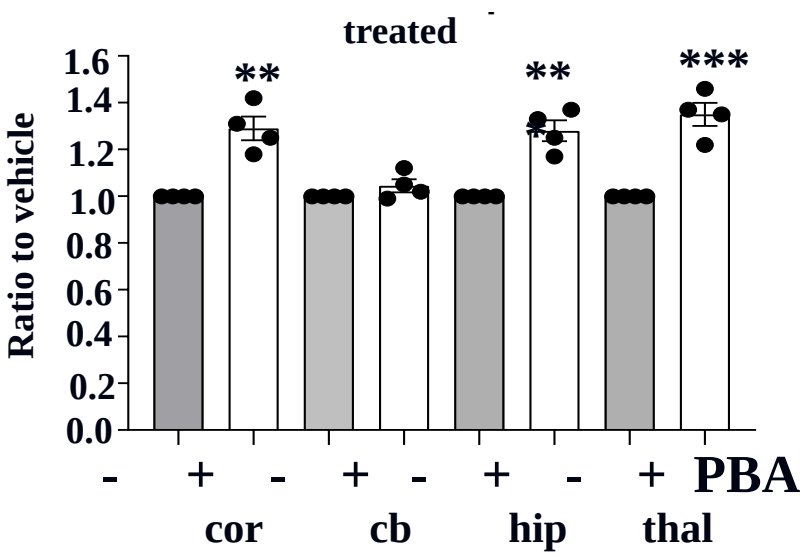
F *Slc6a1*$^{+/A288V}$



G *Slc6a1*$^{+/S295L}$



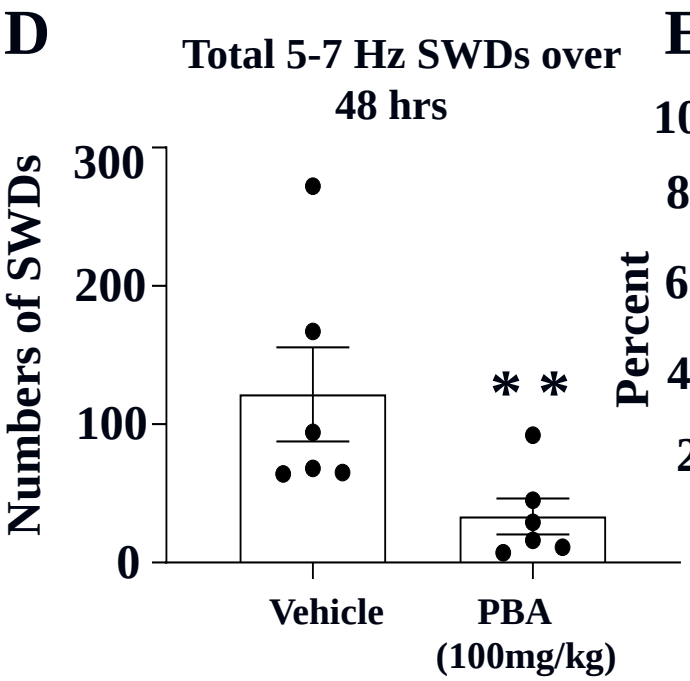
H *Slc6a1*$^{+/A288V}$



PBA alone (acute treatment) reduced seizures (>70%) in the heterozygous mutant mice

Experiment for EEG recording

EEG surgery (30 min) → recovery (7 days) → Baseline recordings (48 hrs) → PBA (7 days) → EEG recordings (48 hrs)



Nwosu et al., 2022, Brain Commun

We have a large program on SLC6A1 mutations

Our goal: Find a CURE!

Provide proof-of-principle guidance for treatment development from pre-clinical patient derived cell (iPSCs) and mouse models

Take home messages:

- Partial or complete loss of function is common across SLC6A1 mutations regardless of clinical phenotypes
- The mutant GAT-1 is retained inside endoplasmic reticulum in both neurons and astrocytes
- PBA alone can restore GABA uptake and mitigate seizures
- PBA can be beneficial for most of patients if not all
- PBA can upregulate the function of the wildtype allele
- The effect of PBA on the mutant allele is mutation-dependent

Acknowledgements

Everyone in Kang lab (previous trainees Felicia, Marshall)

SLC6A1 Connect

Many patients & parents

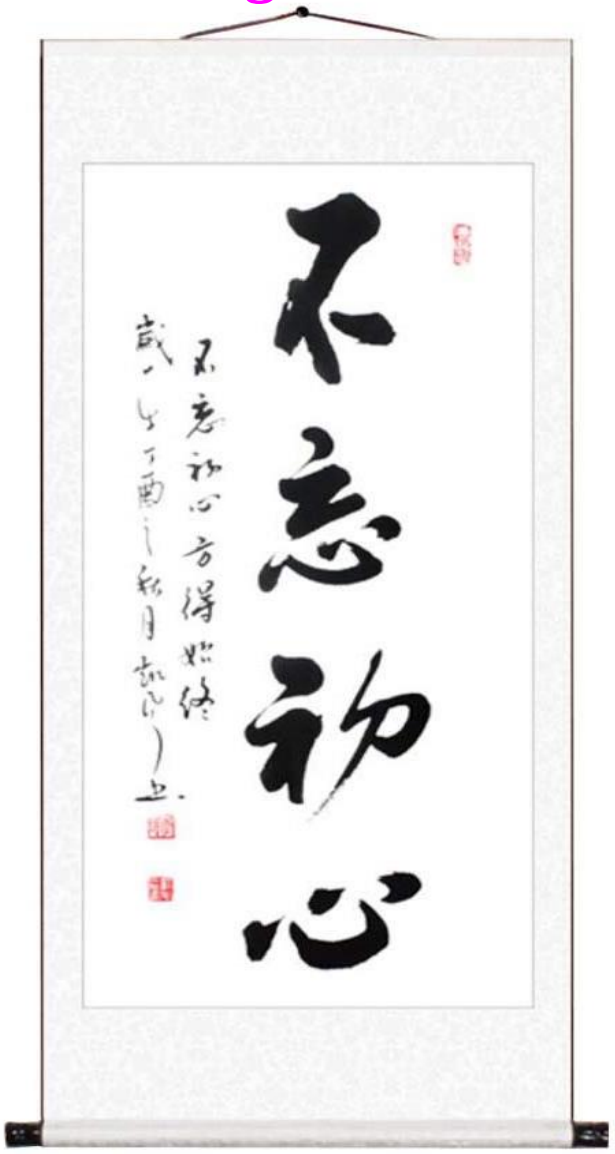
Industry partners: UCB, Roche, BioMarin
Taysha gene Therapies

Many physicians: Drs. Zachery Grinspan,
Kim Goodspeed,
Scott Demarest,
Robert Carson,
Inna Hughs
Emma Grace,
Many Chinese physicians

NIH National Institute of Neurological Disorders and Stroke
Reducing the burden of neurological disease...

Do not forget the original intention

Bu wang chu xin



You all must work hard to find a better drug!


Welcome to Music City!