SLC6A1 mutations in cell and mouse models

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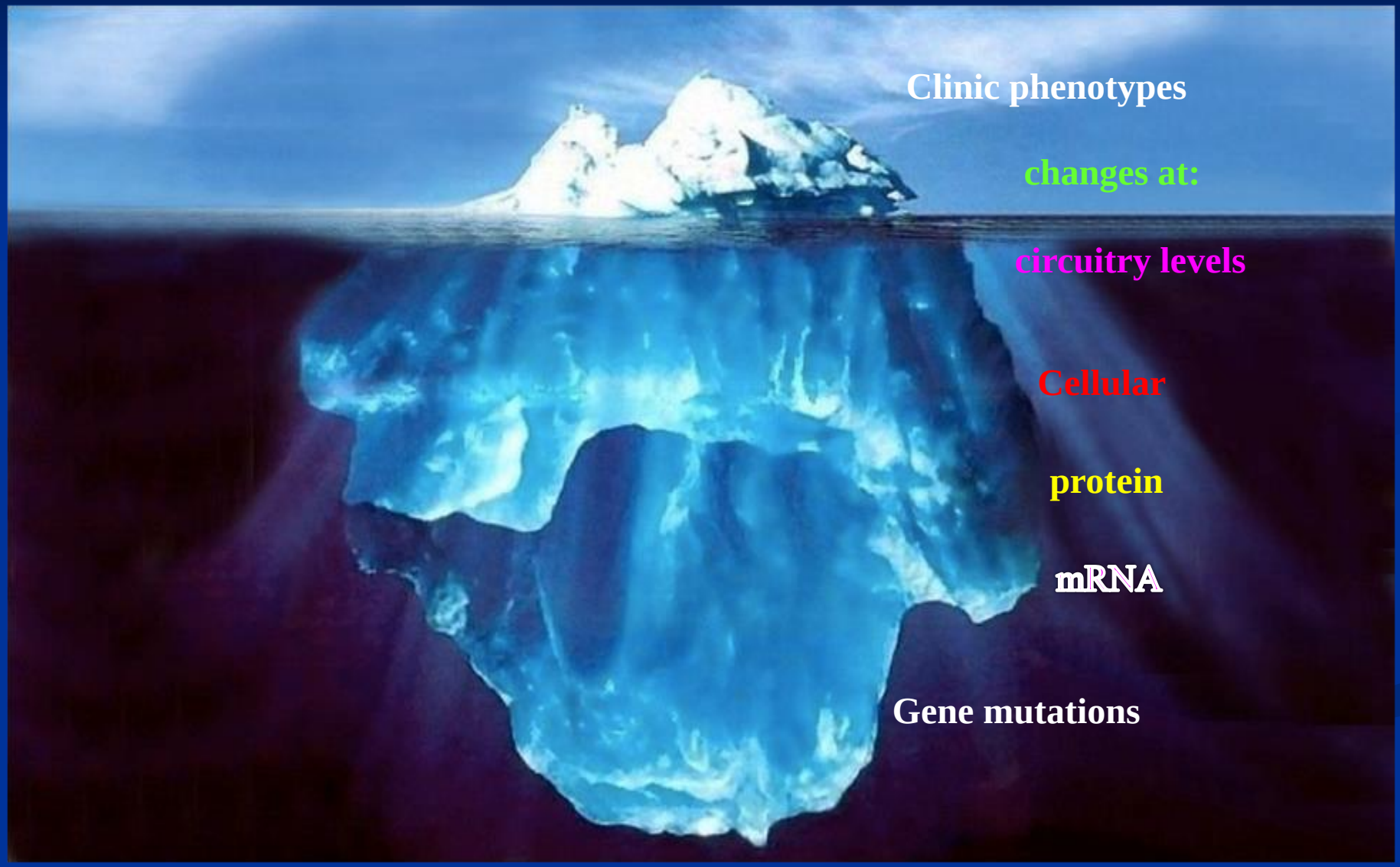
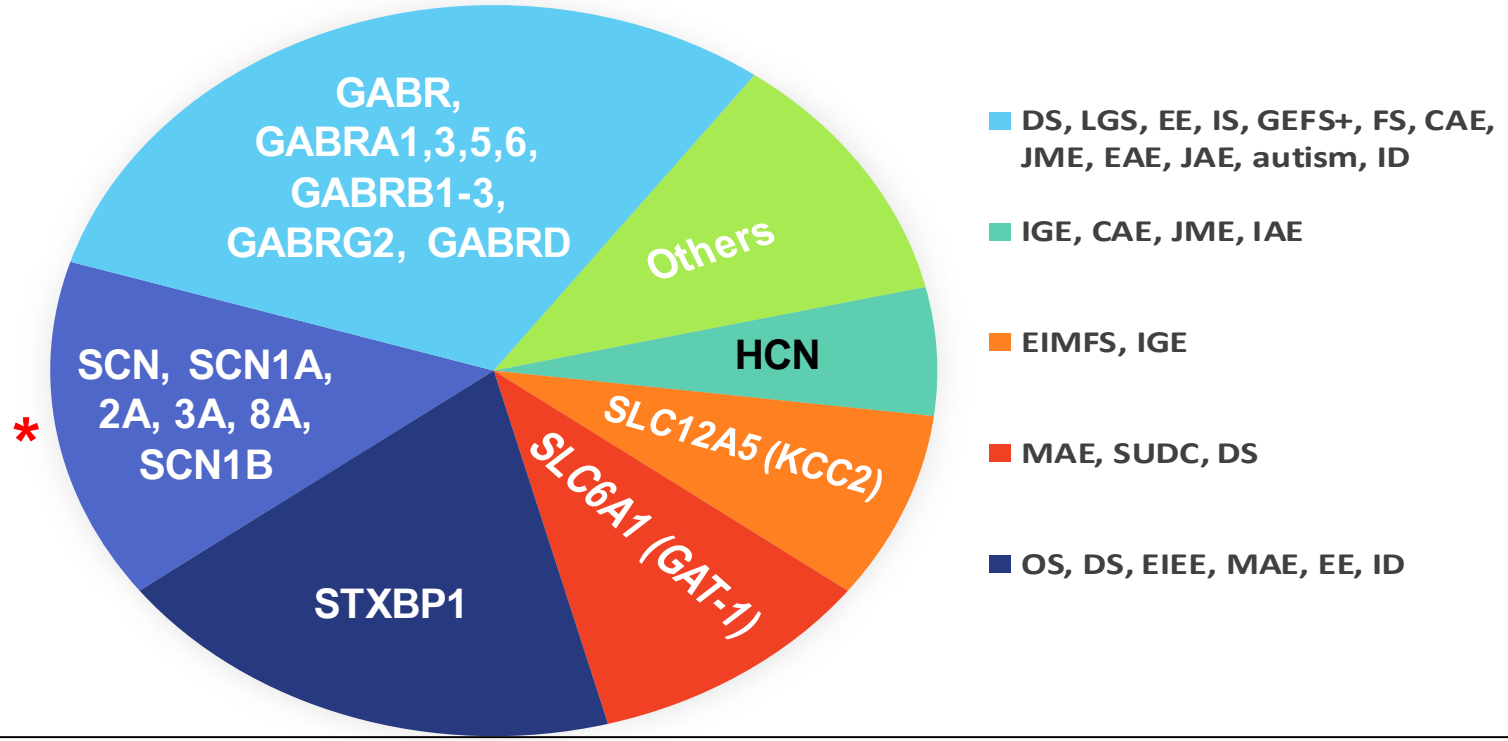
What is epilepsy?



Mutations in genes proposed in the study and the associated epilepsy syndromes



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=Idiopathic absence 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

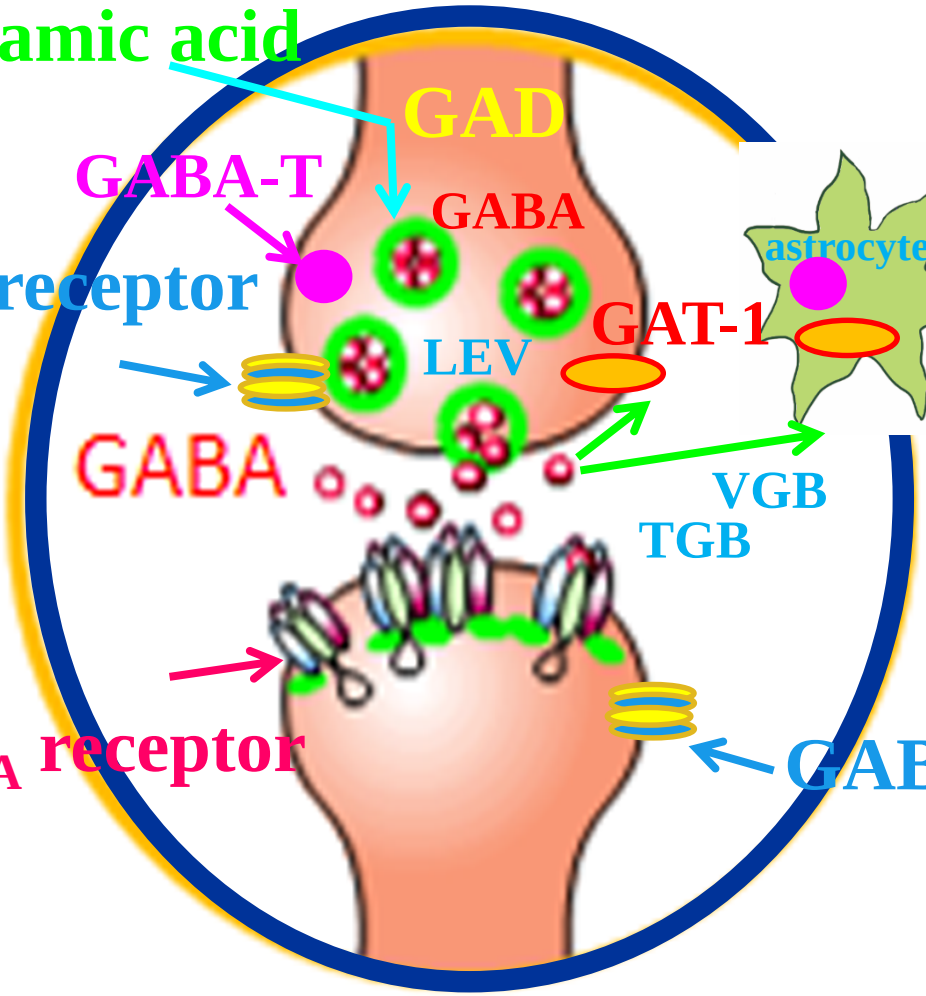
MAE=Myoclonic astatic epilepsy

OS=Ohtohara syndrome

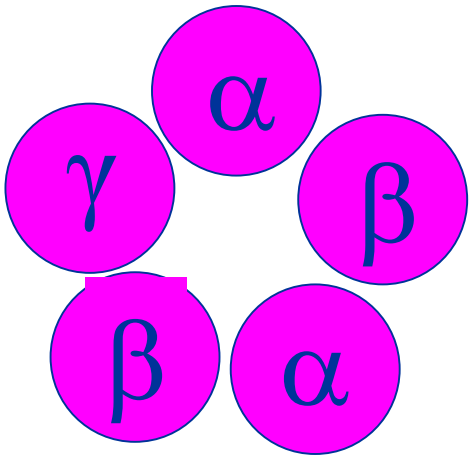
SUDC=Sudden unexplained death in childhood

