Novel models to study chronic neurologic symptoms in branched chain amino acid disorders

Rebecca Ahrens-Nicklas, MD, PhD

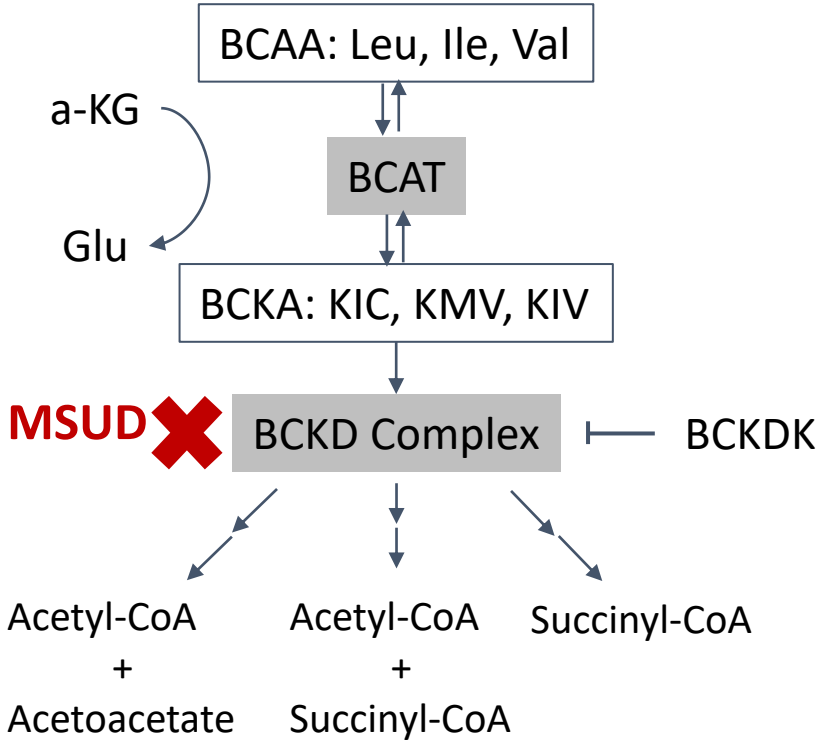
Division of Human Genetics, Section of Metabolism

The Children's Hospital of Philadelphia

Children's Hospital of Philadelphia®

How does MSUD alter the development and function of the brain?

- Extreme elevations of BCAAs and BCKAs
- Infants have encephalopathy, coma, seizures, death due to acute leucine toxicity.
- Treatment with low-BCAA diet and / or liver transplantation improves BCAA in plasma.
- Unfortunately, even well-controlled patients often develop cognitive impairment and neuropsychiatric illness. Likely due to ongoing abnormal BCAA metabolism in brain.
- Previously available mouse models do not survive long enough to investigate chronic neurologic phenotypes



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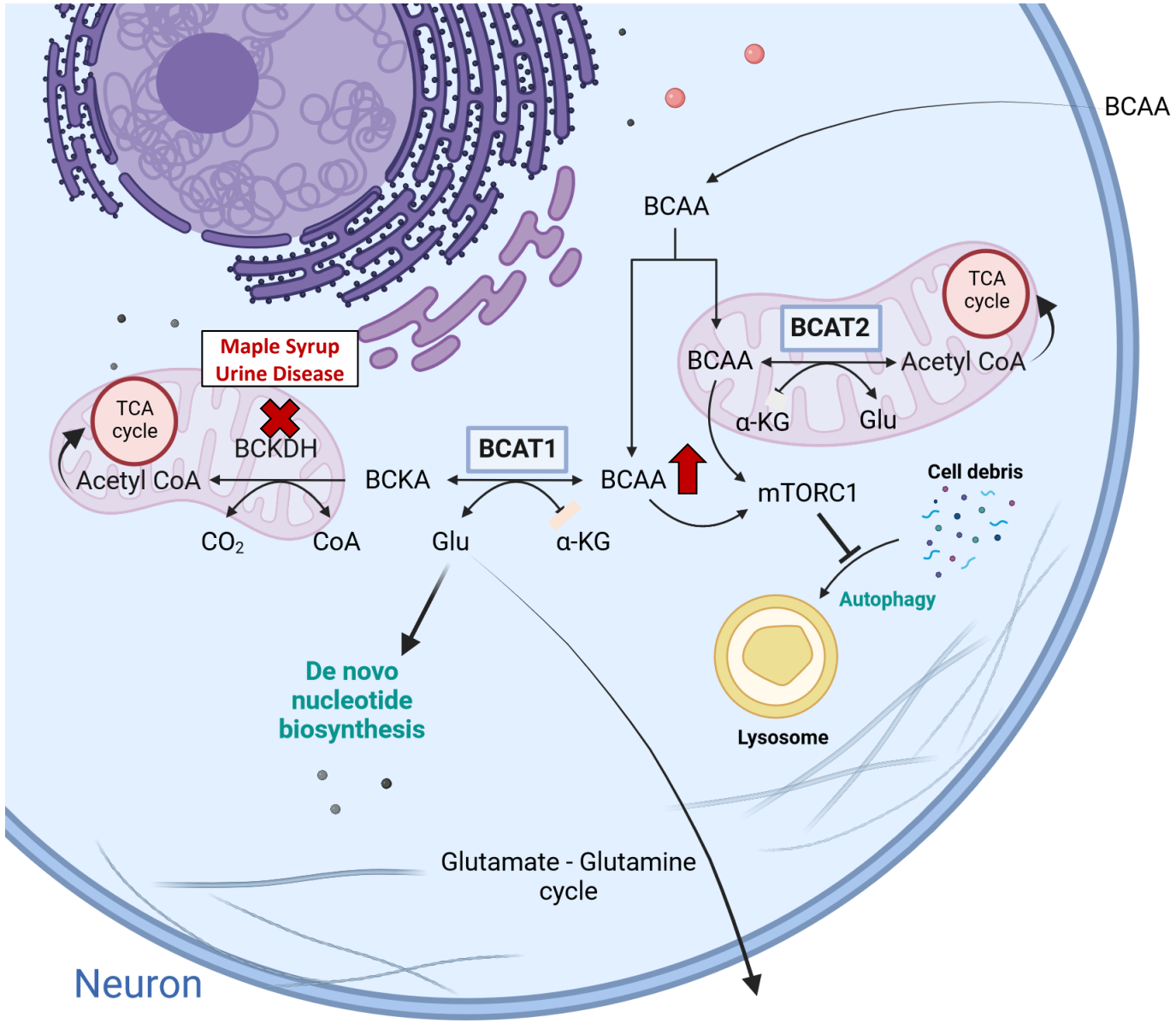
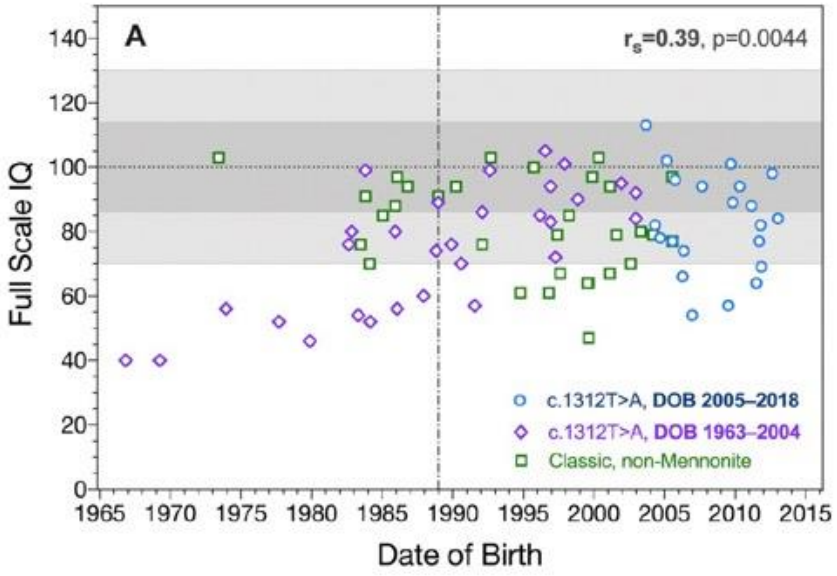
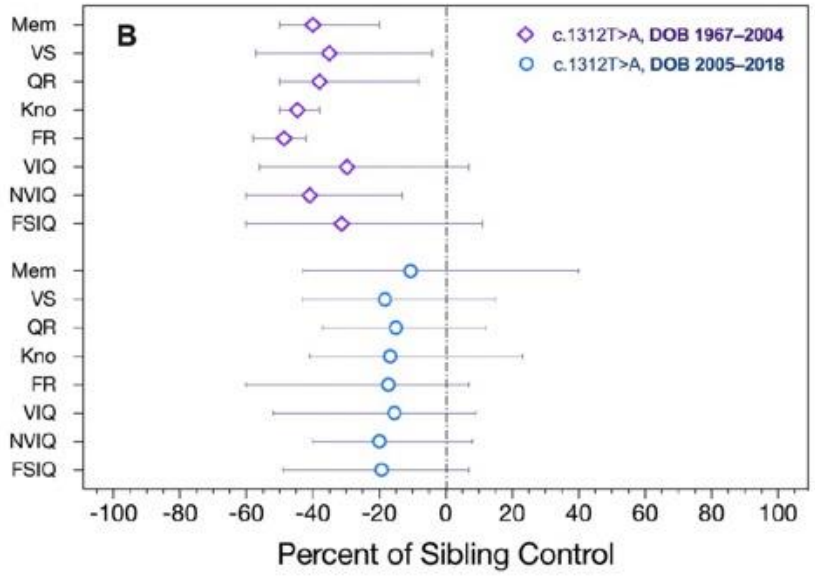
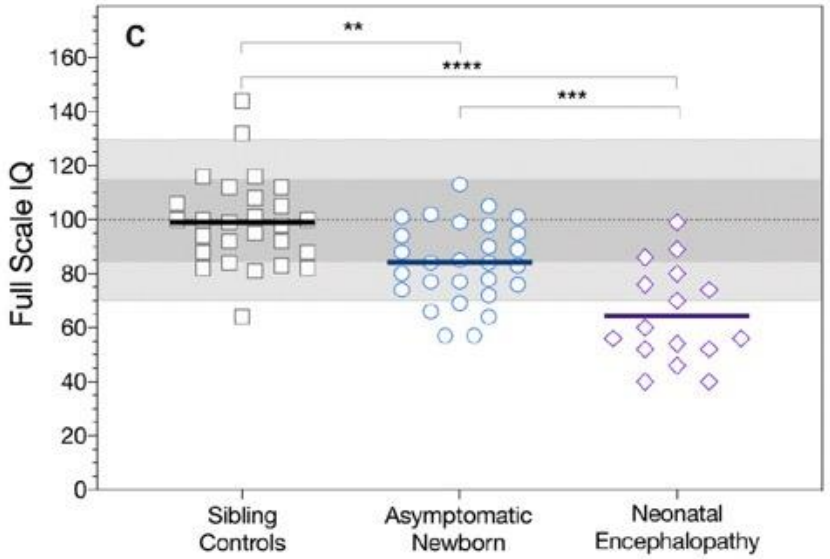