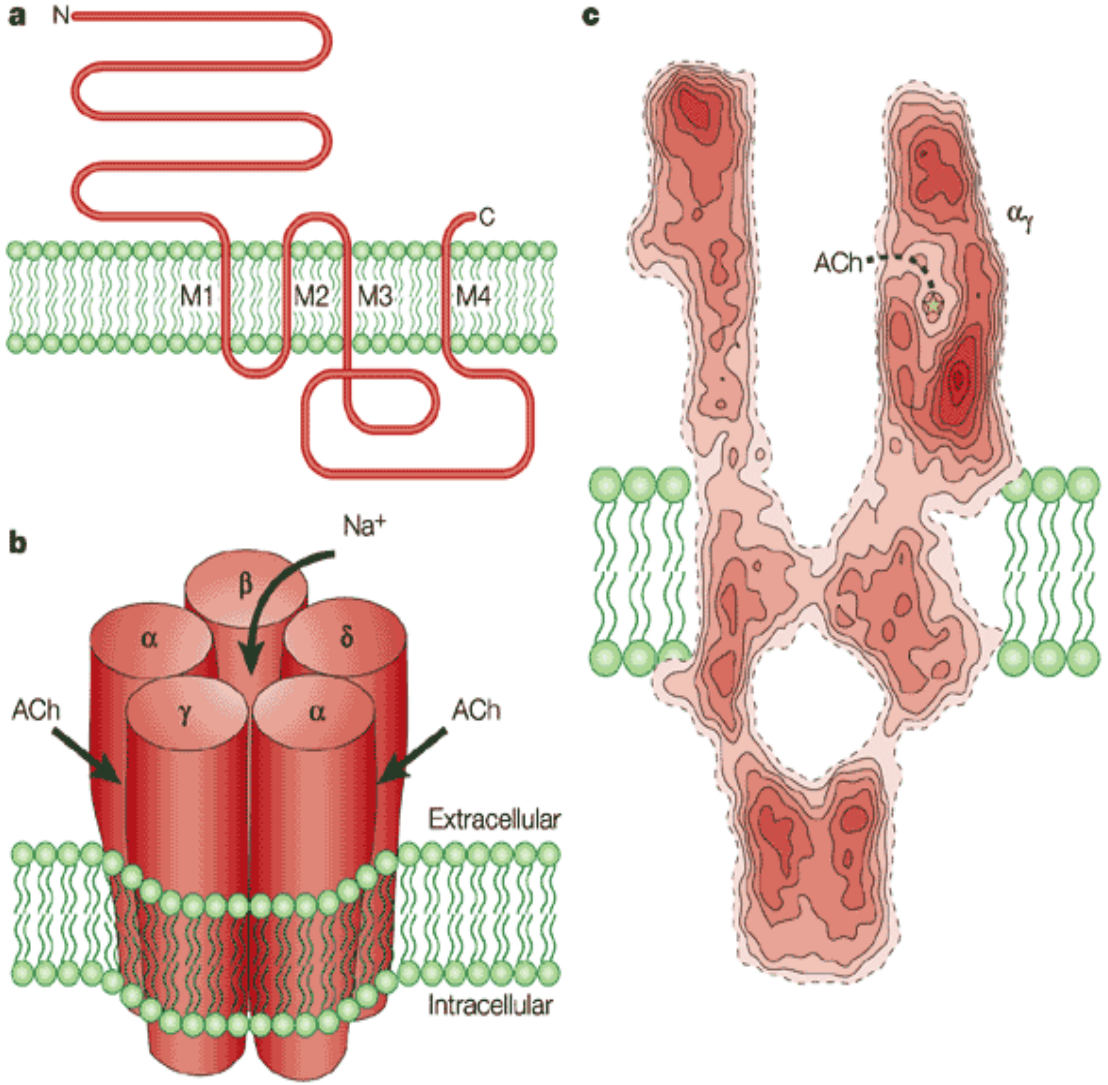
*We will not study SCN

GABAergic neurotransmission is a major pathway for epilepsy



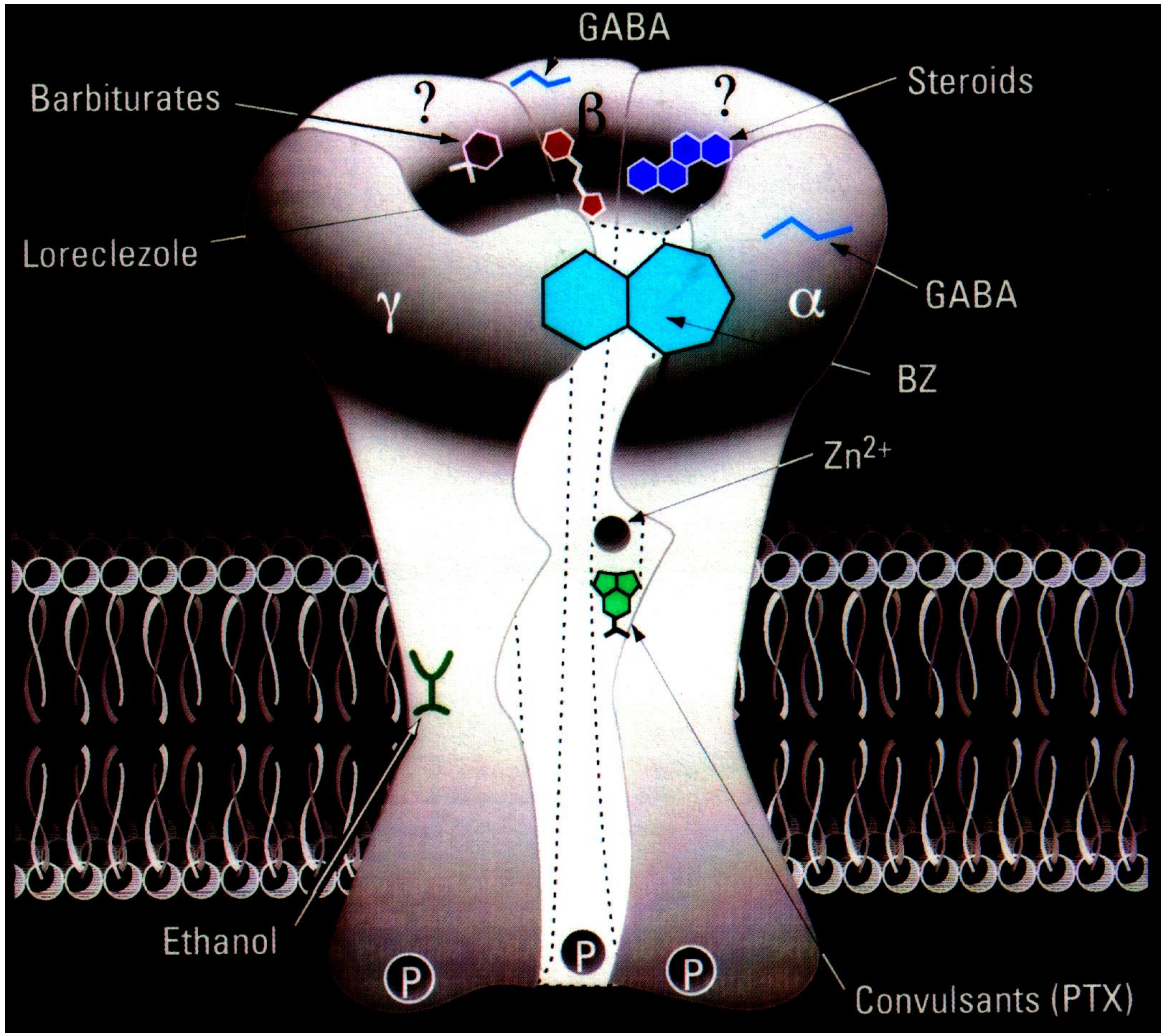
Our previous work has mainly focused on $GABA_A$ receptor





Many sedative, anticonvulsant drugs work by modulating $GABA_A$ receptors

$GABA_A$ receptor binding sites



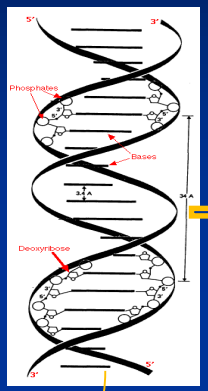
Our research focused on catastrophic epilepsies, ~30% resistant to current AEDS

Genes have been identified to be related to epilepsy in $GABA_A$ receptors and GABA transporters

Receptors	Transporter
GABRG2	GAT-1
GABRA1	
GABRB2	
GABRB3	

Our research

We try to identify mechanism-based therapies for genetic epilepsies

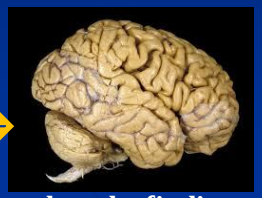


Mutations identified in Epilepsy patients

Characterizations in the dish using recombinant receptors

Mutation Knockin

Generation of mutation harboring knockin mice and characterize the heterozygous mutant mice

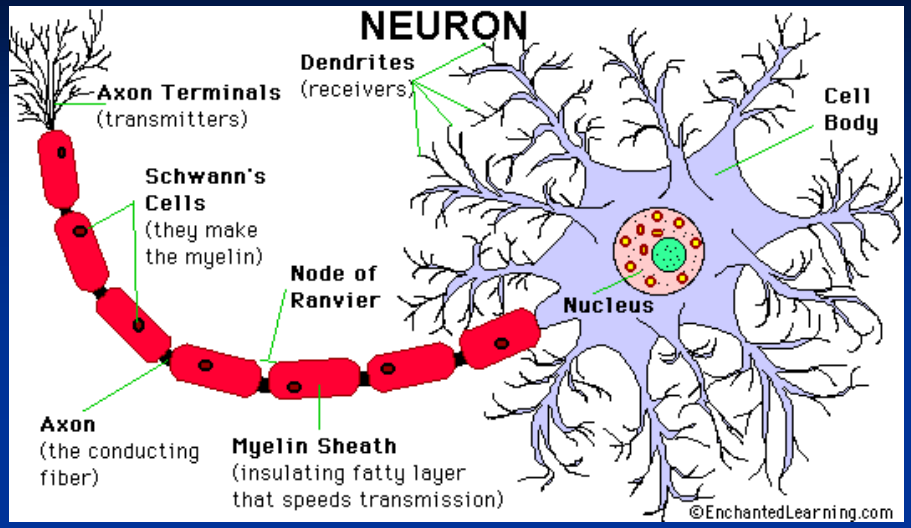


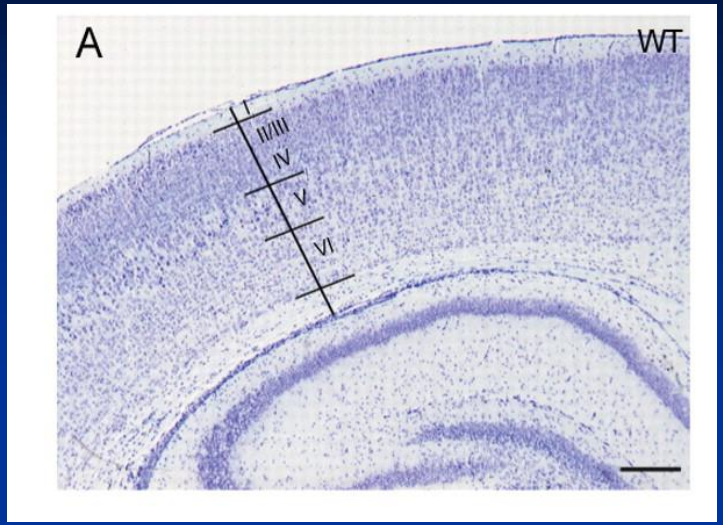
Correlate the findings with human brain patho-physiology and find mechanism based therapy

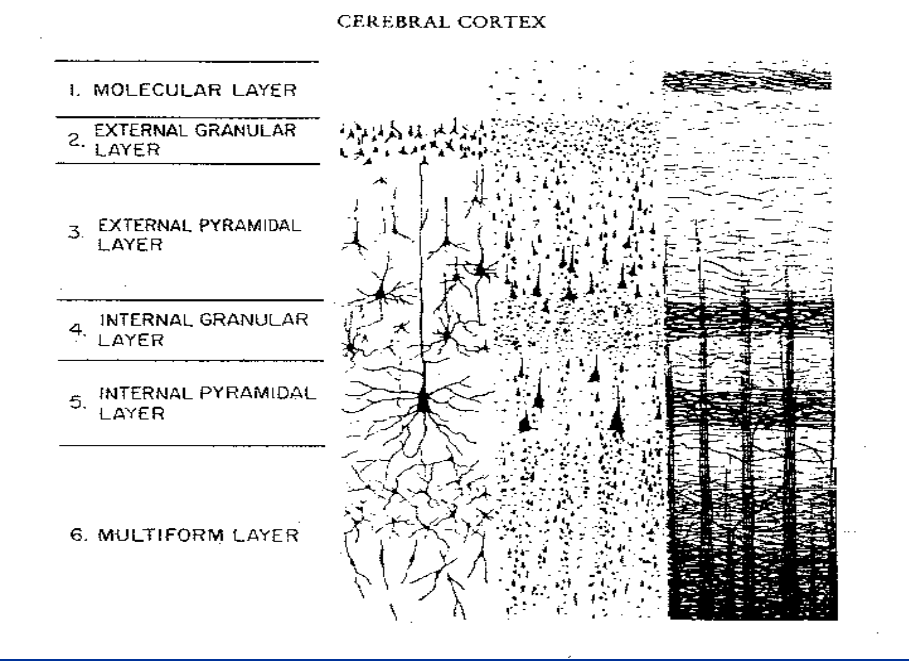
Ongoing research

GABRA1 knockout
GABRG2 knockout
GABRB3 knockout
GABRA1(A322D) knockin : JME
GABRG2(Q390X) kncokin: GEFS+/DS
GABRB3(P11S) knockin: autism, CAE
GABRB3(D110N) knockin: IS
GABRB3(N120D) knockin: LGS
GABRG2(K328M) knockin :FS, SUDEP

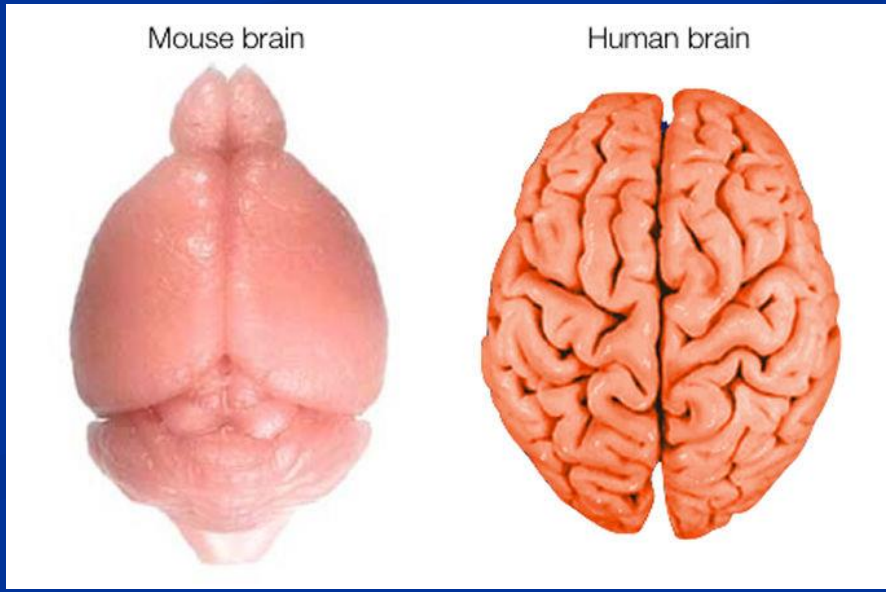
Neuron is very complicated and each neuron has many synapses to communicate with other neurons



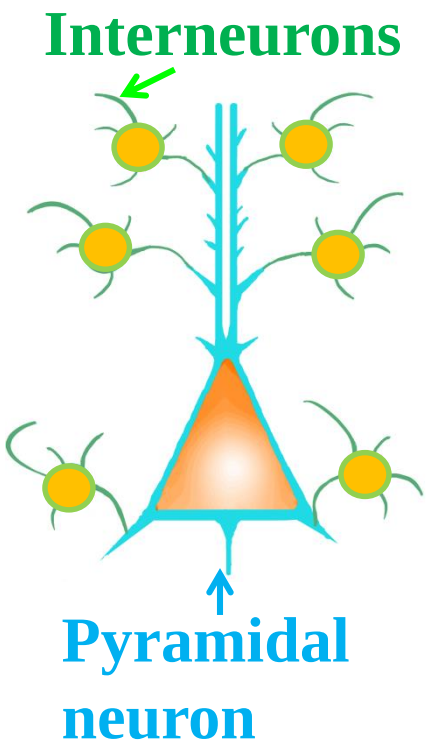


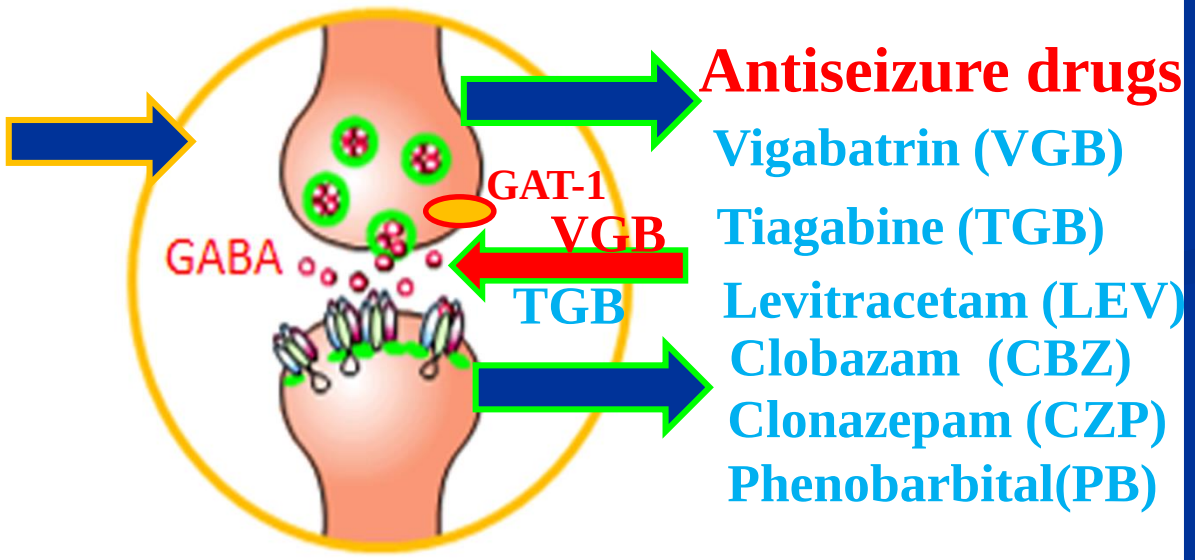


Where is GAT-1 ??