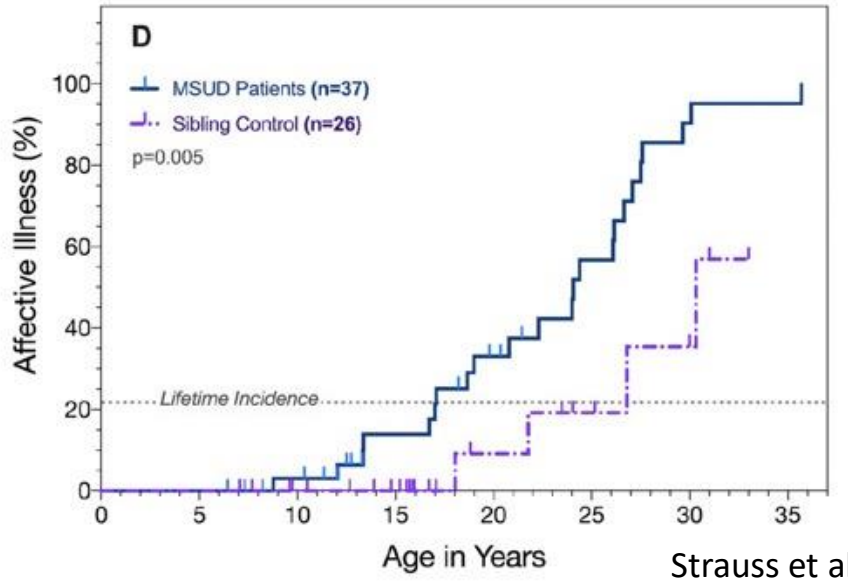
- Alterations in energy production in the mitochondria
- Changes in the glutamate / glutamine cycle
- Alterations in mTOR signaling
- Disruption of de novo purine and pyrimidine synthesis

What are the clinical effects of neuronal dysfunction in treated MSUD?