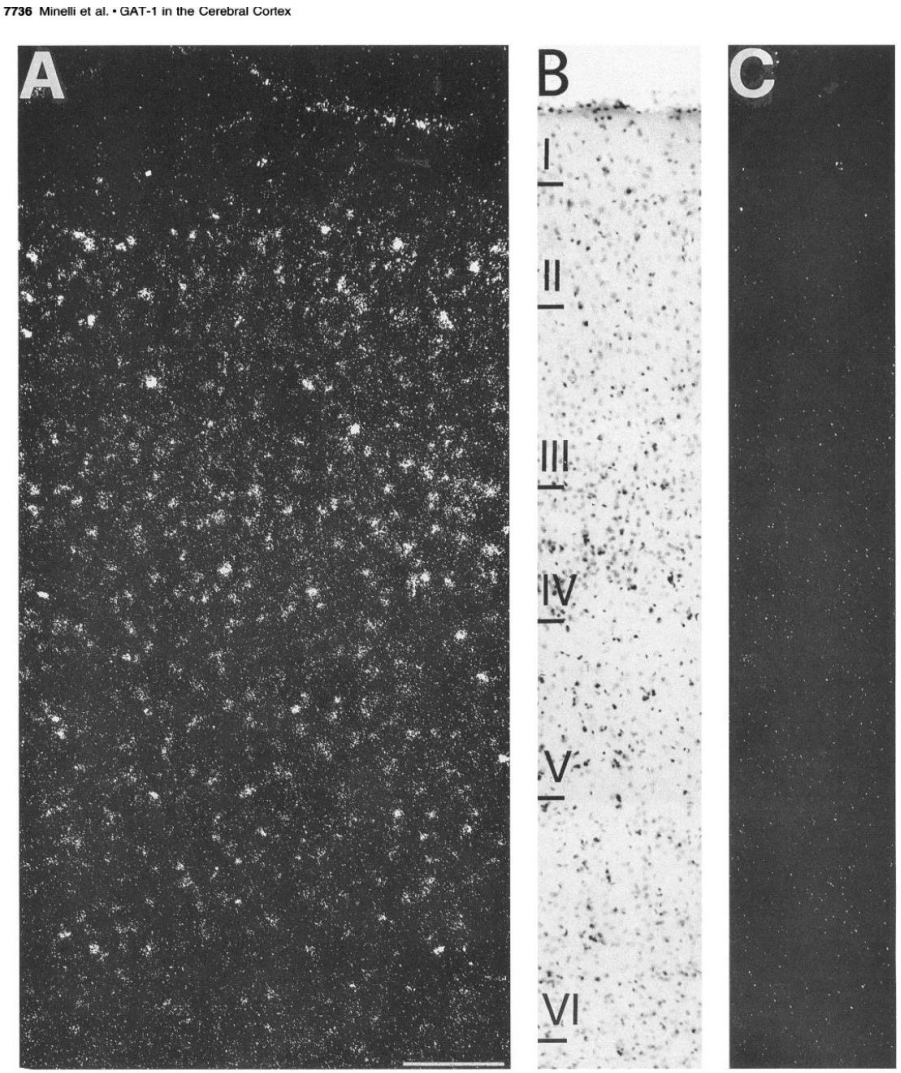


Our previous work has identified cortical GABAergic interneurons are autophagic in *Gabrg2*$^{+/Q390X}$ mice. This is likely due to the accumulation of the γ2 subunits are accumulated in GABAergic interneurons.





where is GAT-1 expressed?



GAT-1 transcripts in the first somatosensory cortex of the adult rat. The high level of hybridization signal in layer IV.

GAT-1 and GAD67 are often colocalized.

GAT-1 is also expressed in some pyramidal neurons

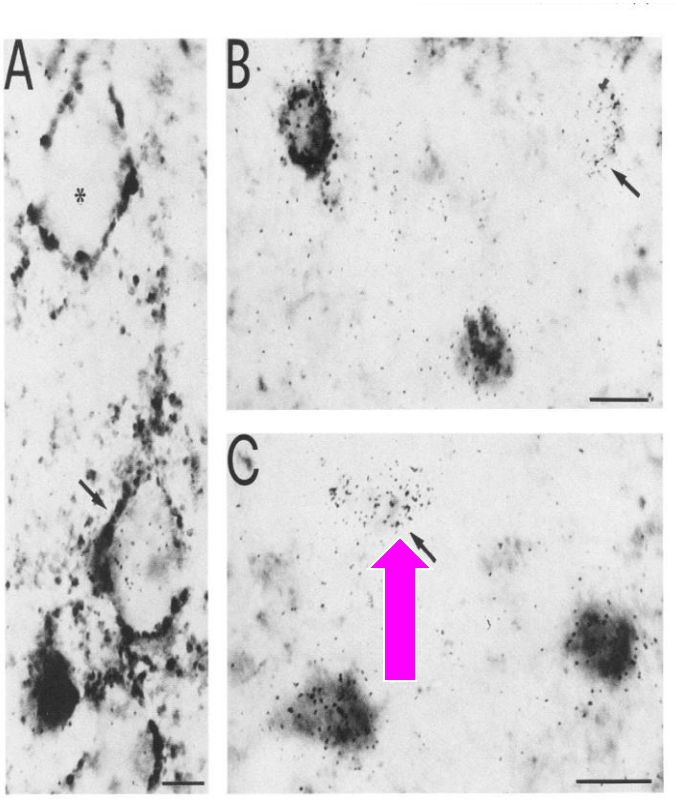


Figure 5. Simultaneous visualization of GAD67-ir and GAT-1 mRNA in rat cerebral cortex shows that all GAD67-positive cells express GAT-1 mRNA, but that not all GAT-1 mRNA expressing cells contain GAD67-ir (*arrows*). Of these, some are pyramidal neurons, as revealed by the dense clustering of GAD67-positive axon terminals on their somata and proximal dendrites (*A*). In *A*, *asterisk* indicates a pyramidal neuron not expressing GAT-1 mRNA. Exposure time: 15 d. *A*, Layer V; *B* and *C*, II. Scale bars: 20 μm.

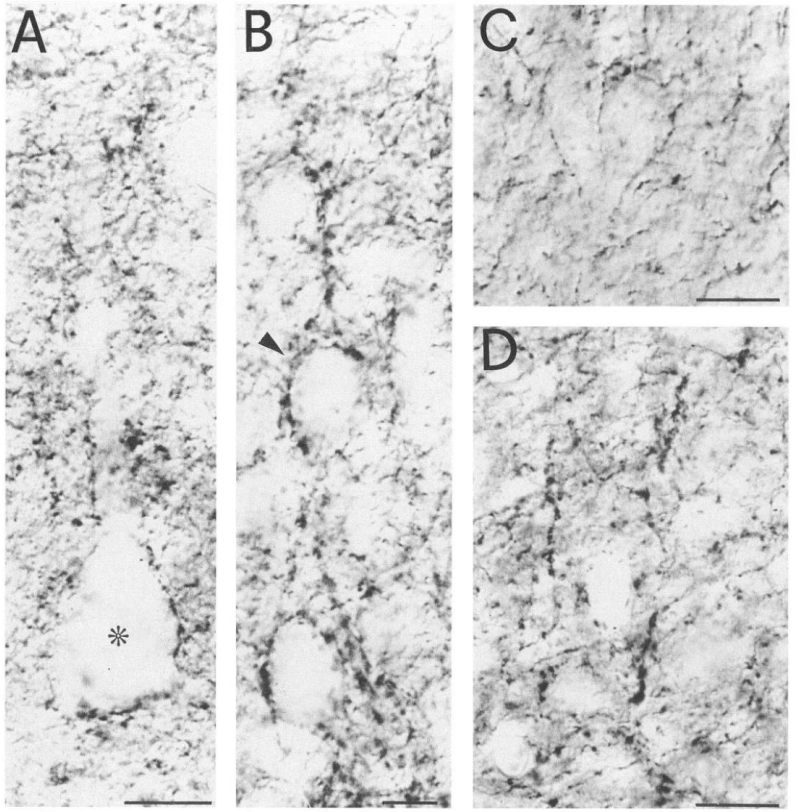
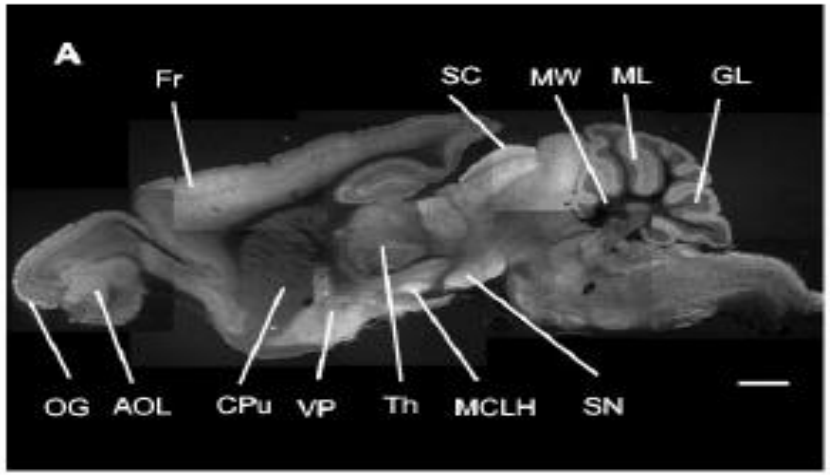
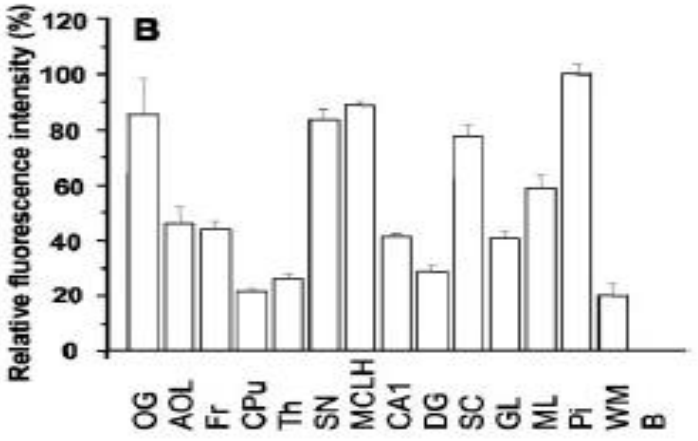


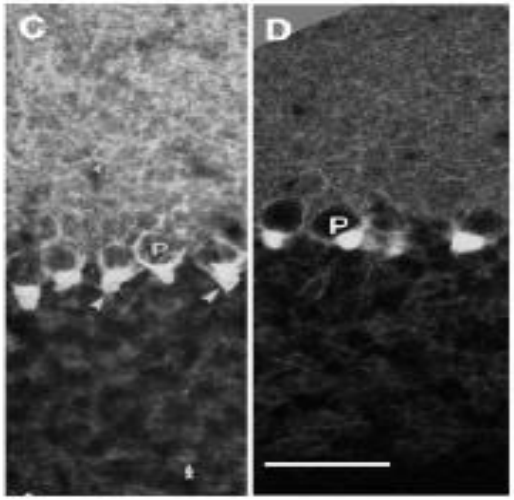
Figure 8. GAT-1 immunoreactivity within punctate structures that represent probable axon terminals (*A* and *B*) and in some fibers (*C* and *D*). The cell bodies and primary dendrites of both pyramidal (*asterisk*) and nonpyramidal (*arrowhead*) cells are outlined by stained axon terminals. Scale bars: *A*, 15 μm; *B*, 10 μm; *C* and *D*, 20 μm.

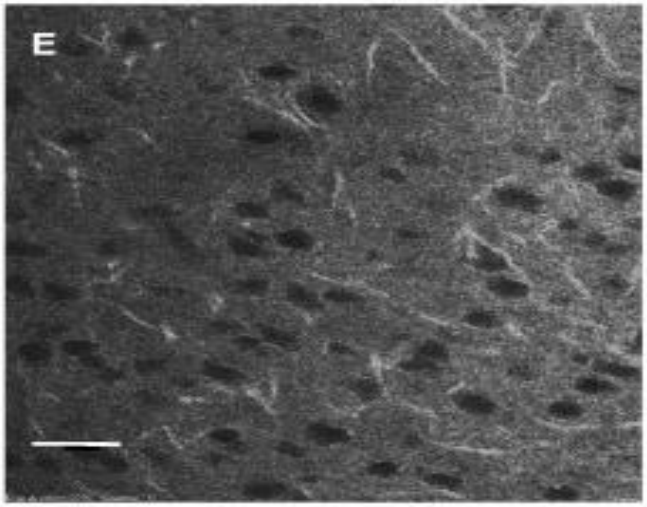
Number, Density, and Surface/Cytoplasmic Distribution of GABA Transporters at Presynaptic Structures of Knock-In Mice Carrying GABA Transporter Subtype 1–Green Fluorescent Protein Fusions

Chi-Sung Chiu,[1] Kimmo Jensen,[4] Irina Sokolova,[1] Dan Wang,[3] Ming Li,[1] Purnima Deshpande,[1] Norman Davidson,[1] Istvan Mody,[4] Michael W. Quick,[3] Stephen R. Quake,[2] and Henry A. Lester[1]







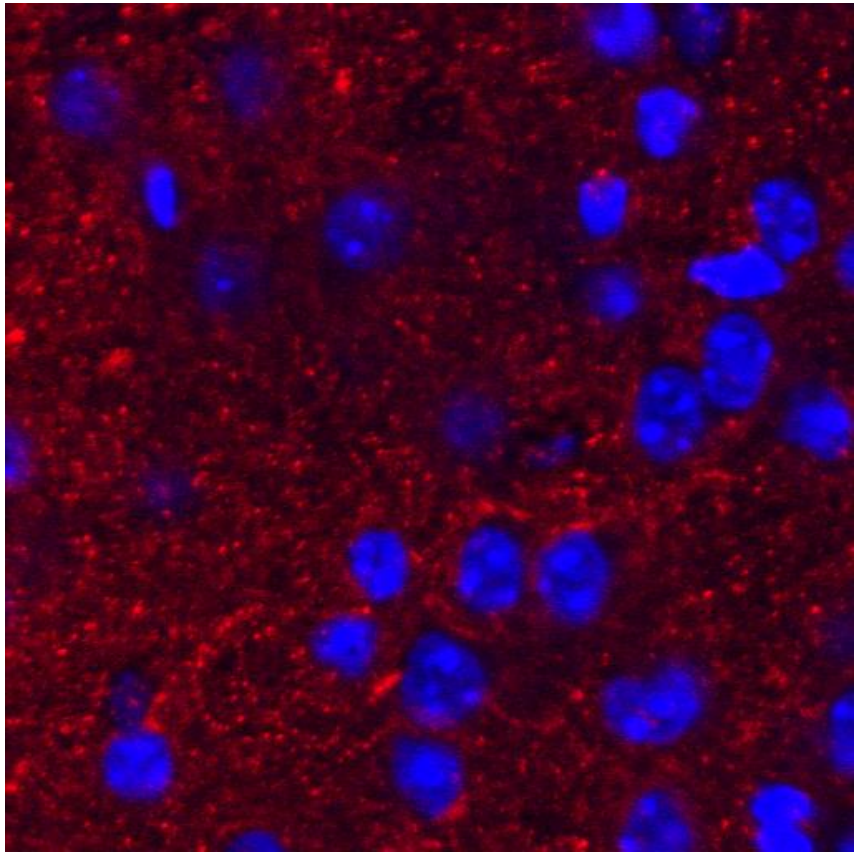


SN=substant
Nigra
MCHL=Mag
cellular
pineaux
Nucleus of
lateral
hypothalamu
Pi=Pineaux

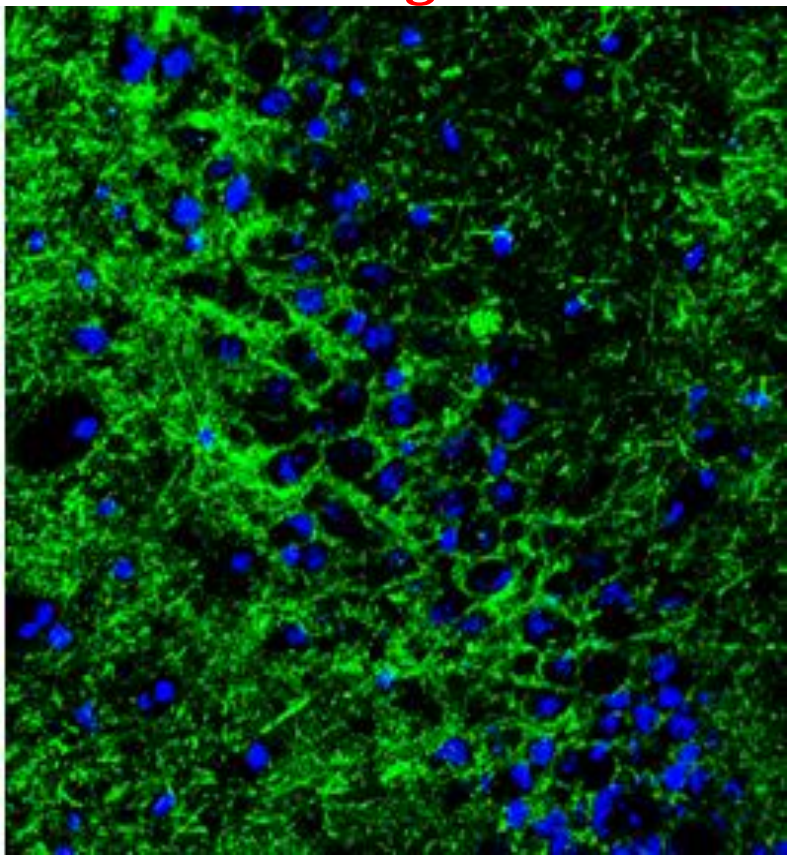
Where is GABA transporter?