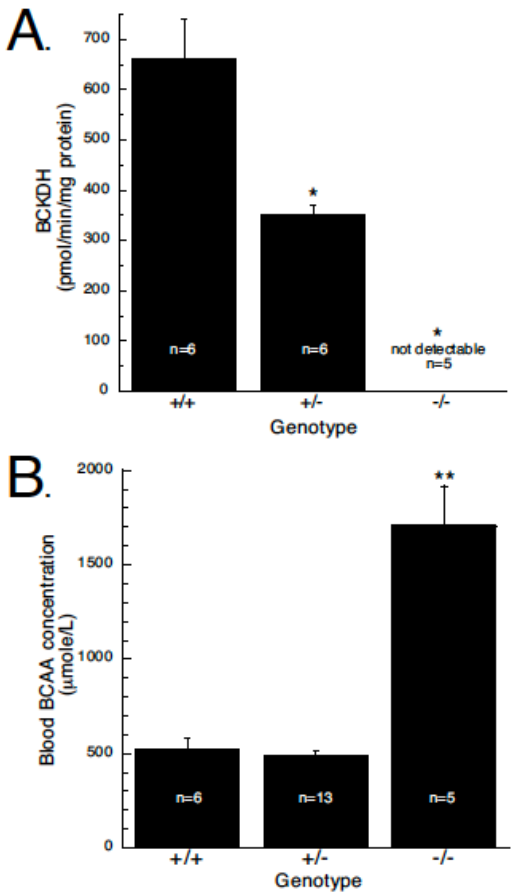


Strauss et al. MGM. 129 (2020) 193-206

Existing MSUD mice: Limited use in studies of chronic neurologic symptoms

Whole body DBT KO

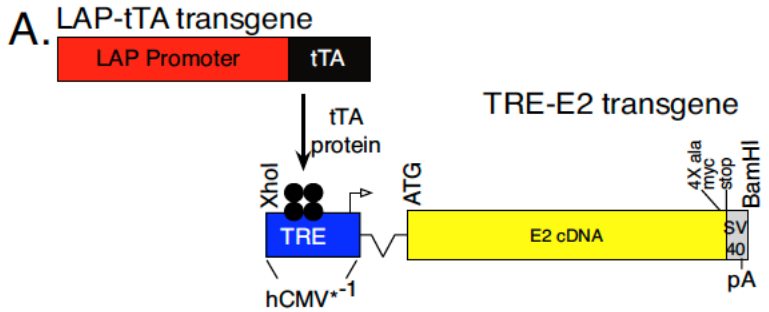
All pups die within 72 hours

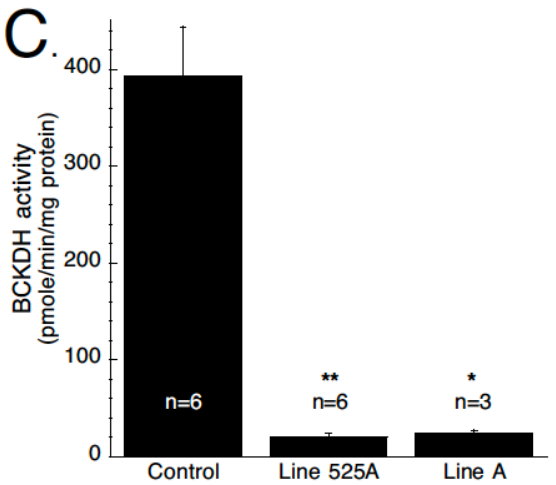


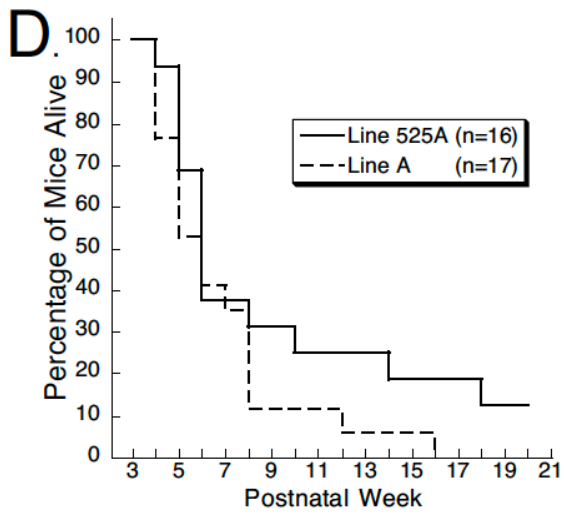
Intermediate MSUD mouse

-Human DBT gene knocked in expressing at 5-6% activity

- Mice live until a median of 6 weeks but are small and have motor defects



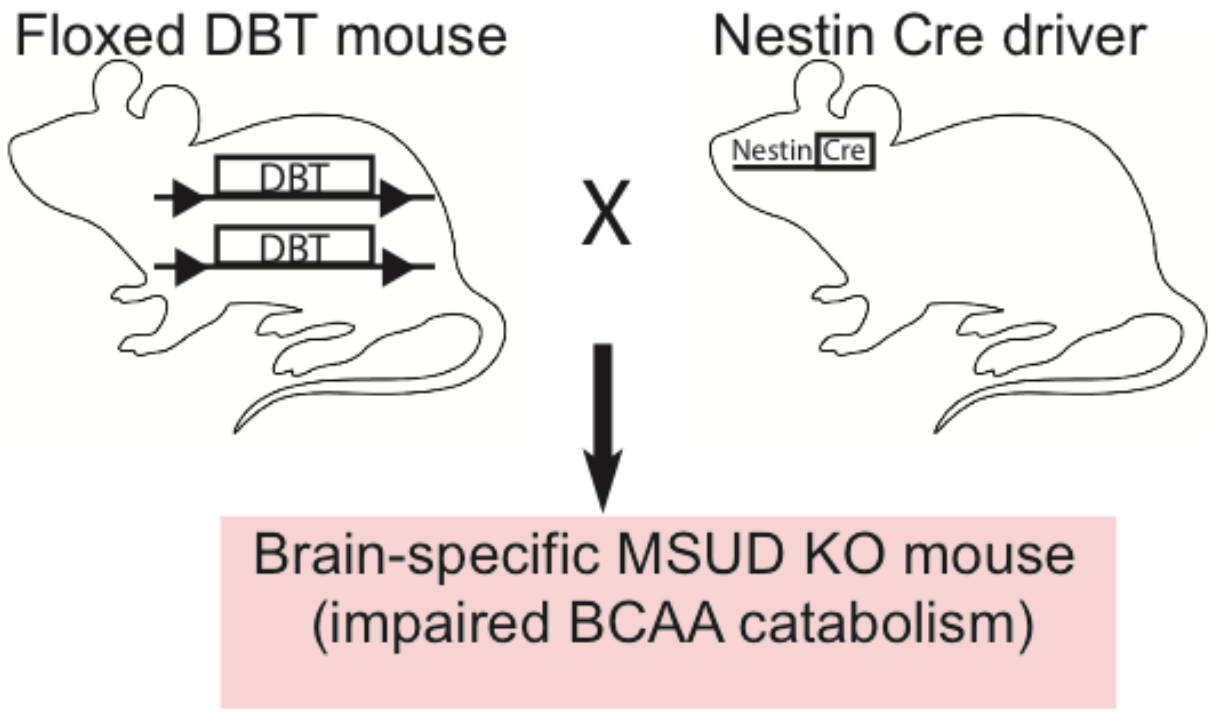




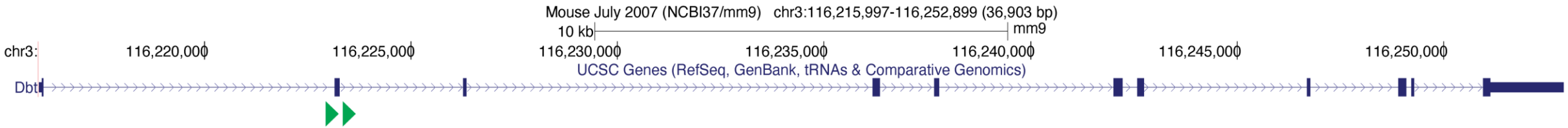
BMC Medical Genetics 2006, **7**:33

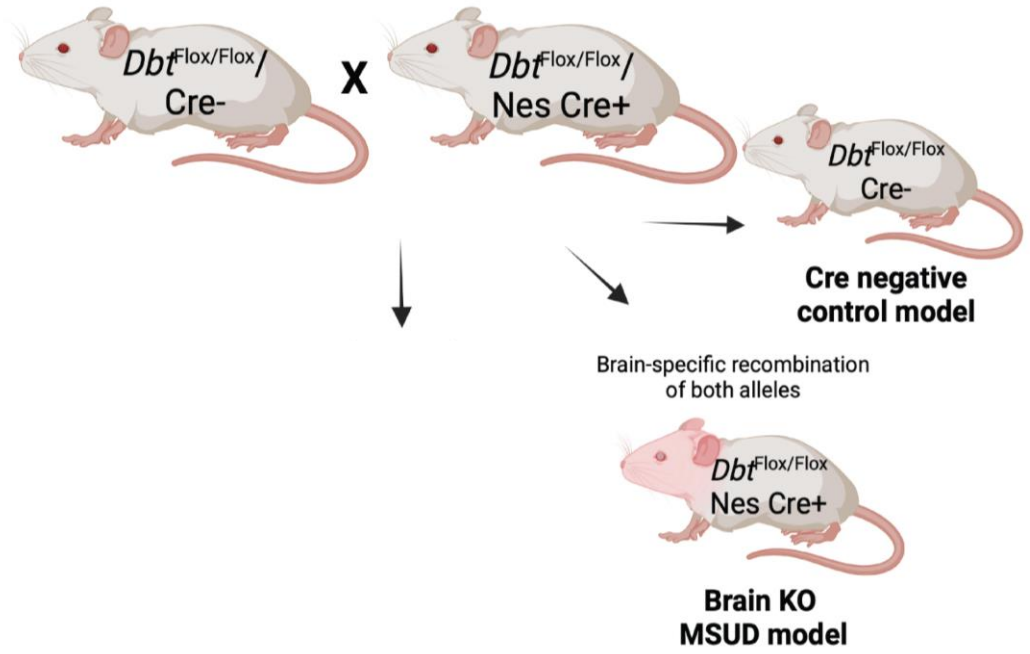
How does impaired BCAA metabolism in brain disrupt neuronal function?

Developing tools to answer this question



Developing a brain-specific *Dbt* knockout model





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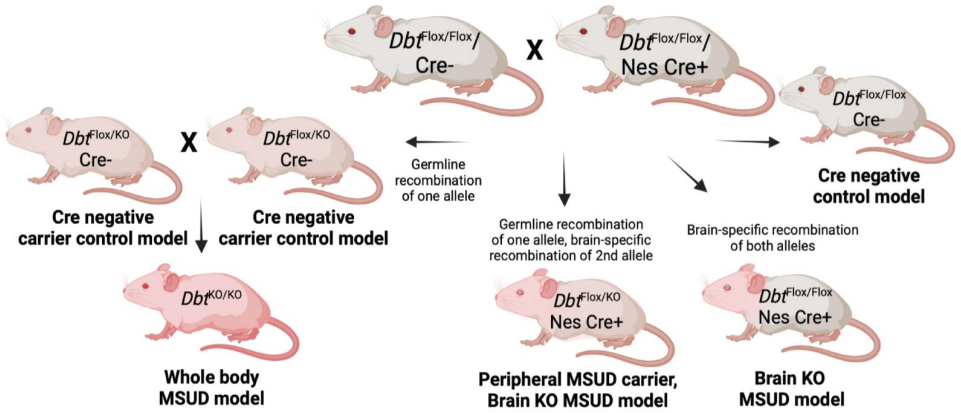
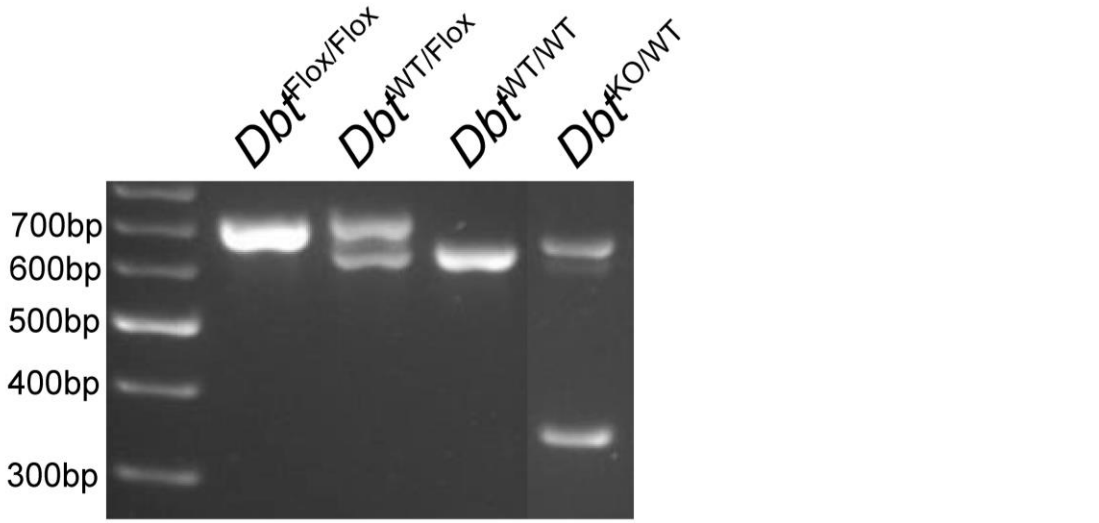
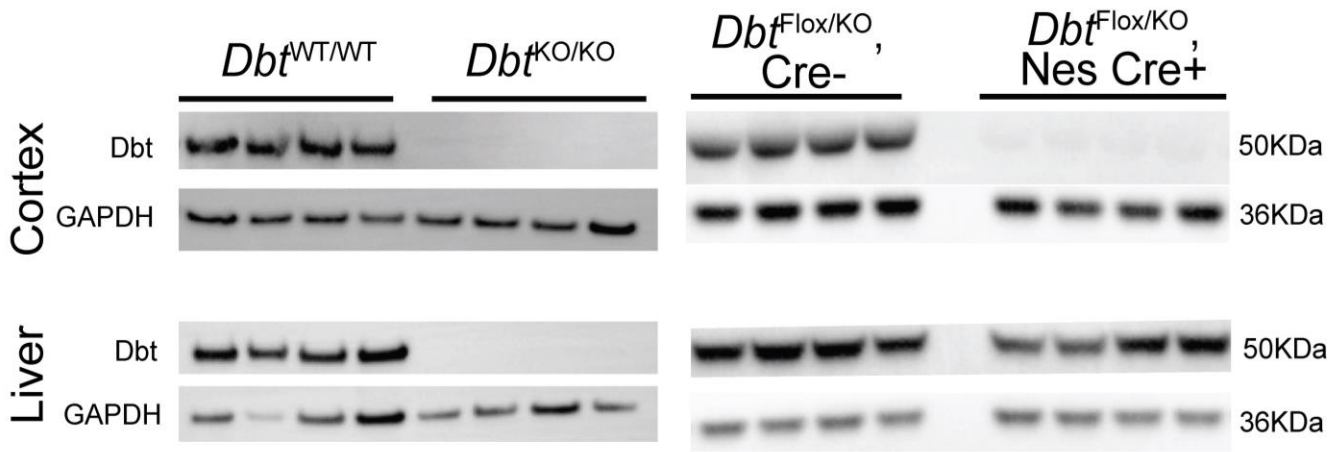
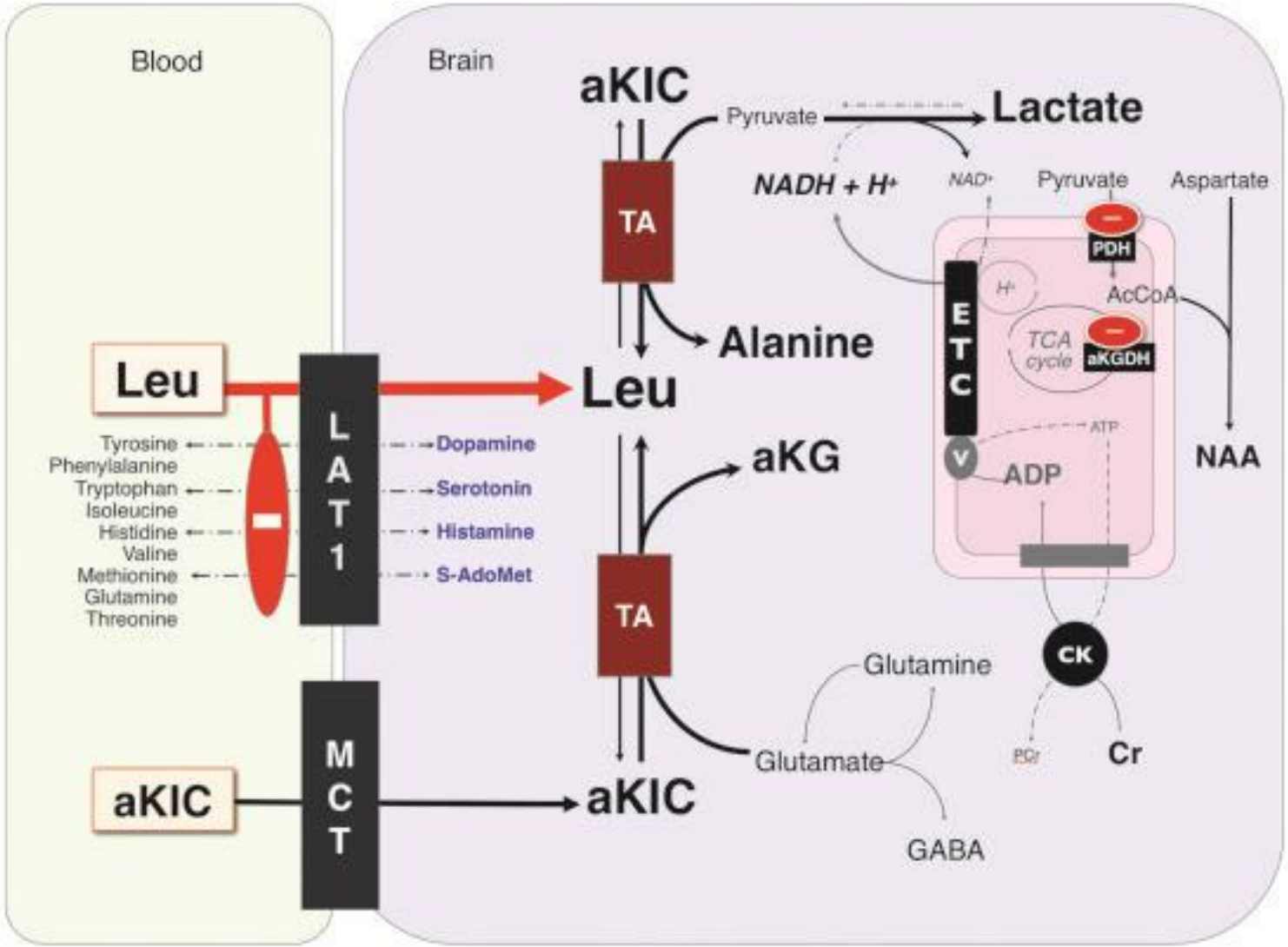




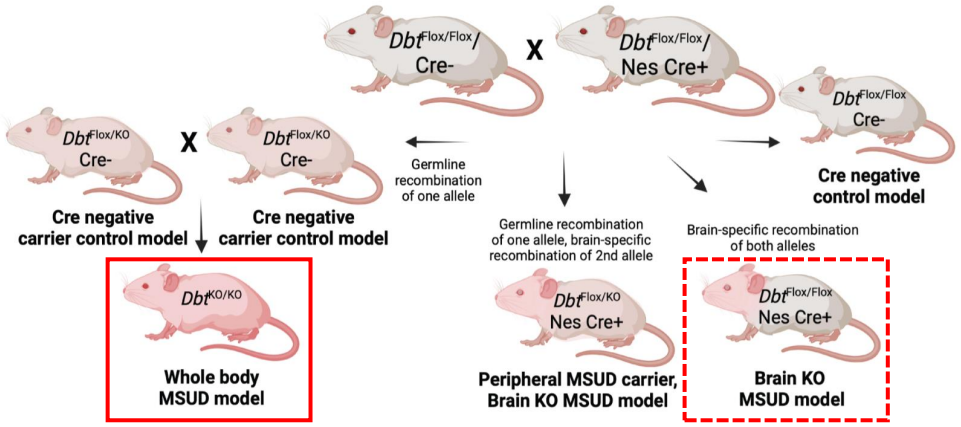


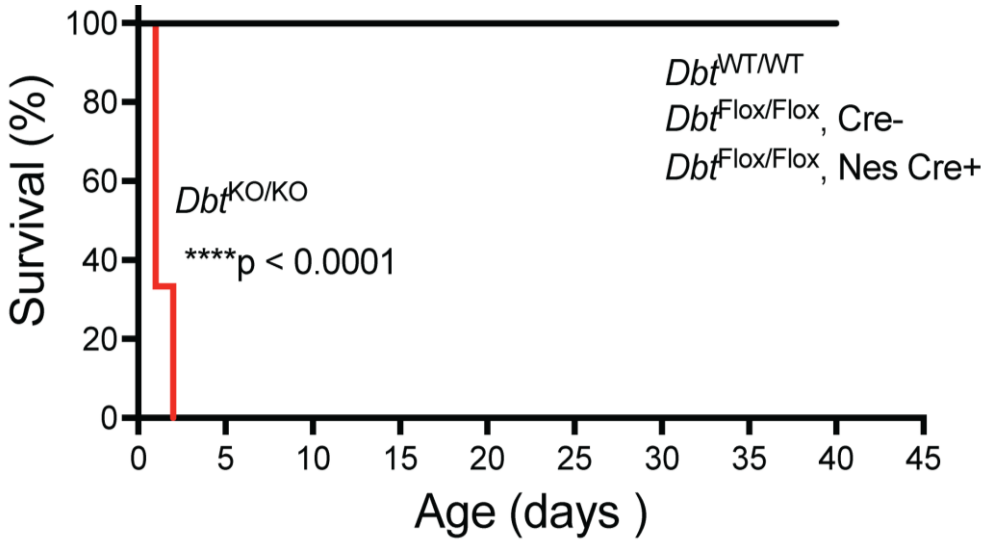
Proposed mechanisms of neuropsychiatric disease in MSUD

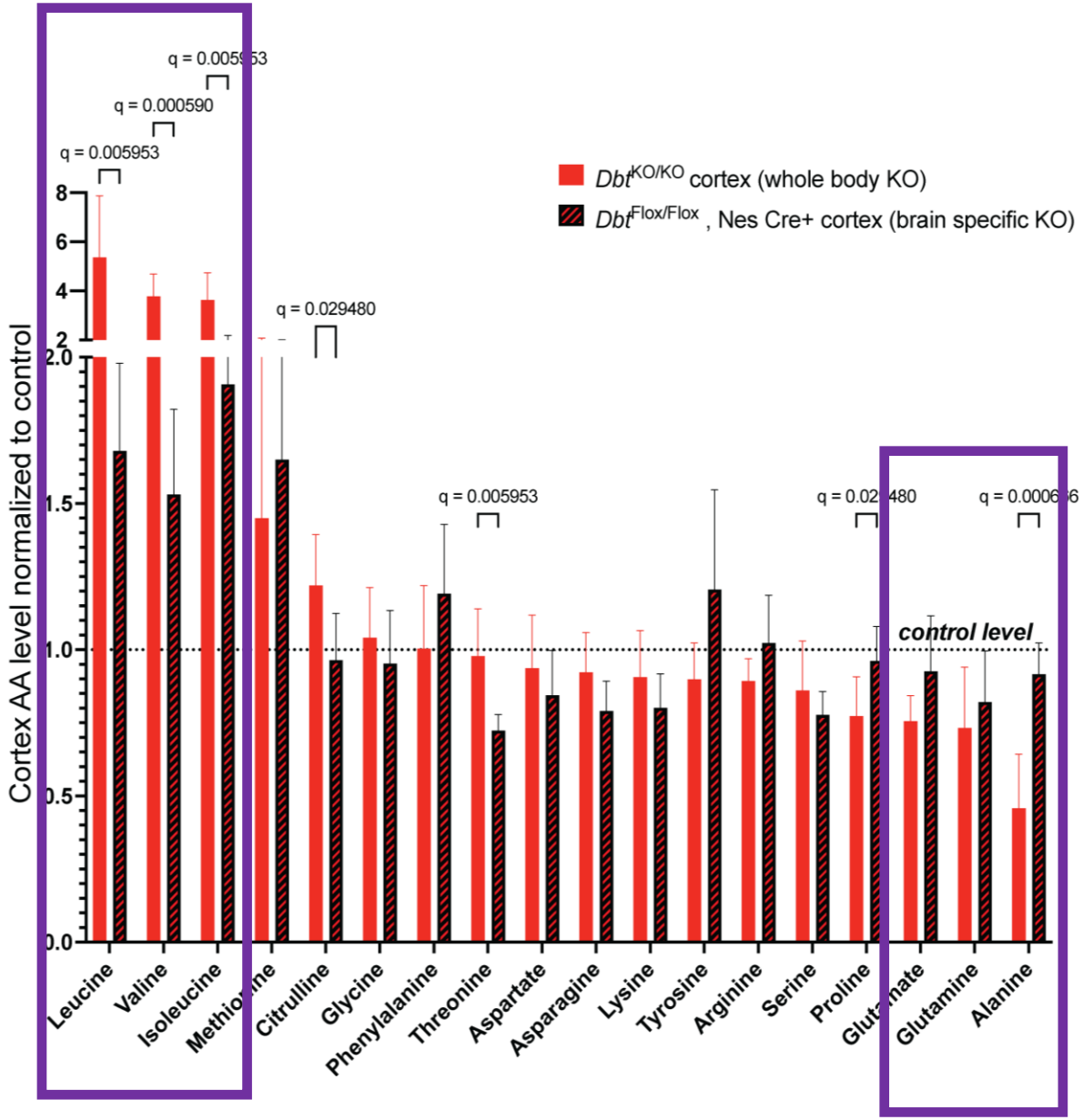


Muelly et al. *J Clin Invest.* 2013;123(4):1809-1820.

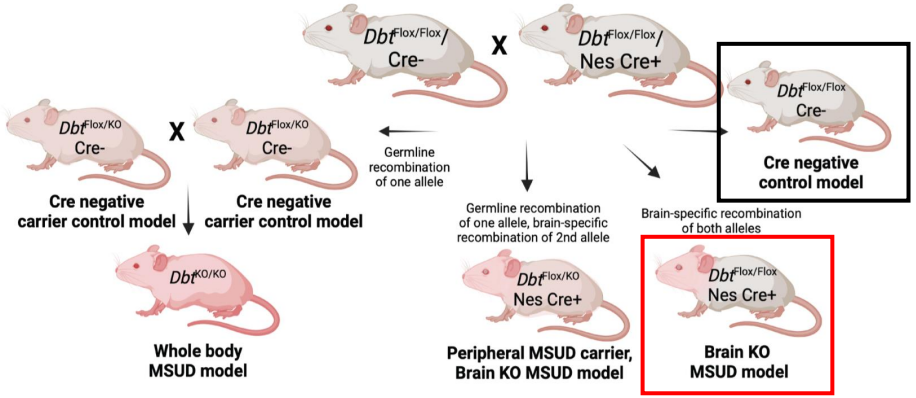
Loss of *Dbt* expression in brain increases branched chain amino acid levels in cortex