GAT-1 immunoreactivity in cortex (red) and hippocampus (green)

3 months old mouse in C57/BL/6J mouse background



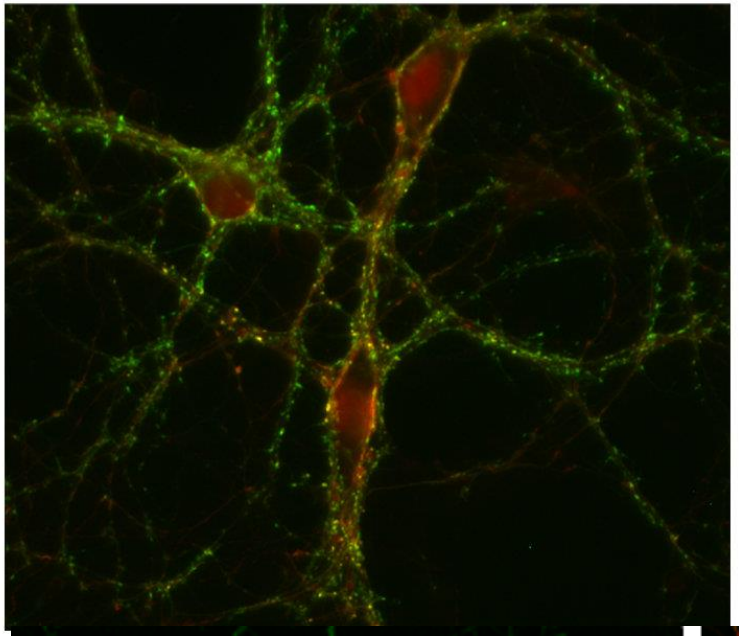
Cortex



Hippocampus

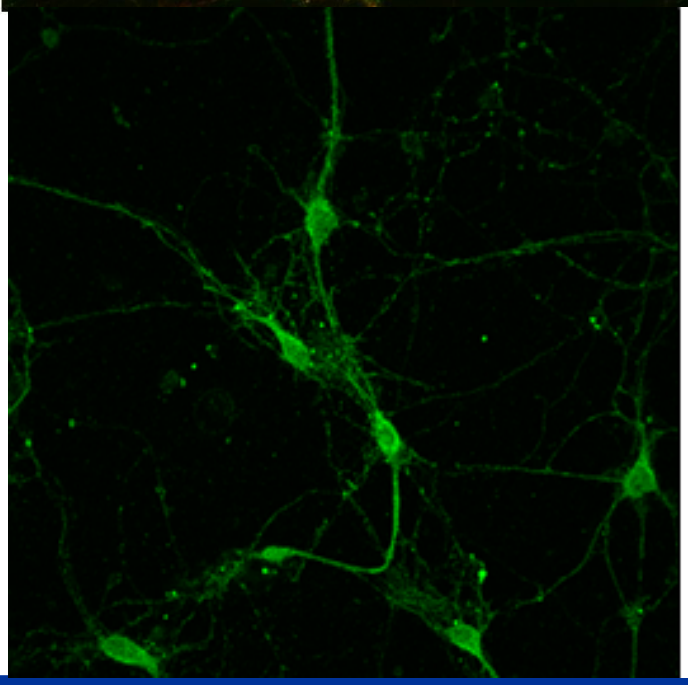
Mouse tissue

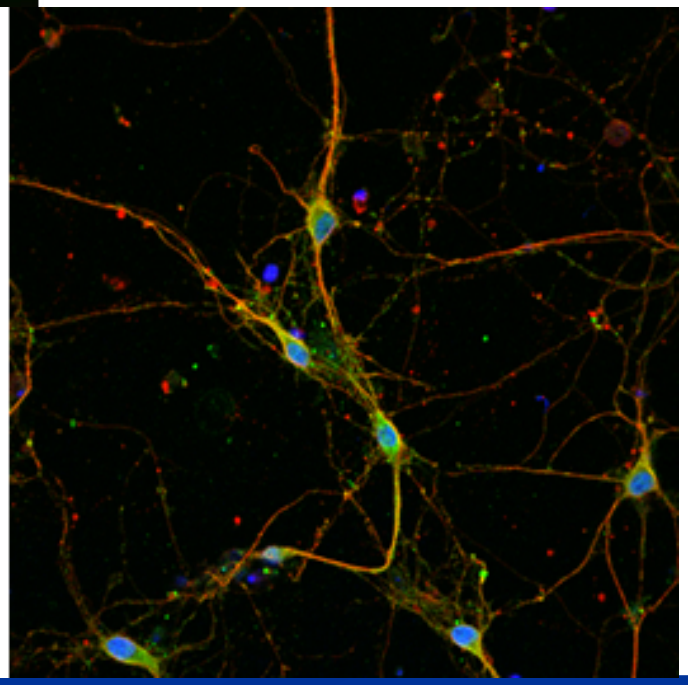
At cell level



In cultured neurons

GAT-1 immunoreactivity in Cultured neurons Green: GAT-1 Red: Tubulin Tuji

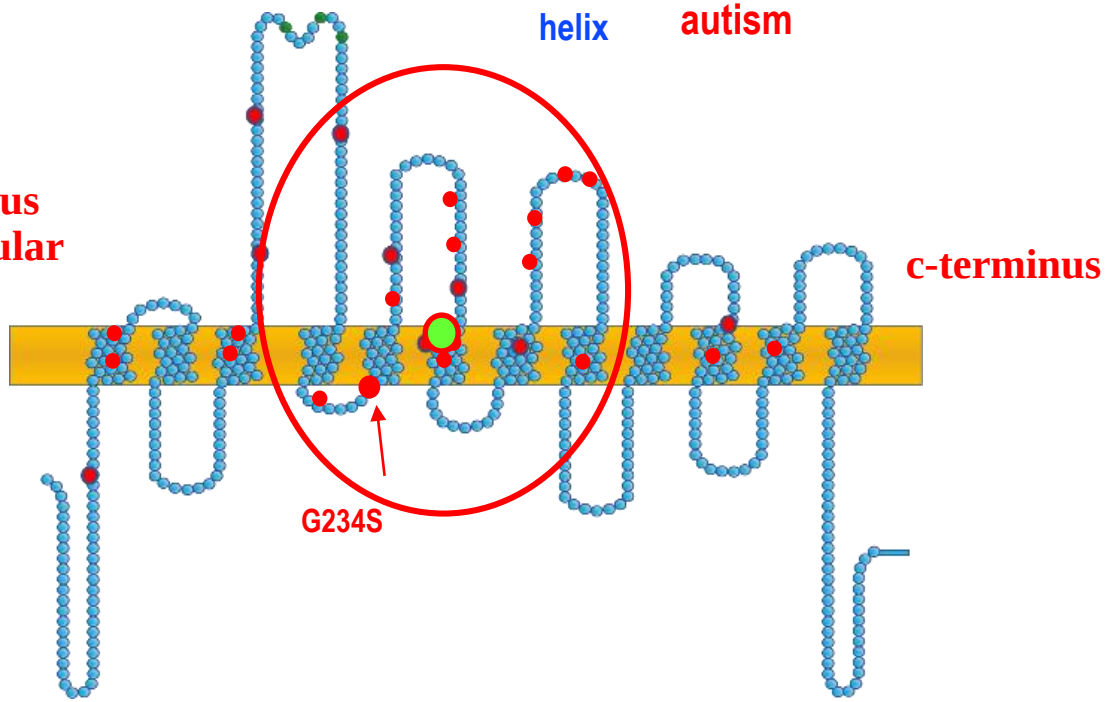




Mutations in *SLC6A1* associated with epilepsy and intellectual disability

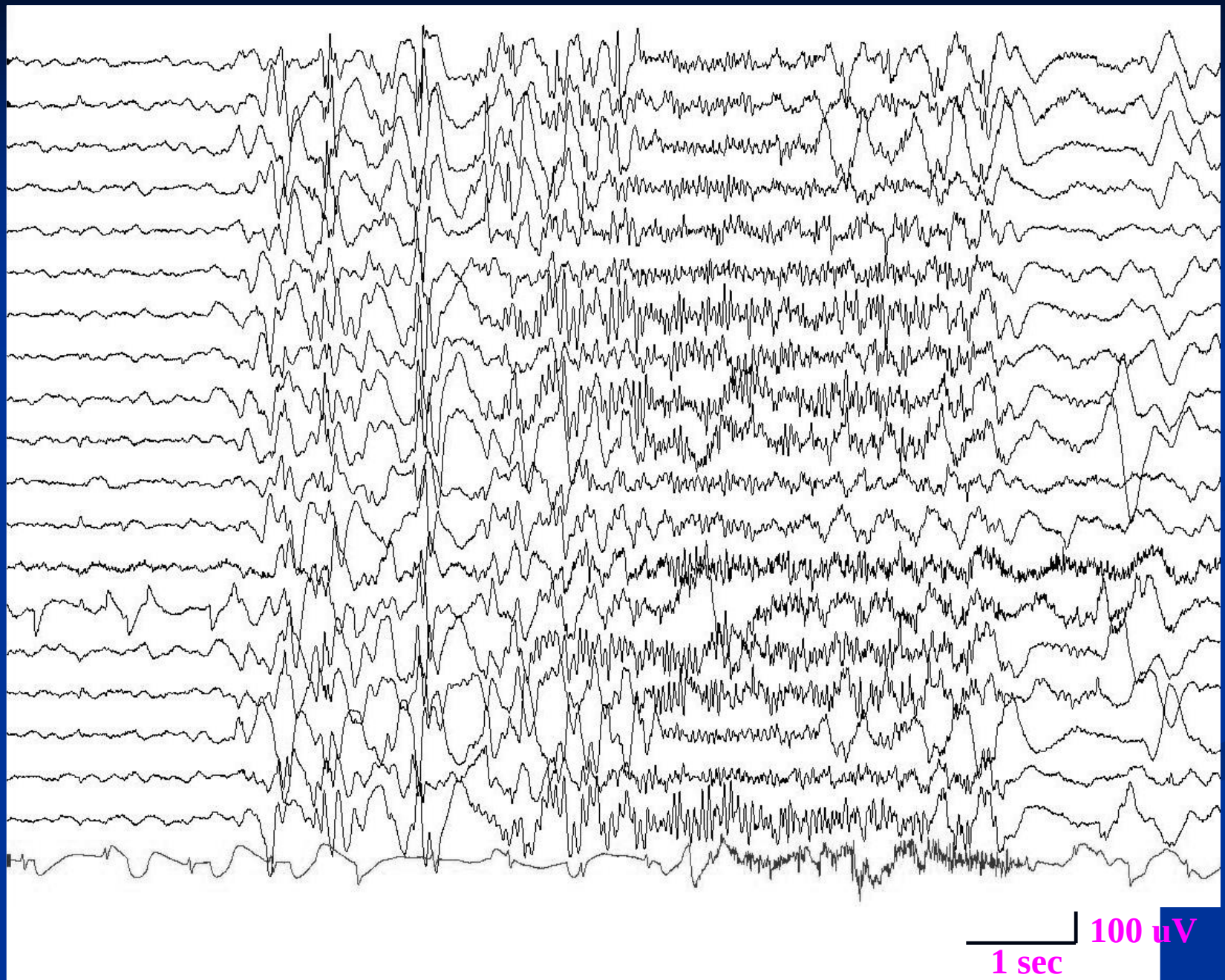
No. of SL6A1 (GAT-1) mutations= 121

Phenotypes:
Myoclonic atonic epilepsy
Lennox-Gastaut syndrome
Absence seizures
Intellectual disability, autism