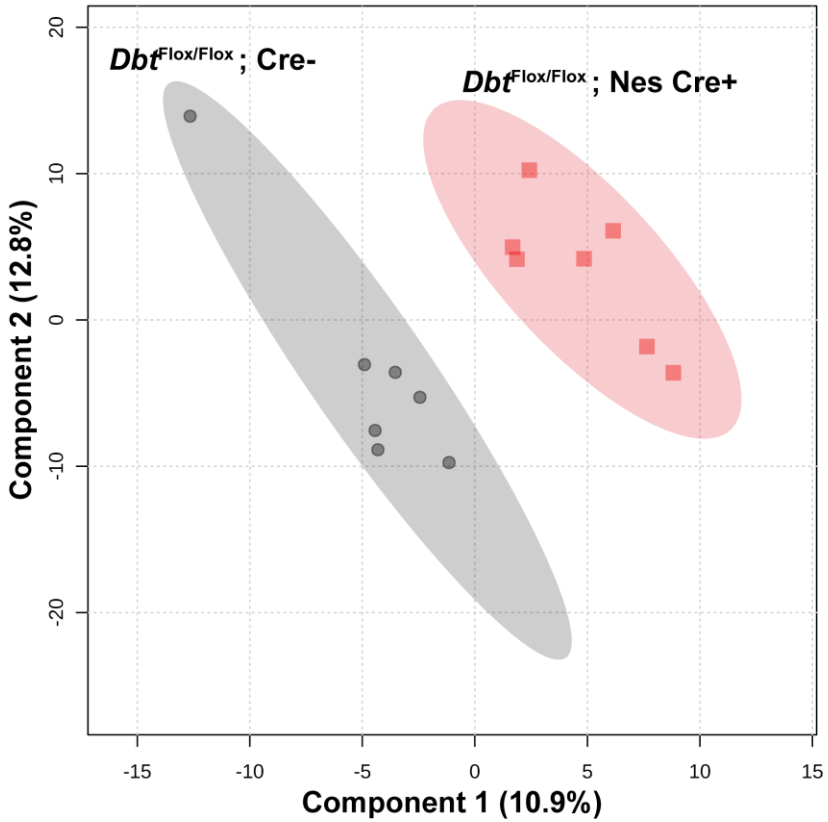


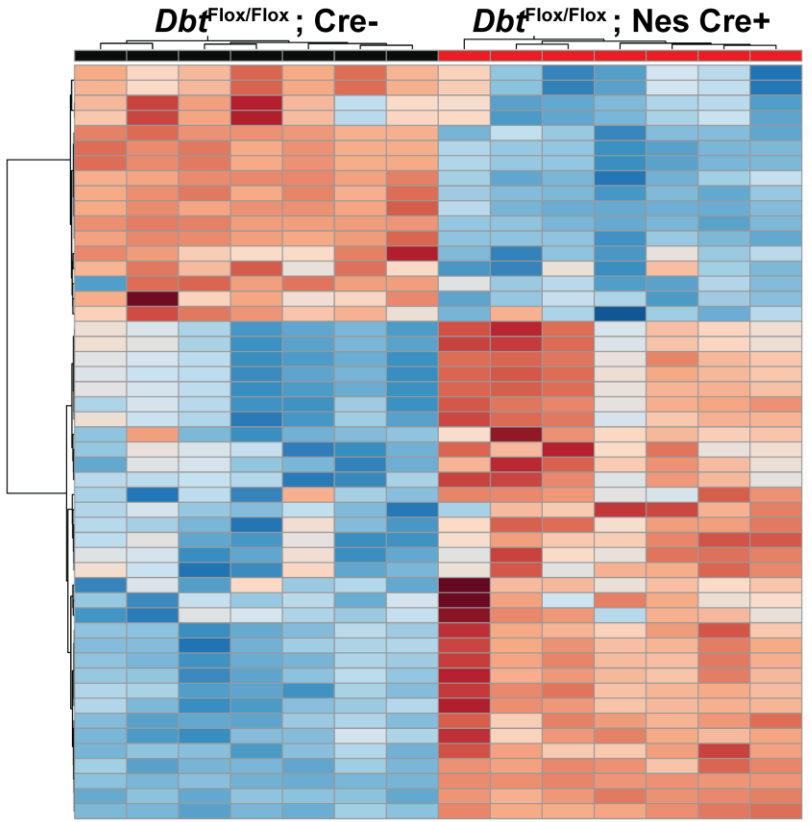




Loss of *Dbt* expression in brain disrupts untargeted metabolomic profiles in cortex







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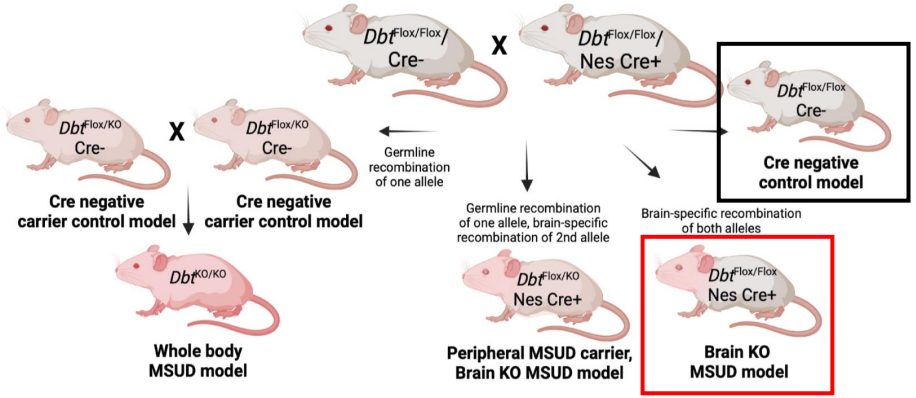
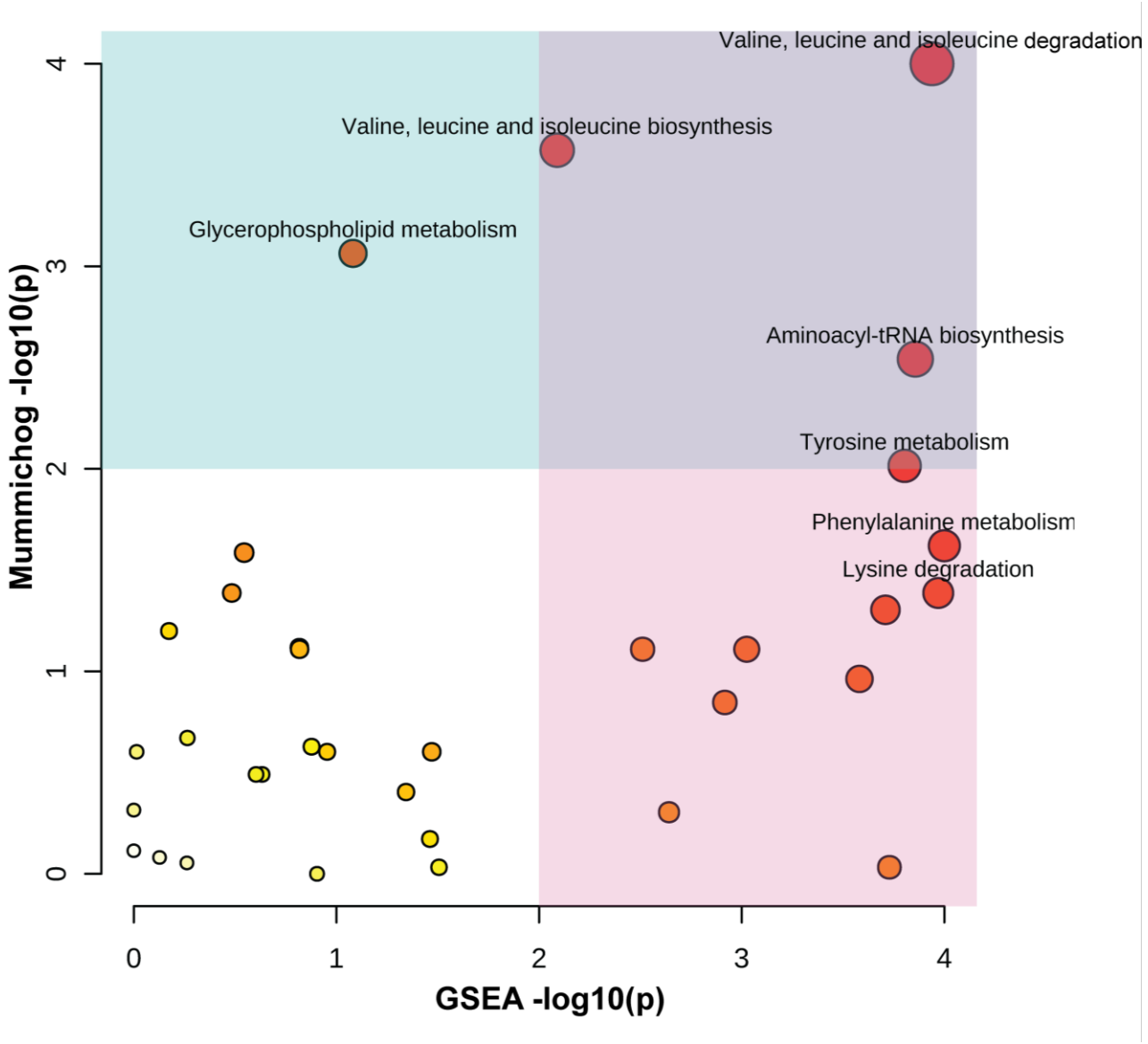
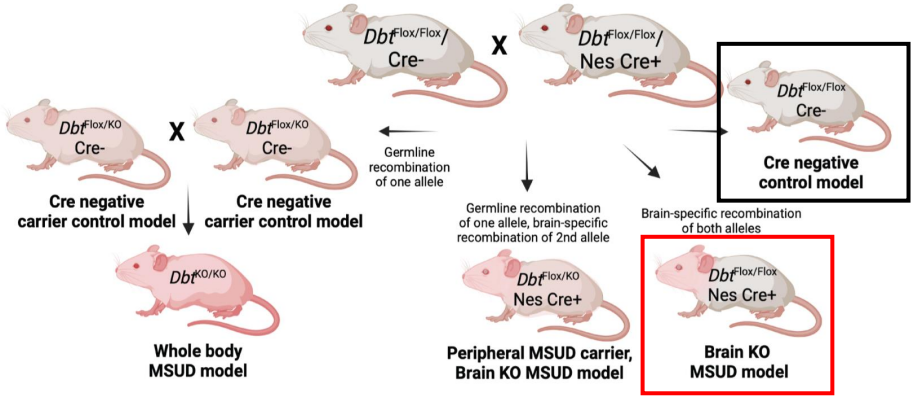
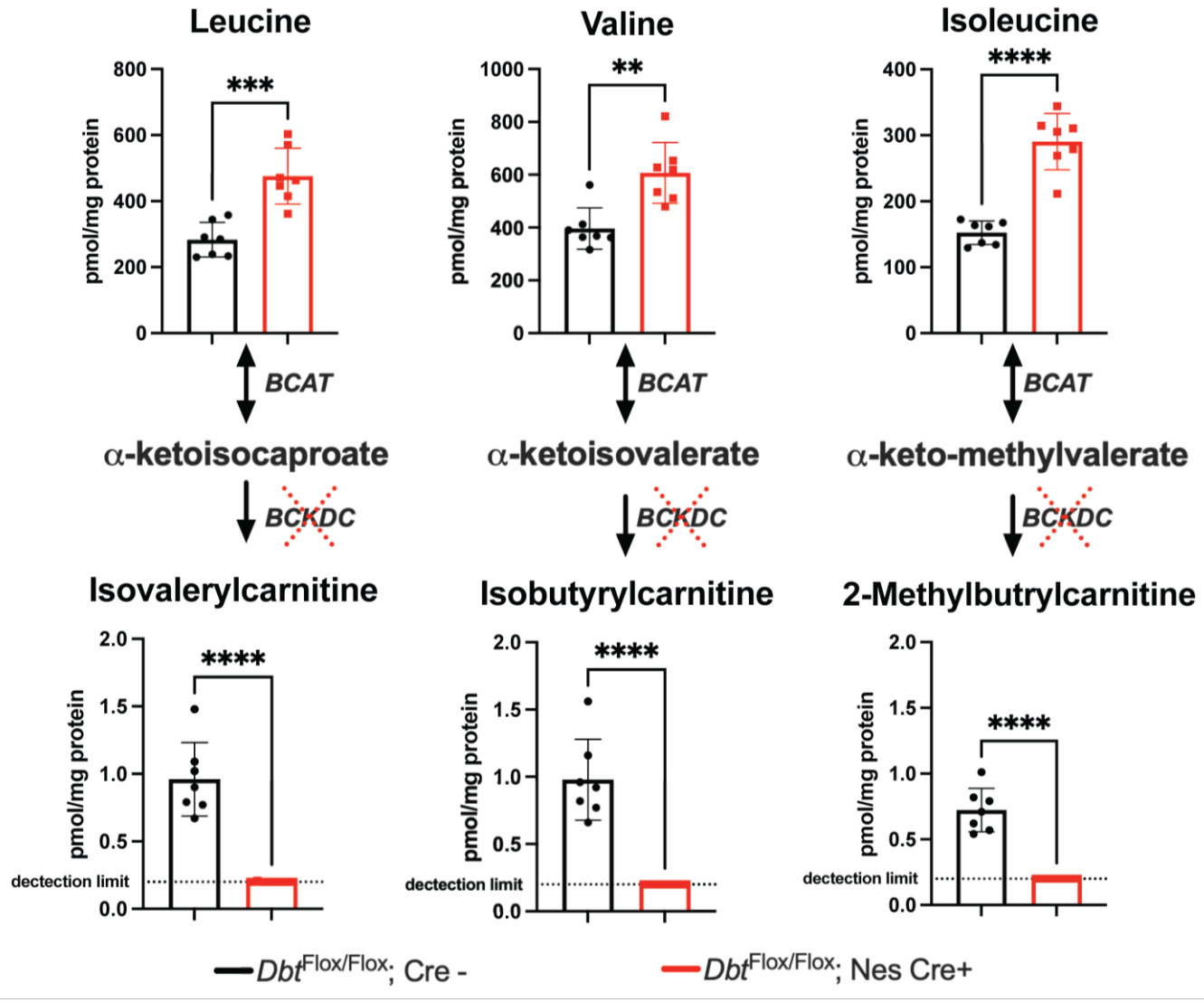


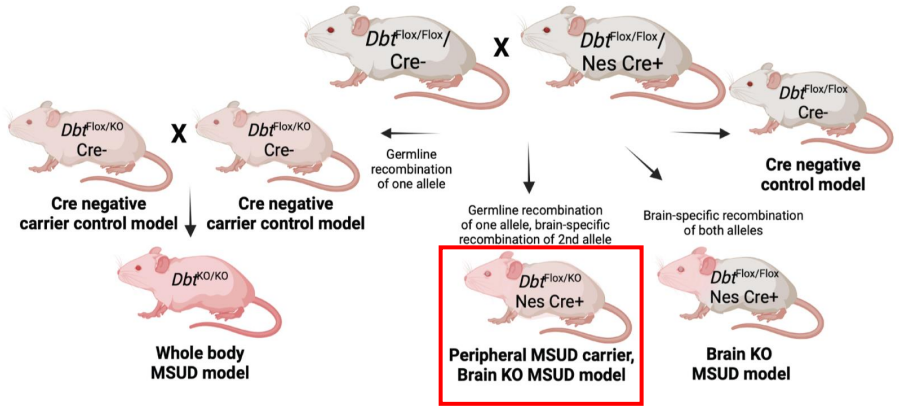


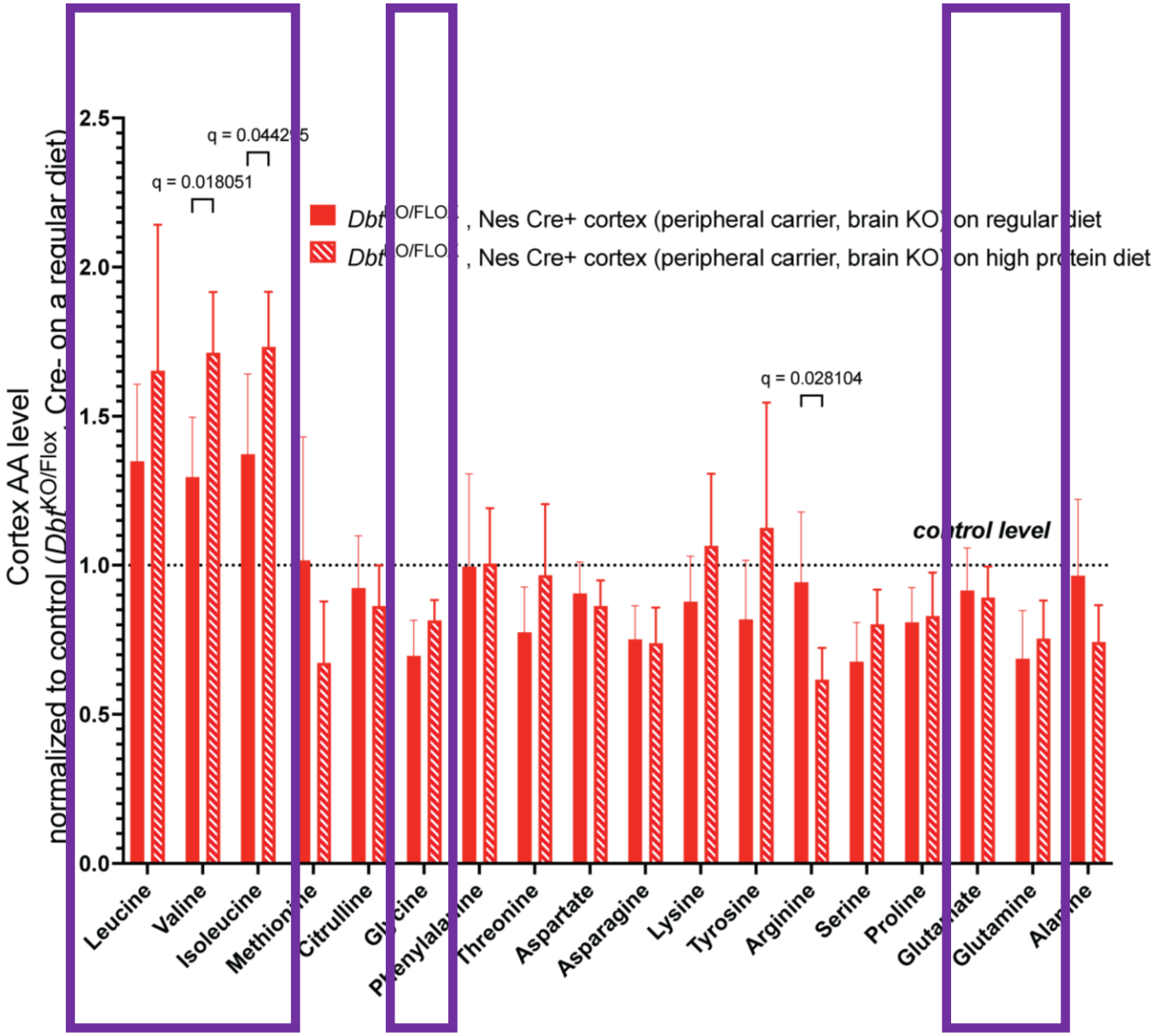
Loss of *Dbt* expression in brain reduces acylcarnitine species downstream of BCKDH





A high protein diet exacerbates metabolic abnormalities in the cortex of peripheral carrier, brain knockout MSUD mice





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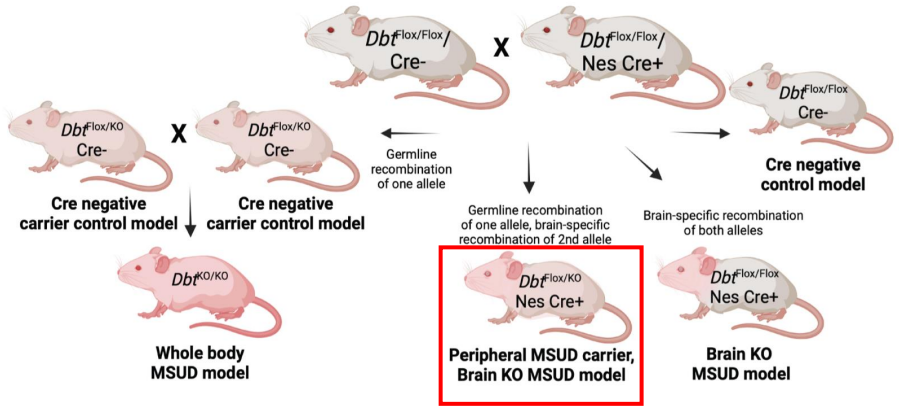
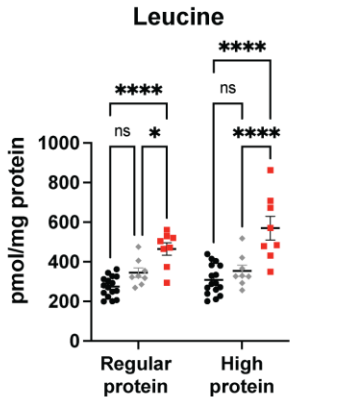
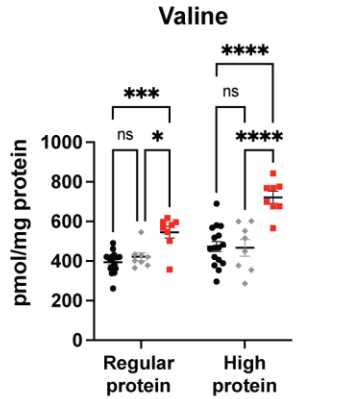
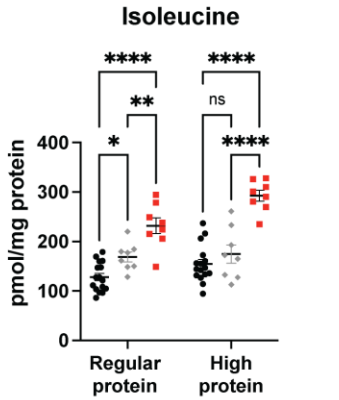




Source of Variation	% of total variation	P value
Interaction	1.911	0.2877
Diet	3.315	0.0400
Genotype	51.42	<0.0001



Source of Variation	% of total variation	P value
Interaction	3.532	0.0825
Diet	13.65	<0.0001
Genotype	43.52	<0.0001



Source of Variation	% of total variation	P value
Interaction	2.423	0.1127
Diet	5.404	0.0024
Genotype	61.03	<0.0001