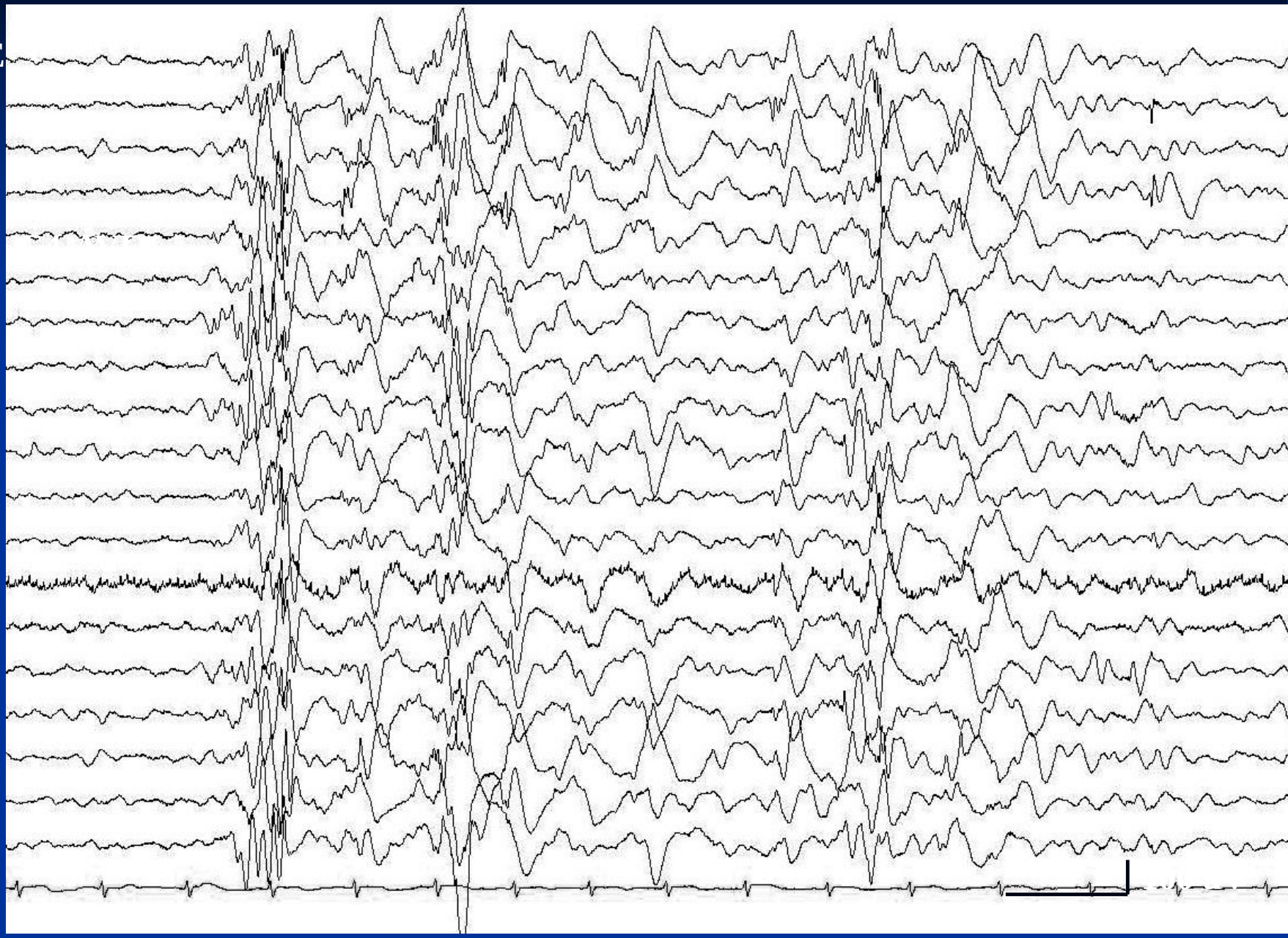


EEG from the patient carrying SLC6A1(G234S) associated with LGS

A



EEG from the patient carrying SLC6A1(G234S) associated with LGS

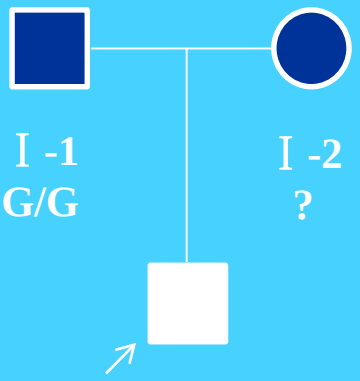


Video showing the patient carrying SLC6A1(G234S) had tonic seizure

Molecular genetics of the patient

A

c.700G>A/p.G234S



Unaffected male

Unaffected female

Affected male

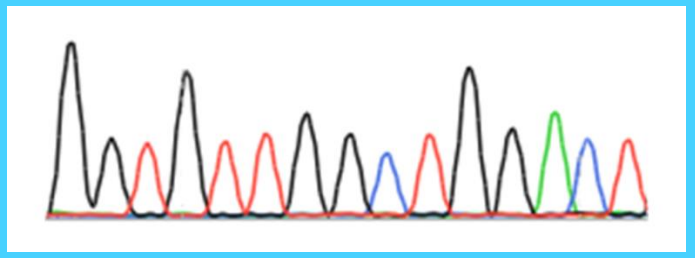
Proband

B

c.700G>A/p.G234S

G	V	S	W	T
G G T G T T **A** G C T G G A C T				

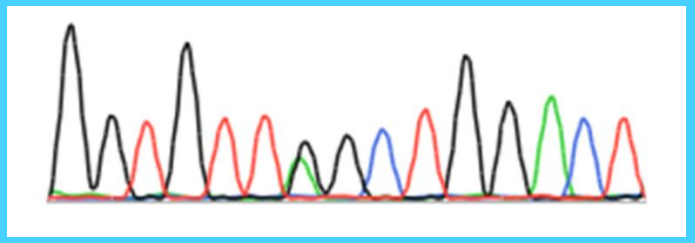
Ⅰ -1



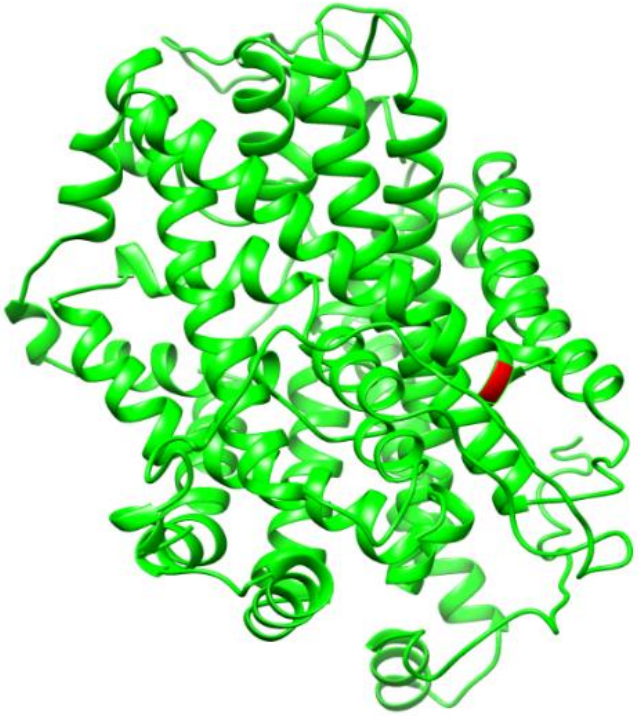
Ⅰ -2

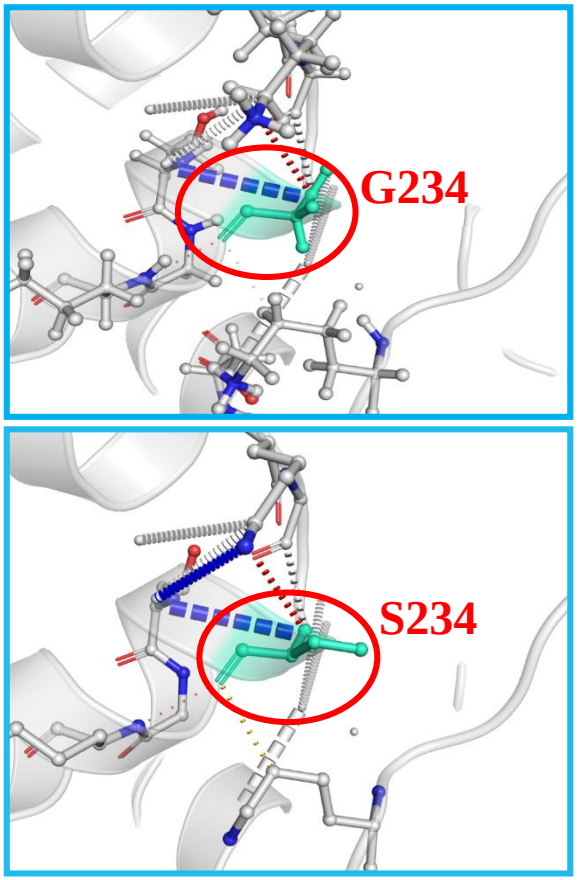
Not available

Ⅱ -1

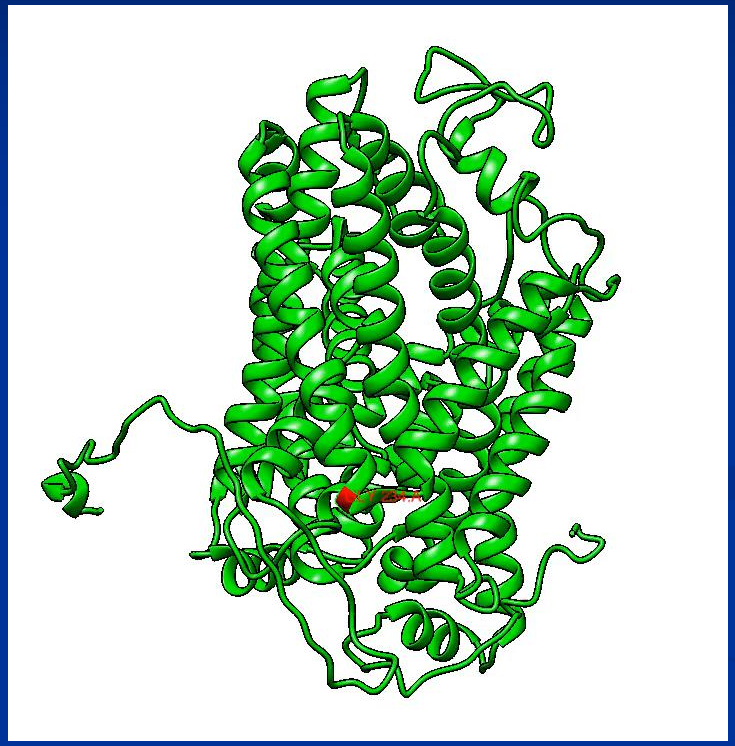


Protein structural modeling and machine learning indicate the S234 destabilize the GAT-1 protein compared to G234

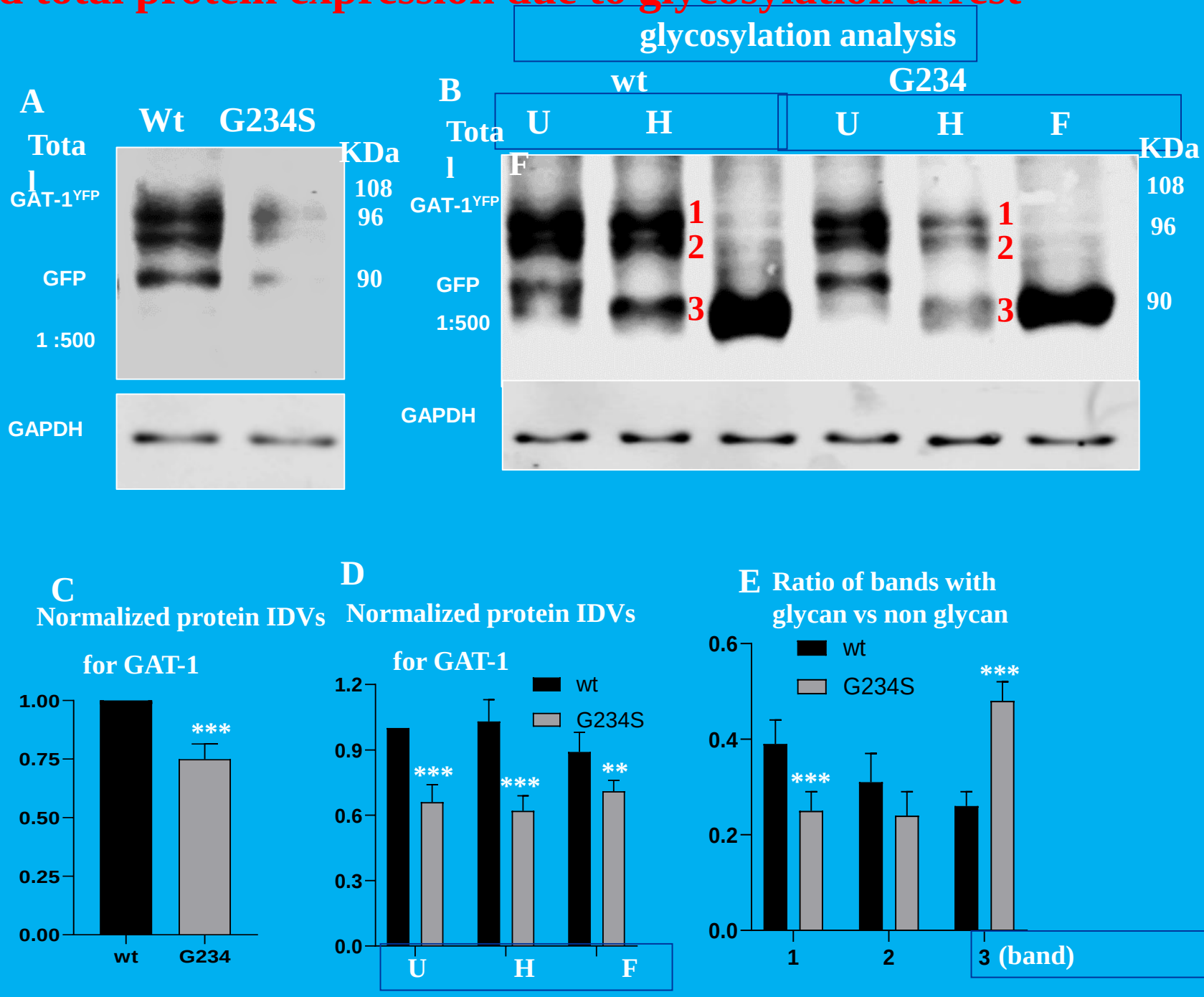




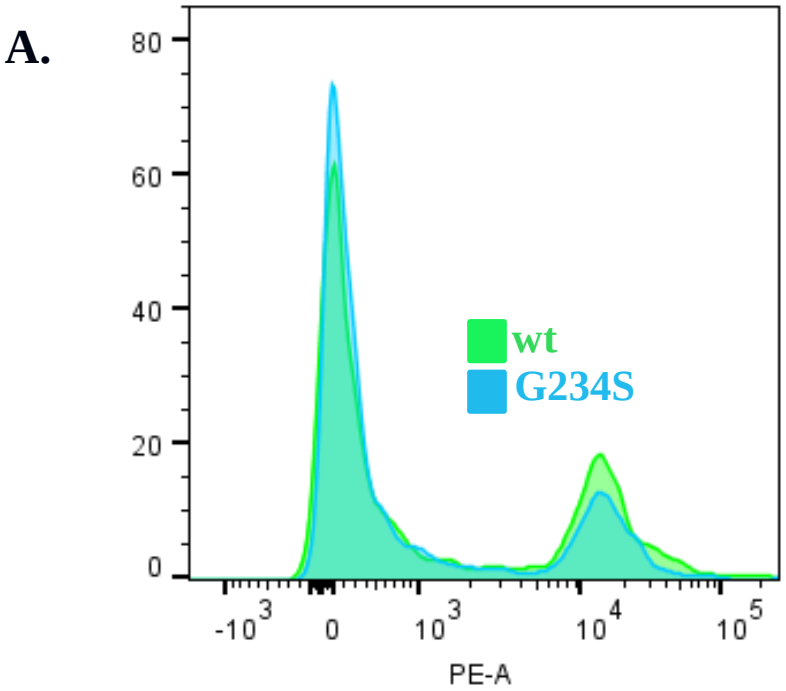
By Juexin Wang

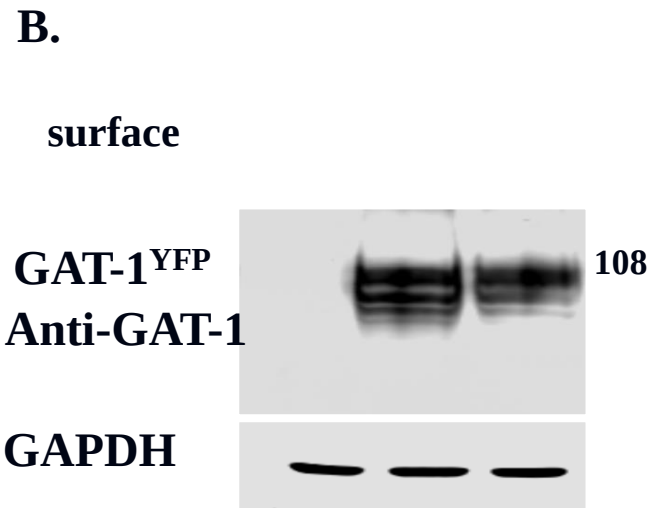


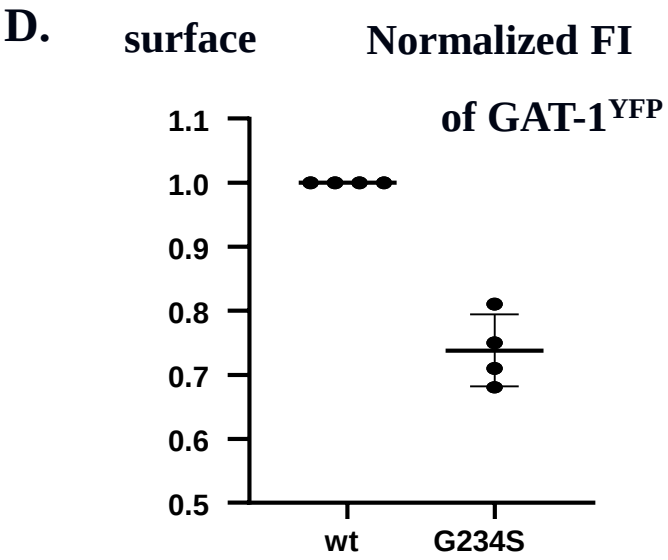
Reduced total protein expression due to glycosylation arrest