BCAT

α-ketoisocaproate

BCKDC

BCAT

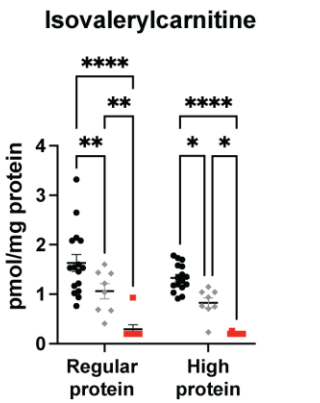
α-ketoisovalerate

BCKDC

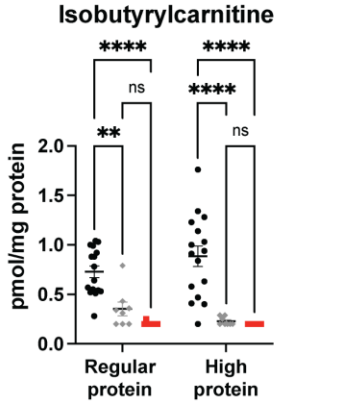
BCAT

α-keto-methylvalerate

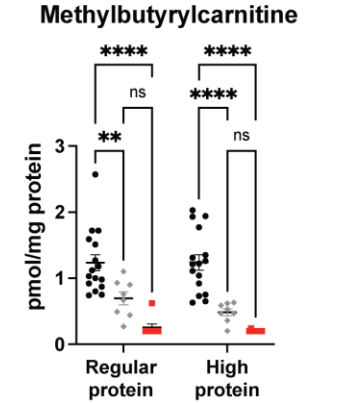
BCKDC



Source of Variation	% of total variation	P value
Interaction	0.4469	0.7093
Diet	2.193	0.0707
Genotype	59.01	<0.0001



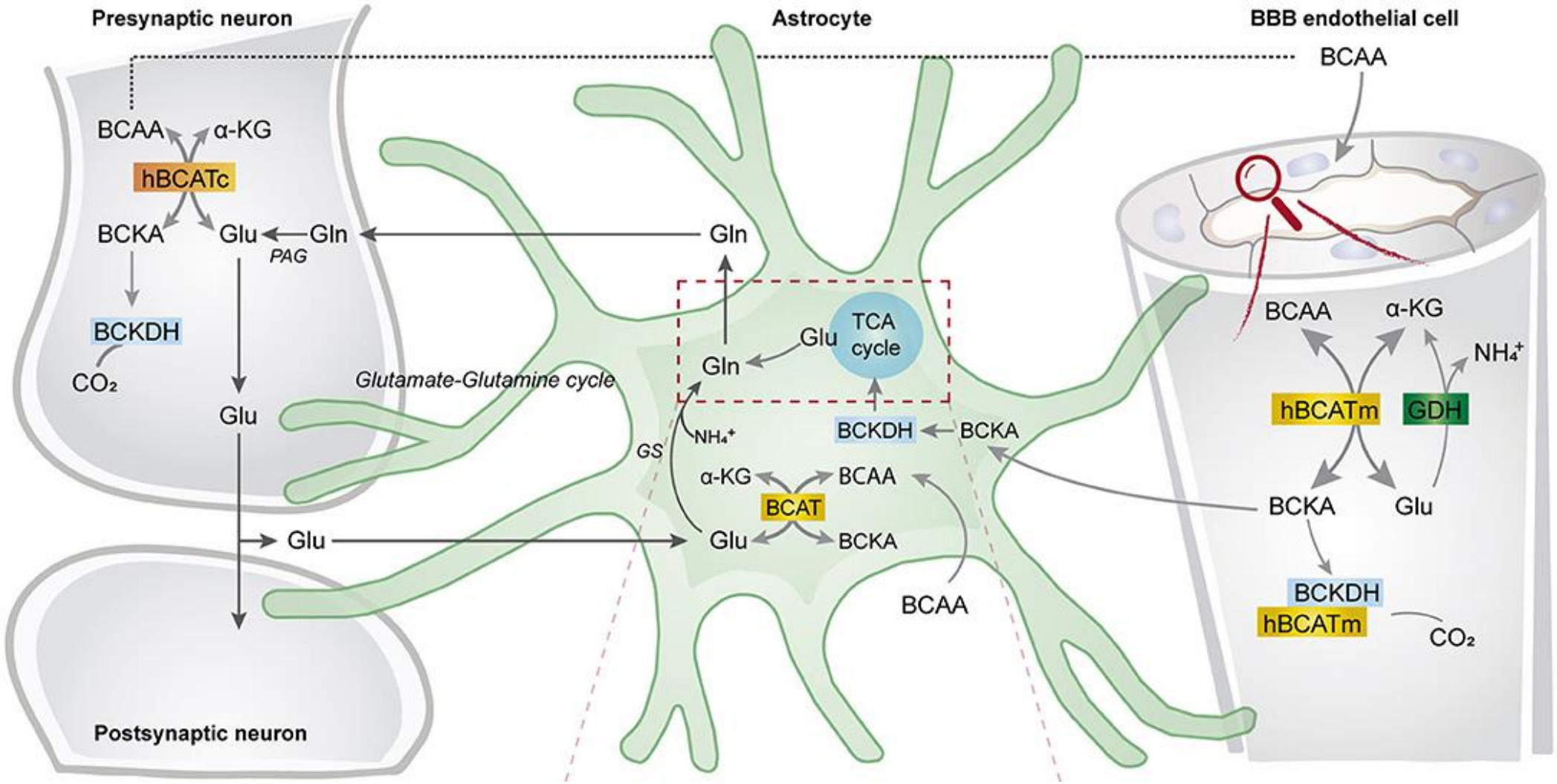
Source of Variation	% of total variation	P value
Interaction	2.480	0.1839
Diet	0.01102	0.9014
Genotype	55.91	<0.0001



Source of Variation	% of total variation	P value
Interaction	0.6327	0.6236
Diet	0.5180	0.3809
Genotype	60.52	<0.0001

● $Dbt^{Flox/Flox}$, Cre- ◆ $Dbt^{KO/Flox}$, Cre- ■ $Dbt^{KO/Flox}$, Nes-Cre+

What happens when there is loss of *Dbt* expression in either neurons or astrocytes alone?



Salcedo C, et al. Front. Aging Neurosci. (2021) 13:736580.

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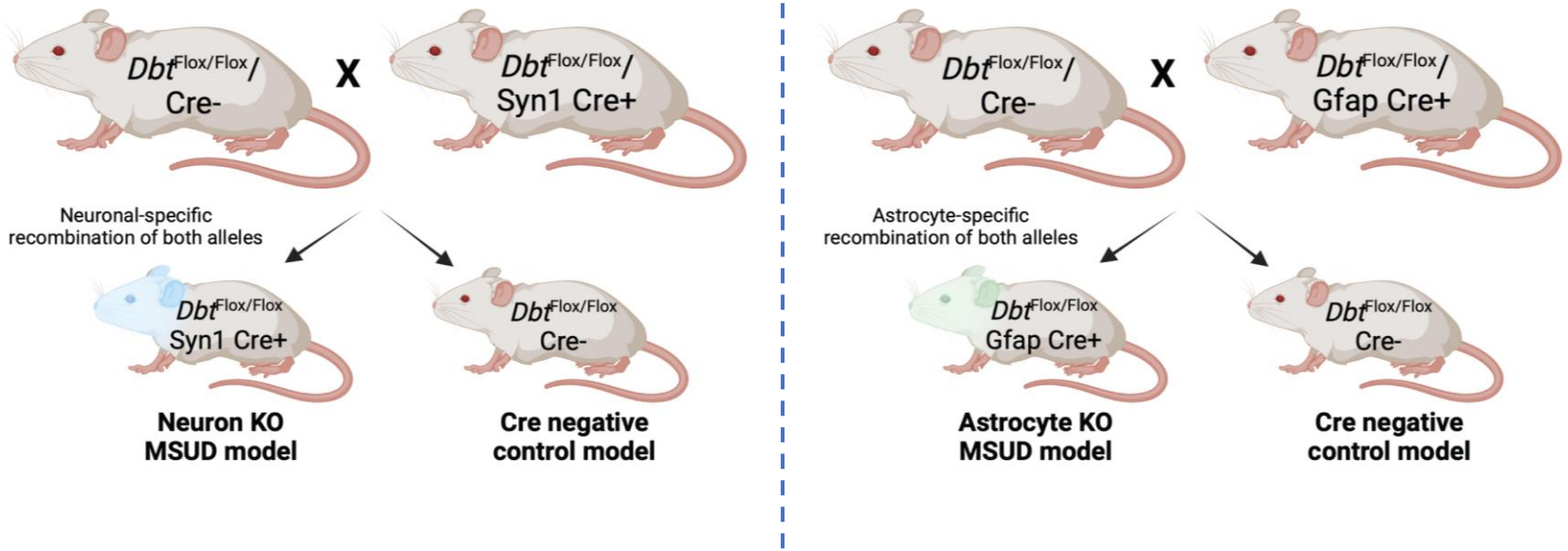