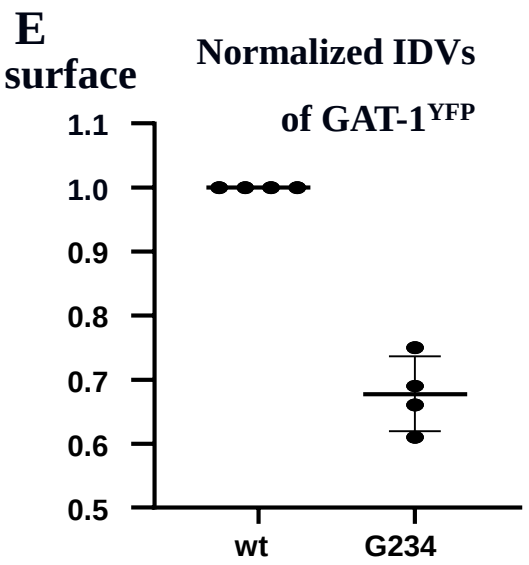


Reduced surface protein expression as evaluated by flow cytometry and live cell surface biotinylation

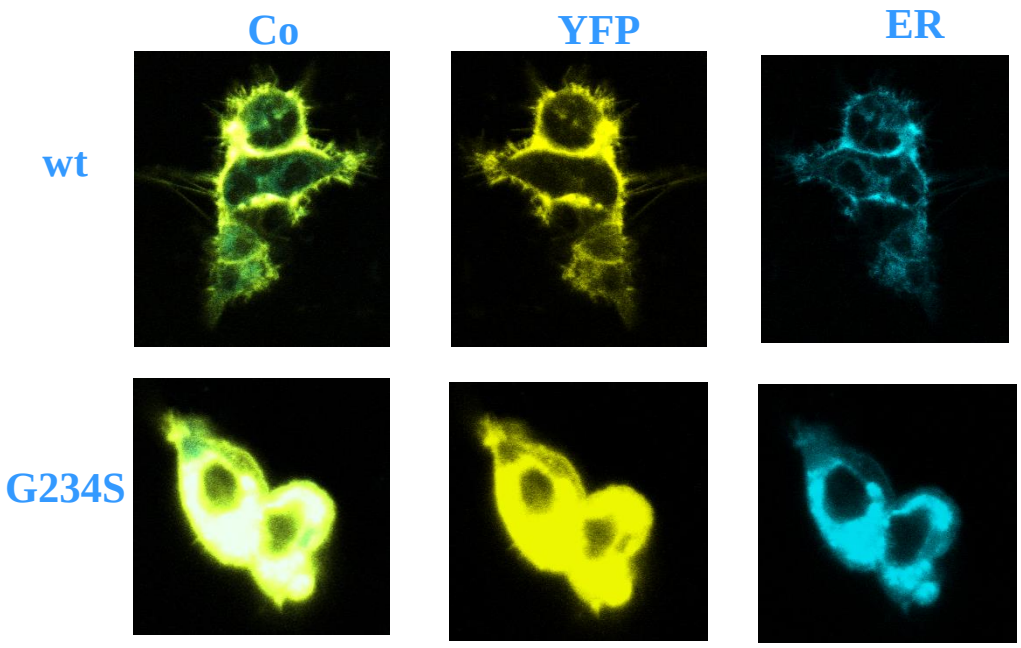






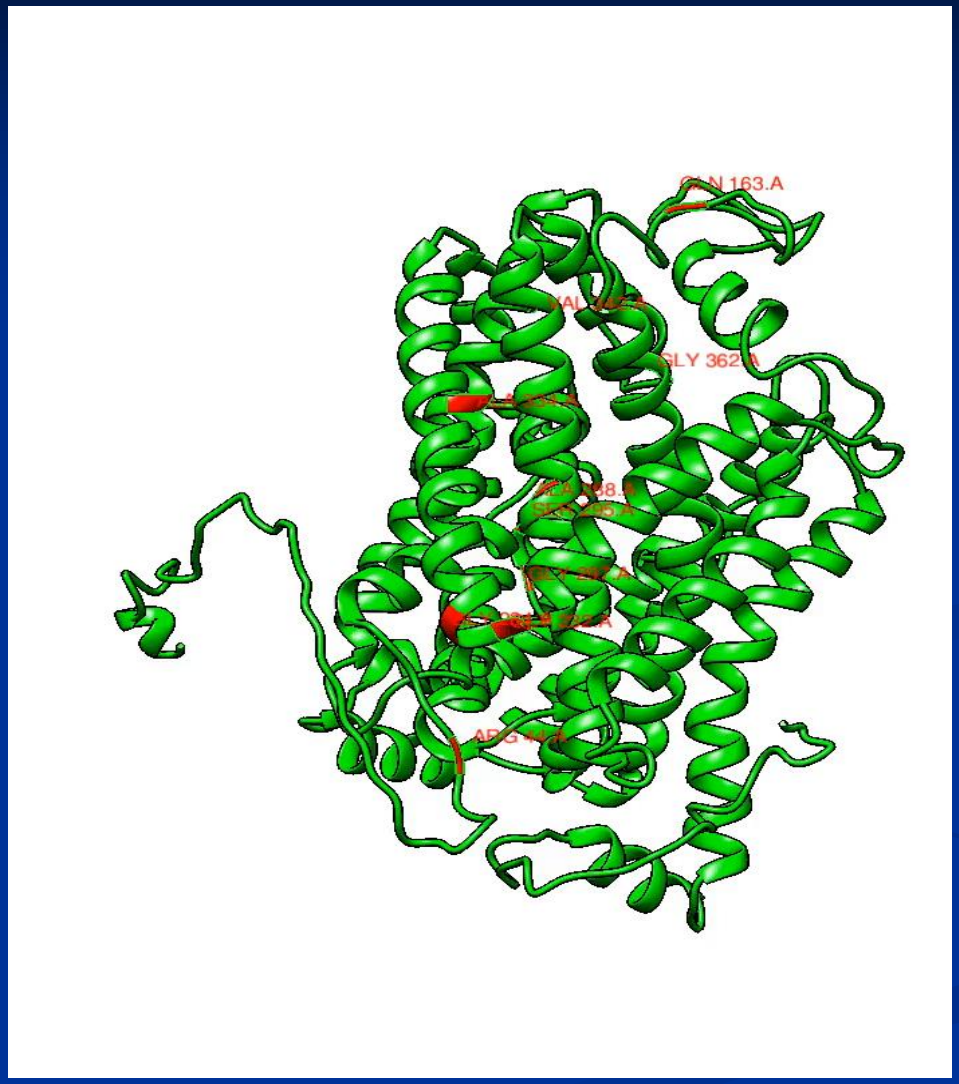


ER retention of the mutant GAT-1 (G234S) transporter

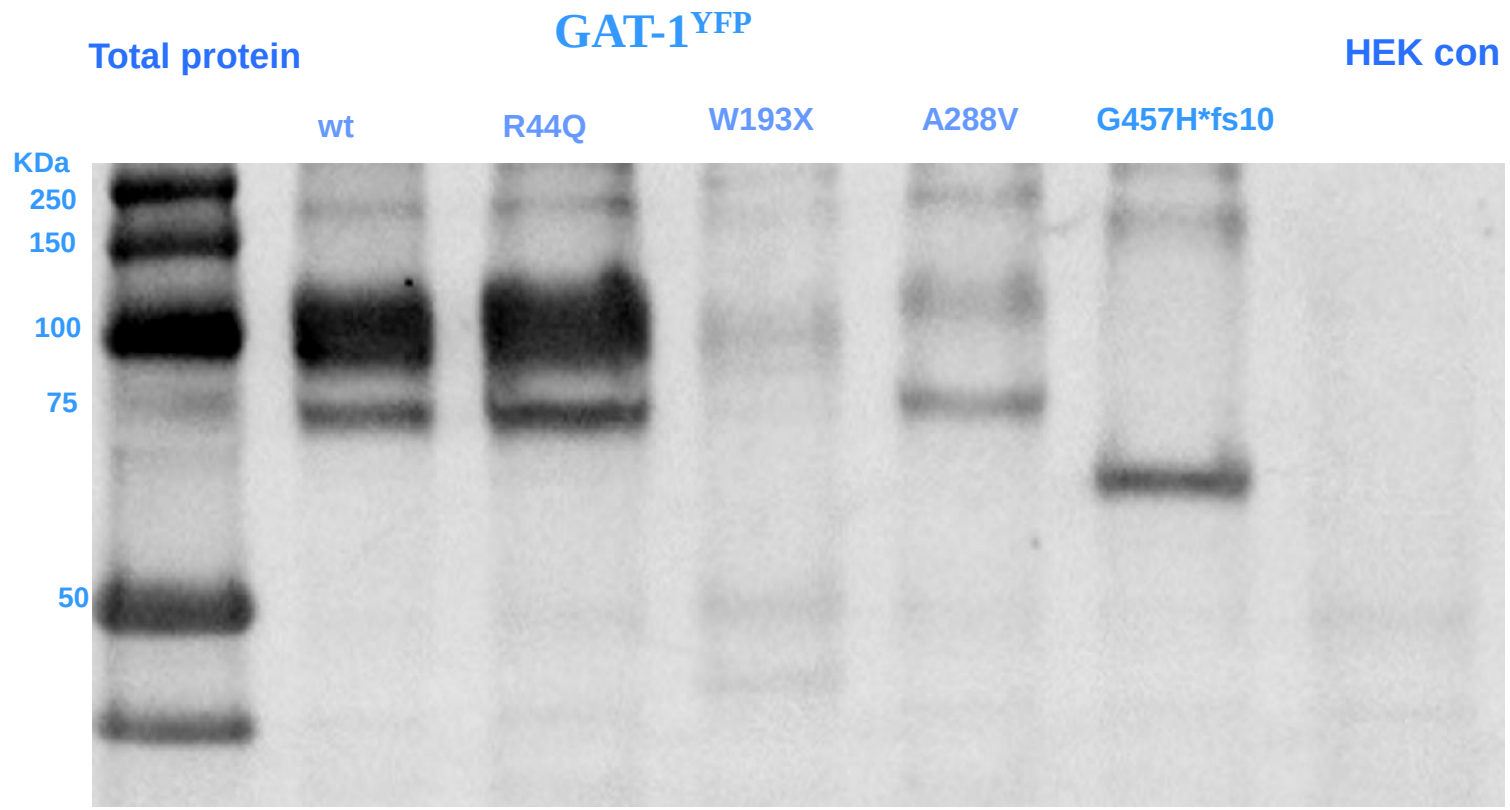


Cai et al., Submitted to JMG on 11-19-2018

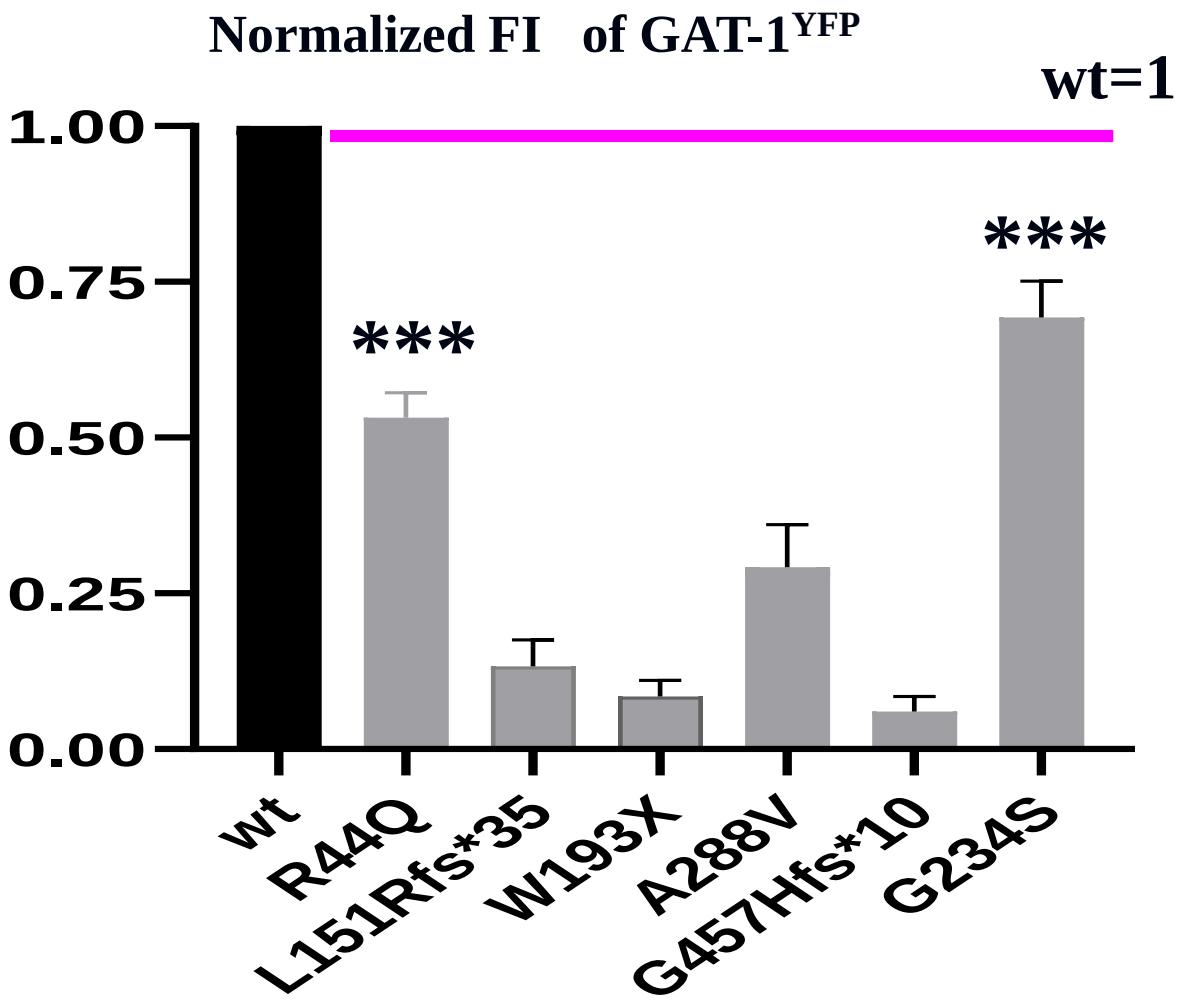
Video showing multiple mutations in GAT-1



Other mutations in *SLC6A1* associated with MAE and intellectual disability

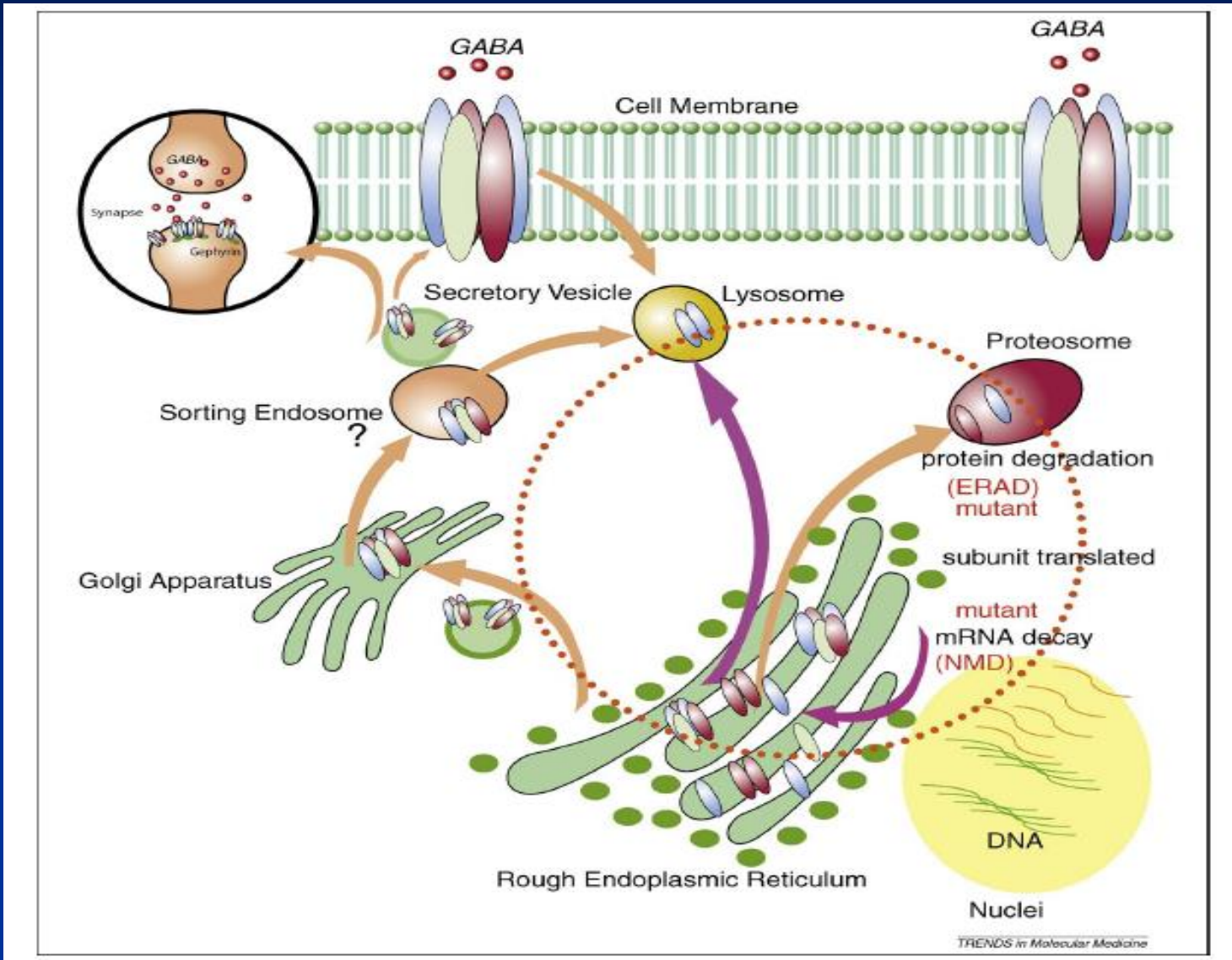


Reduced surface GAT-1 protein expression is a common defect for SLC6A1 mutations



The trafficking for mutant GAT-1 is unknown

1. Normal route of GABA receptor trafficking
2. NMD and ERAD pathways for the nonsense mutants

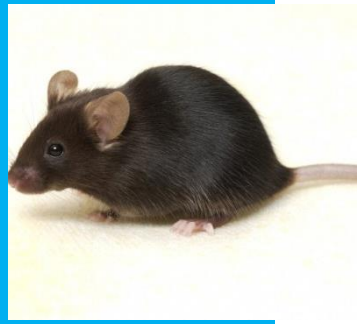


Kang & Macdonald. 2009,Trends Mol Med

Mouse model: $SLC6A1^{+/A288V}$

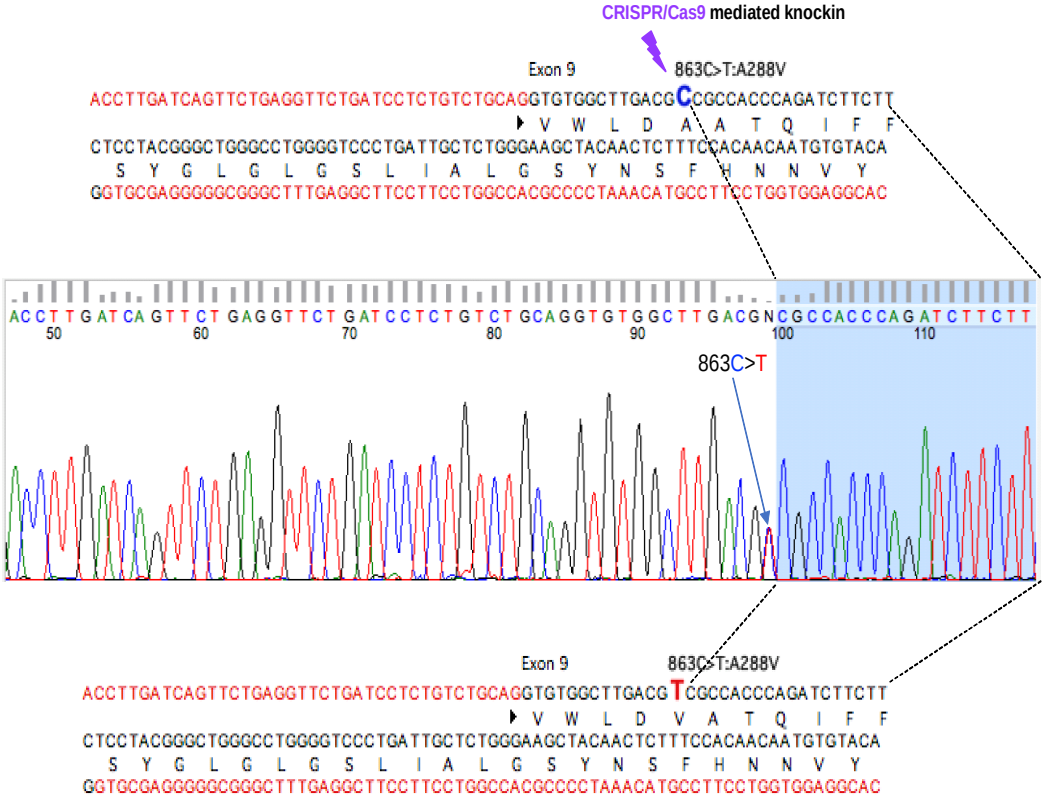
CRISPR/Cas9

Global knockin



MAE, ID

CRISPR/Cas9 mediated Slc6 863C>T:A288V knockin in the Mouse



Compare mouse models carrying mutations in *SLC6A1* and *GABR*

Mouse model:
$SLC6A1^{+/A288V}$

CRISPR/Cas9

mutant GAT-1

mutant GABR

$SLC6A1^{+/A288V}$
CAE, unclassified
generalizedMAE, ID

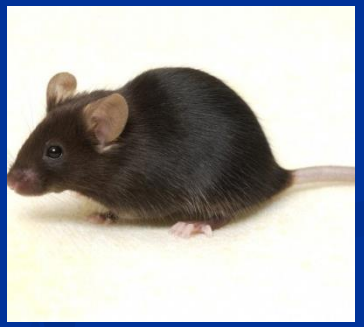
$GABRB3^{+/N328D}$

LGS

Slc6a1$^{+/A288V}$ mutant pups have better survival rate than *Gabrb3*$^{+/N328D}$ pups

SLC6A1$^{+/A288V}$

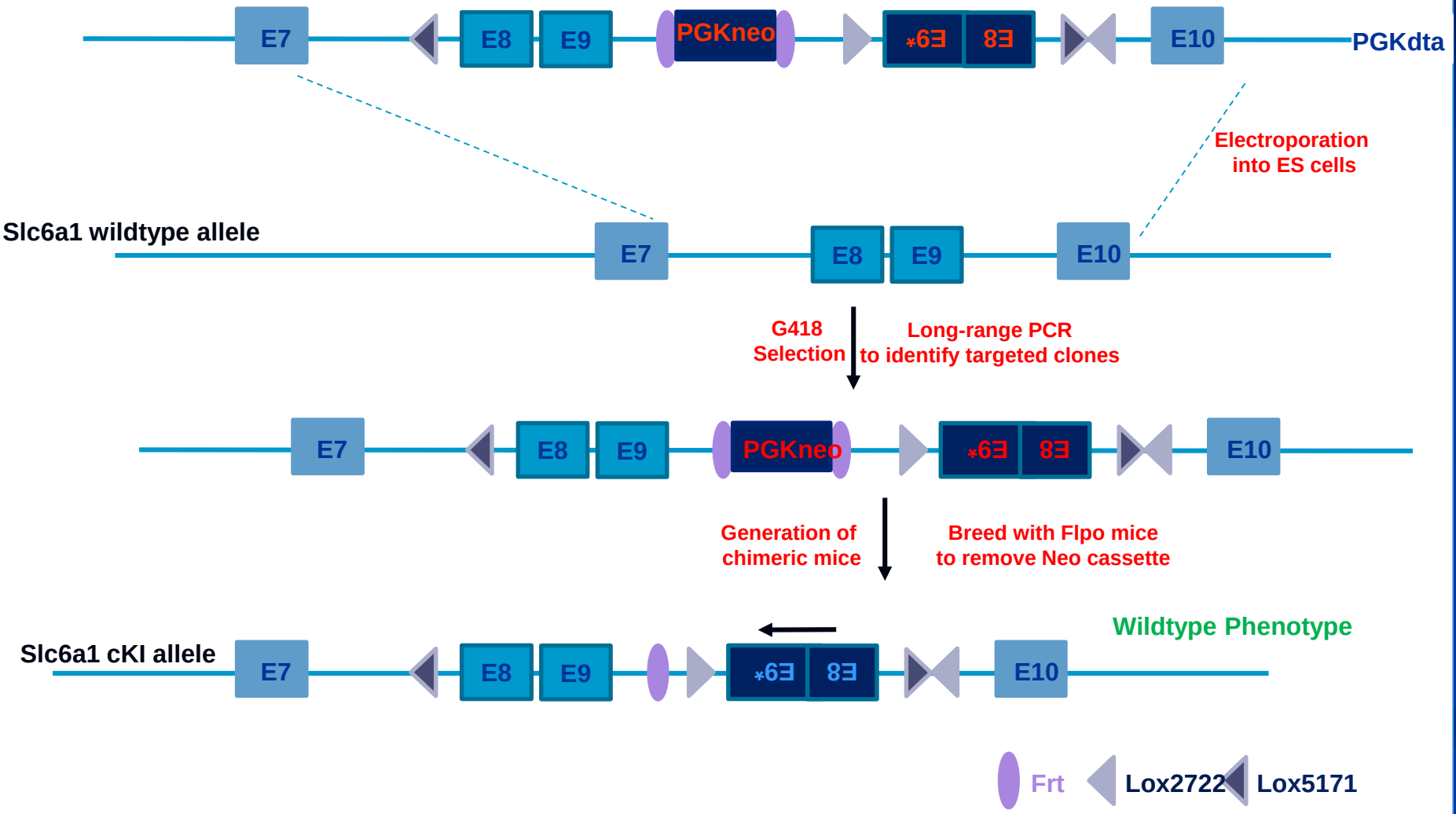
Gabrb3$^{+/N328D}$

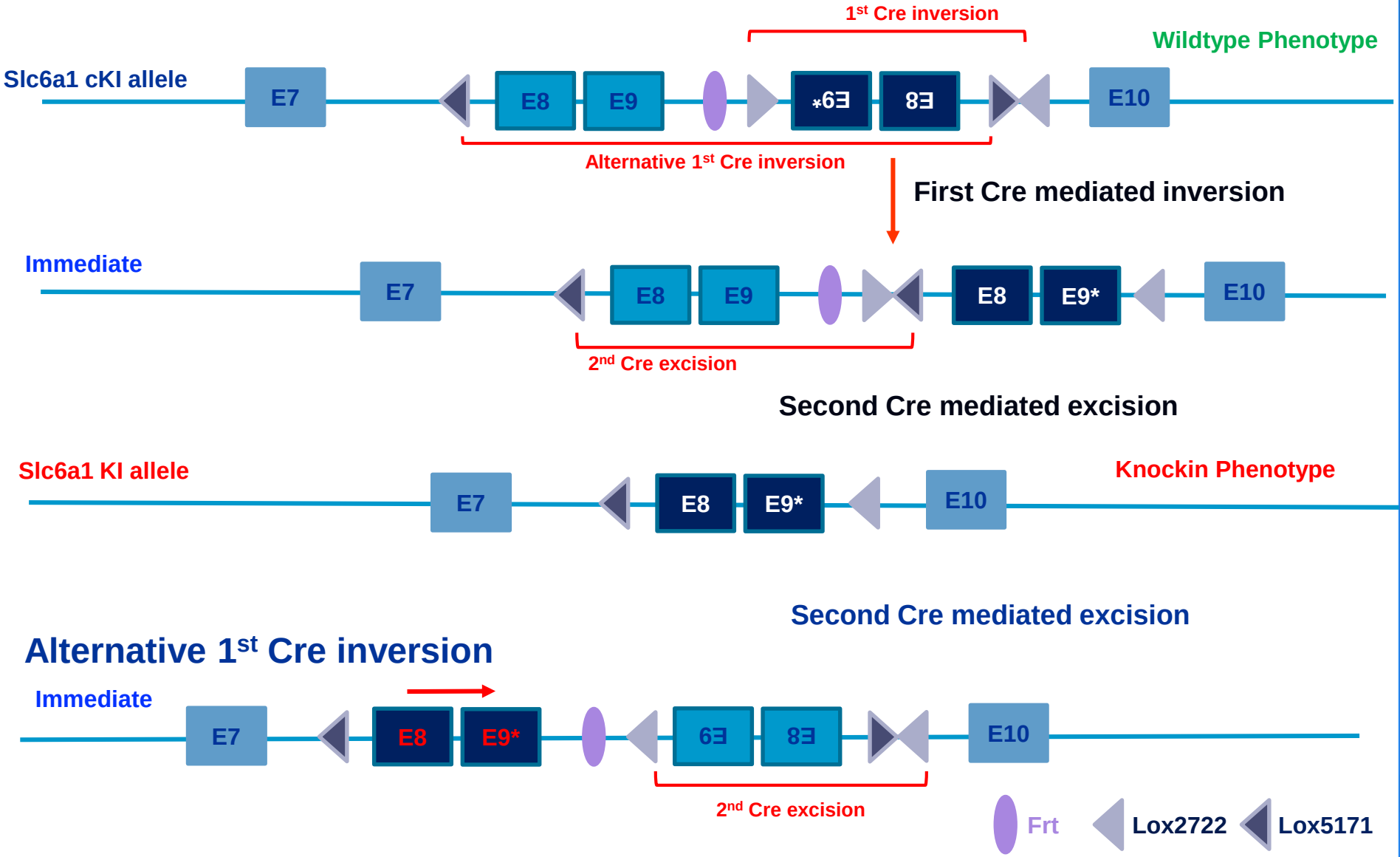


mutant GABR

Generation of Slc6a1 (X) Conditional Knockin Mice using FLeX Targeting Vector

X=A SLC6A1 mutation with different molecular defect than A288V mutation




1st Cre inversion
Wildtype Phenotype
Slc6a1 cKI allele
E7
E8
E9
*6Ǝ
8Ǝ
E10
Alternative 1st Cre inversion
First Cre mediated inversion
Immediate
E7
E8
E9
E8
E9*
E10
2nd Cre excision
Second Cre mediated excision
Slc6a1 KI allele
Knockin Phenotype
E7
E8
E9*
E10
Second Cre mediated excision
Alternative 1st Cre inversion
Immediate
E7
E8
E9*
6Ǝ
8Ǝ
E10
2nd Cre excision
Frt
Lox2722
Lox5171

Advantages and Features of Global Knockin:

- **Efficient to generate using CRISPR mediated gene editing to introduce mutation directly into fertilized egg and therefore allow initial characterization of the effect of the mutation in a relatively short period of time.**
- **Mutation will be present in every cells of the animal and effects as soon as the gene is expressed during gestation.**

Advantages and Features of Conditional Knockin:

- **More complicate, costly and longer time to generate.**
- **Mutation can be activated in a spatiotemporal (specific time and cell type) by breeding with tissue specific CreERT2 mice.**
- **More accurately reflected mutation occurs after conception or later in life in the human.**
- **Better tool to dissect effects of the mutation in a specific cell type or neurons, especially cell-to-cell interaction communication**

With conditional knockin, we can specifically activate the mutation in neurons (different types of neurons) or astrocytes to characterize the disease phenotype.

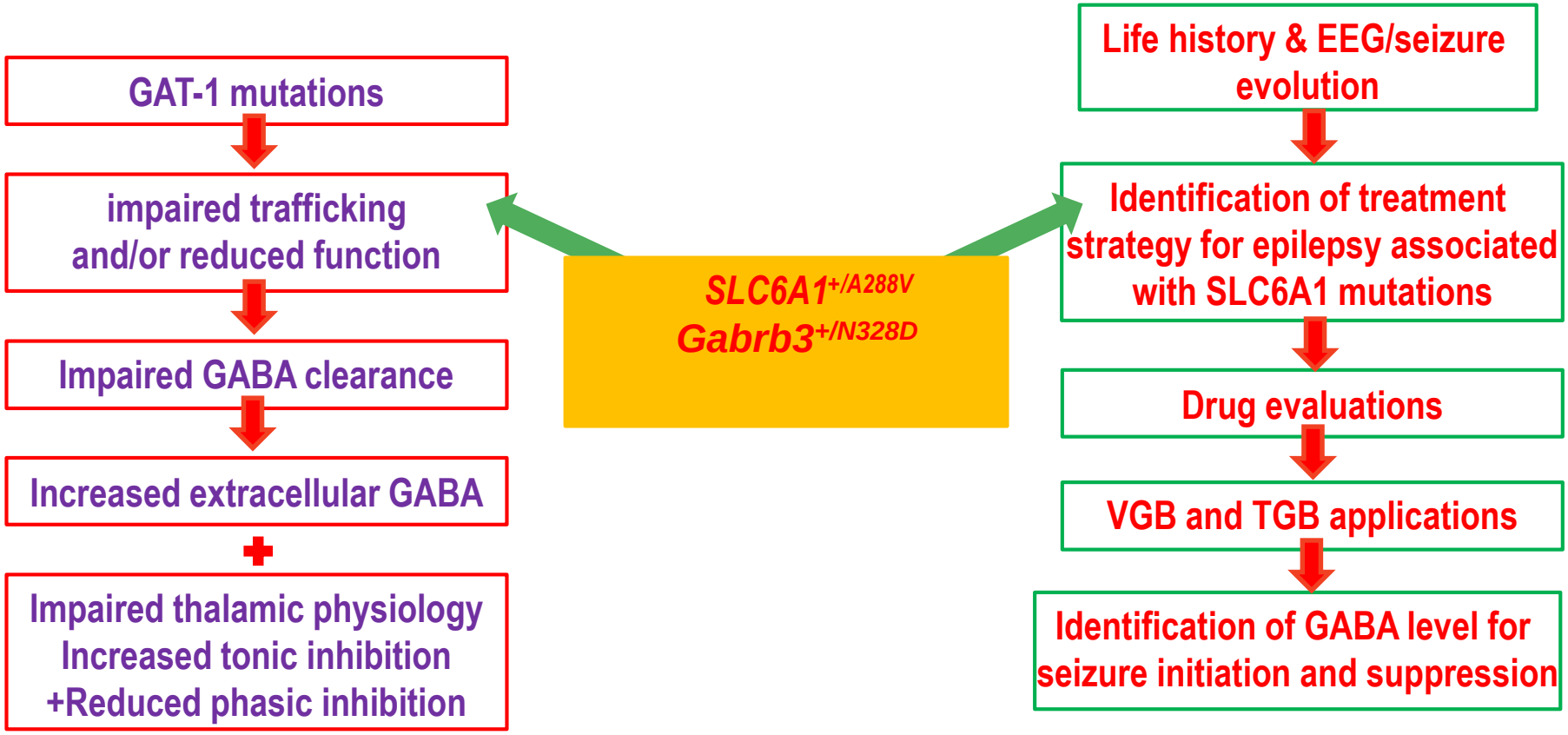
We can compare the phenotypes in mouse with the phenotypes in human iPSC derived neurons or astrocytes.

Compared the effect of treatment with existing antiseizure drugs in mice carrying GAT-1(A288V) and GABRB3(N328D) mutations

In humans: Effective treatment by VPA and LTG in LGS caused by both GABRB3 and GAT-1

In $SLC6A1^{+/A288V}$ and $Gabrb3^{+/N328D}$ mice:????

Our Study Design

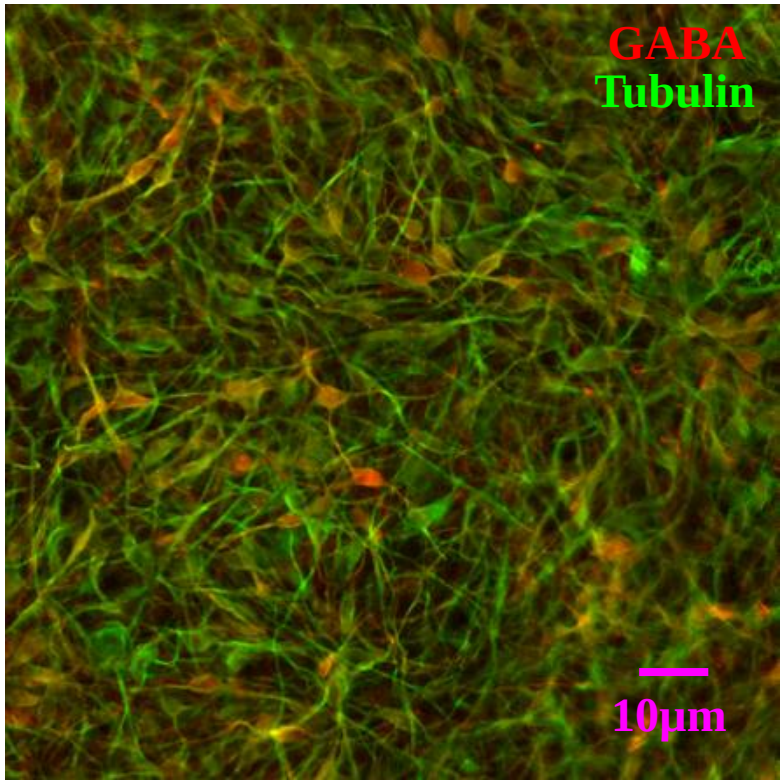


Ongoing work:

In addition to mouse models, we will validate the findings in human iPSC derived neurons and organoids

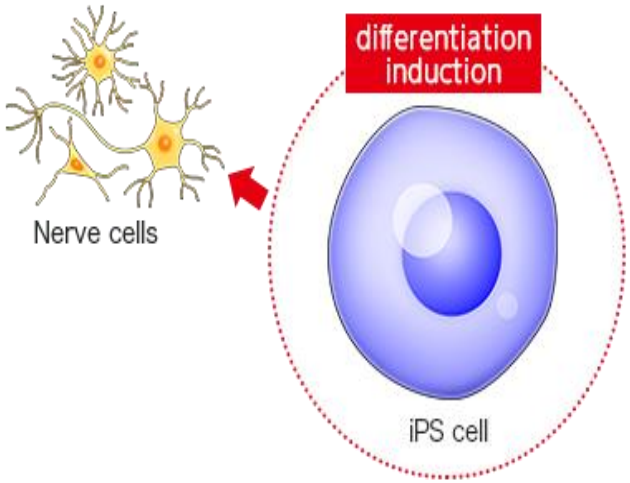
GABAergic neurons derived from patient skin cells

GABAergic differentiated protocol (Maroof et al) was followed. At Day 26 post-plating cultures were fixed and immunostained for GABA (red) and Tuj beta-tubulin III (green).



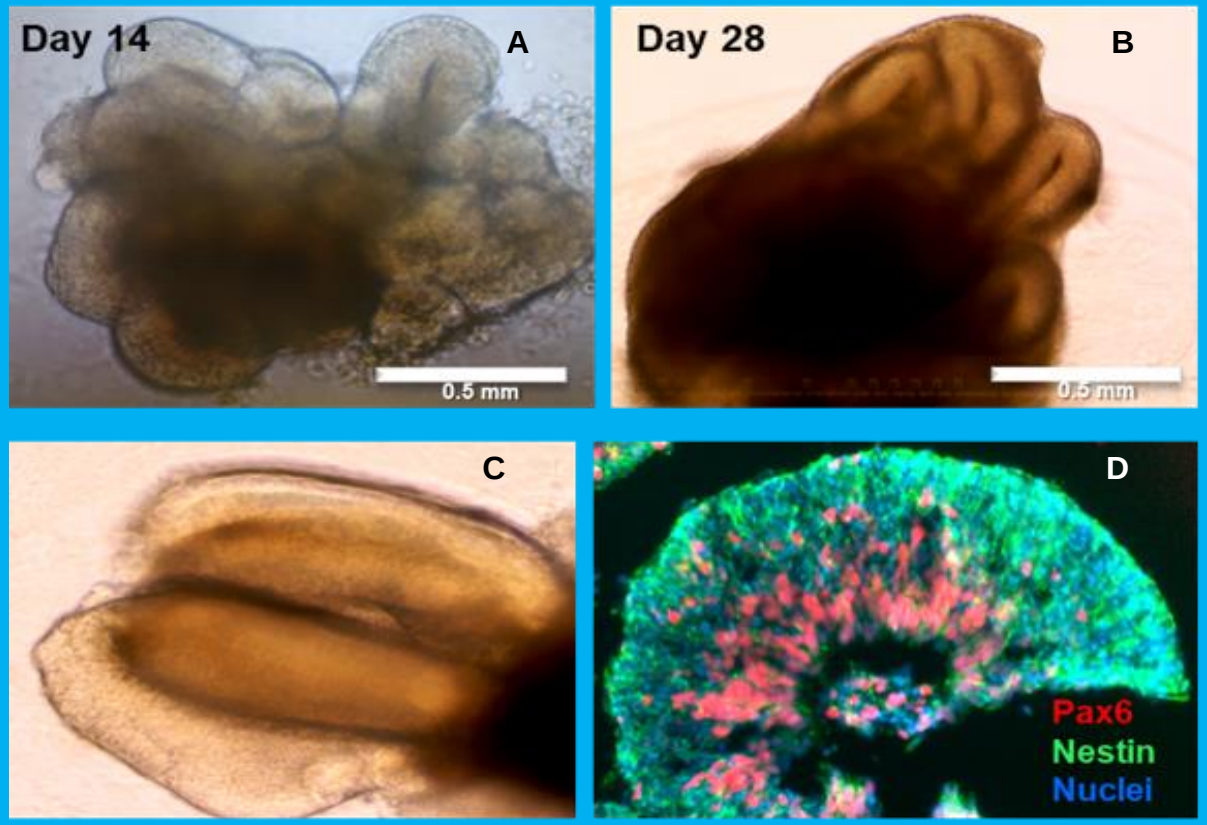
Collaborator: Kevin Ess

Current ongoing work in lab: differentiation of GABAergic interneurons from An Angelman syndrome patient skin cells. The Angelman patient was caused by Deletion of UBE3a and GABRB3. We are comparing the data from cells and GABRB3 knockout mouse.



GABRB3 knockout

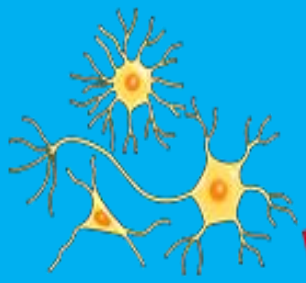
Collaborator: Kevin Ess & Rob Carson

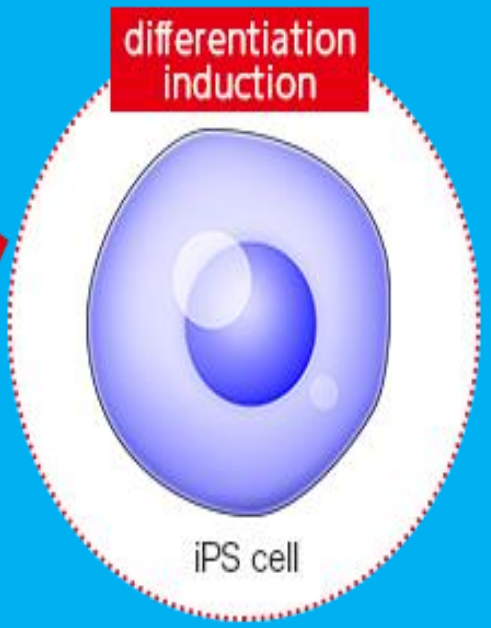


Generation of brain organoids from iPSCs in the Gama Laboratory (using modified protocols from Lancaster, 2016). At day 14 and 28, brightfield images show optically translucent neuroepithelial structures (A, B and C), while IHC staining (D) shows expression of the neural progenitor marker Pax6 (red) and the radial glia cell marker Nestin (green). Images are courtesy of Dr. Vivian Gama.

Collaborator: Vivian Gama

Thanks to all the collaborators, we use multidisciplinary approaches to identify the underlying pathophysiology and mechanism-based therapies for mutations in *SLC6A1*





Brain organoid

Thanks for the invitation!

Musical city convention center in Nashville

End