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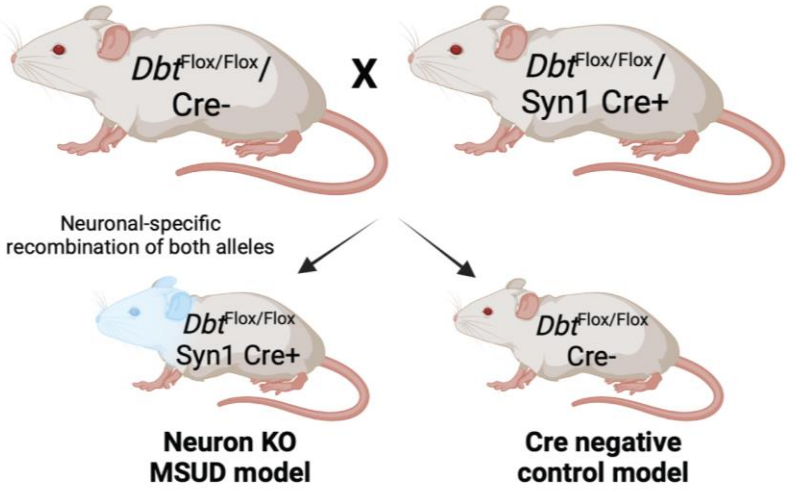
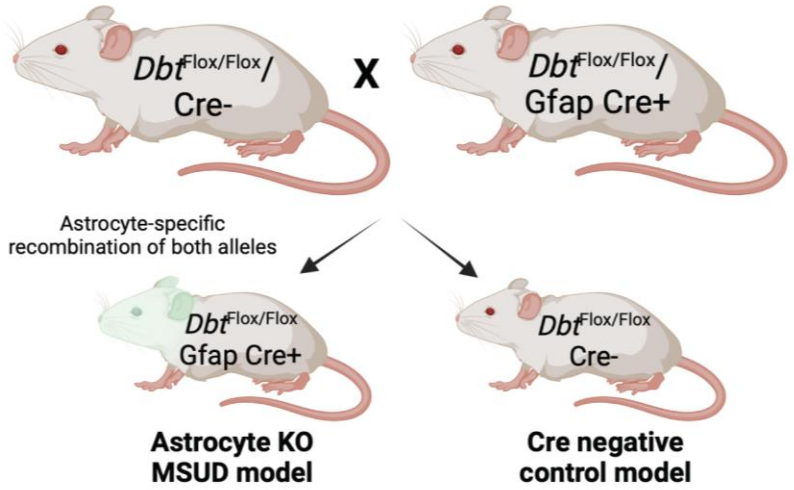
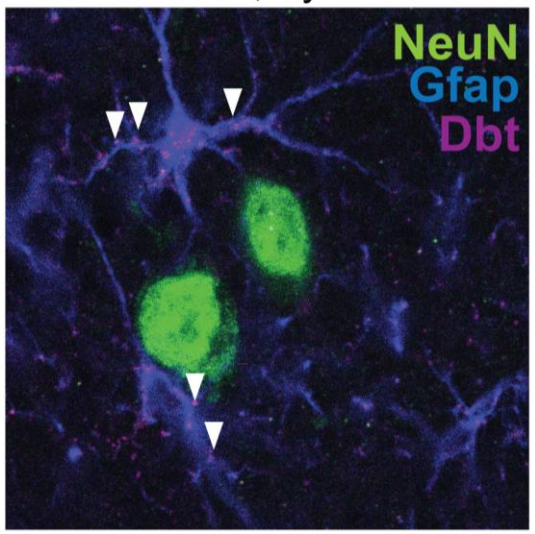
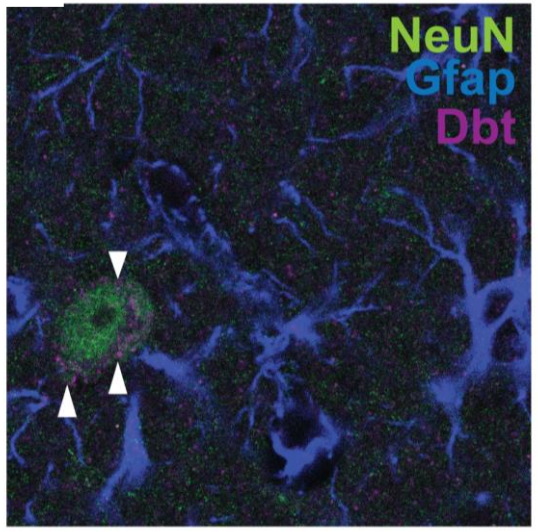




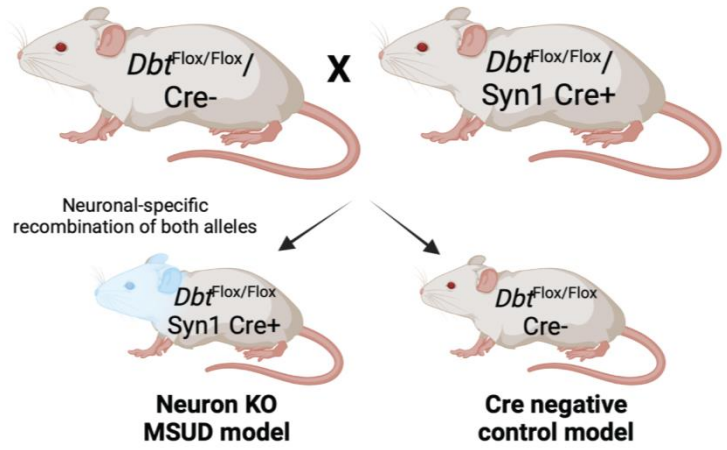
Neuron knockout
Dbt$^{Flox/Flox}$, Syn1 Cre+

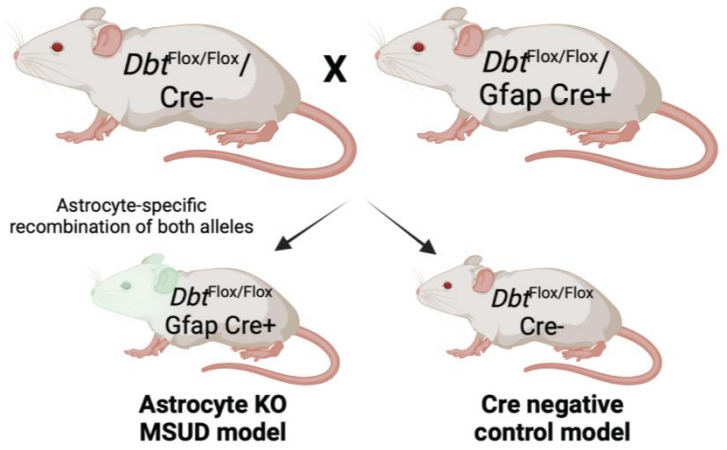


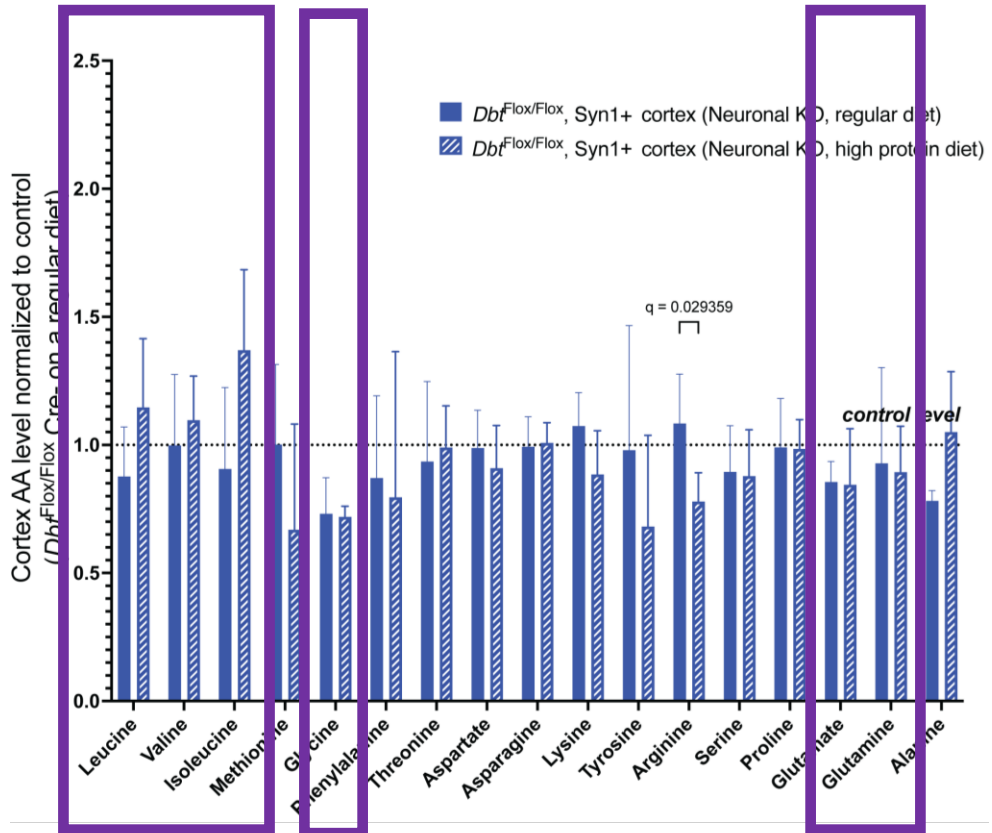
Astrocyte knockout
Dbt$^{Flox/Flox}$, Gfap Cre+

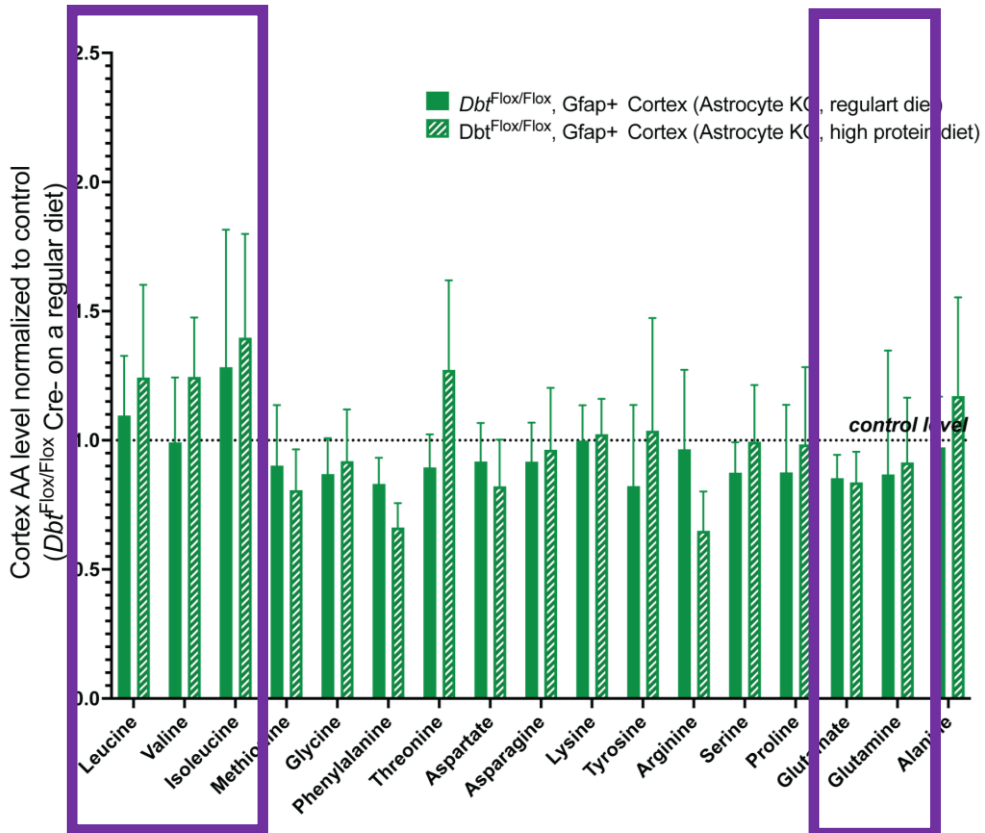


Loss of *Dbt* expression in either neurons or astrocytes alone has only a modest affect on whole brain amino acid levels

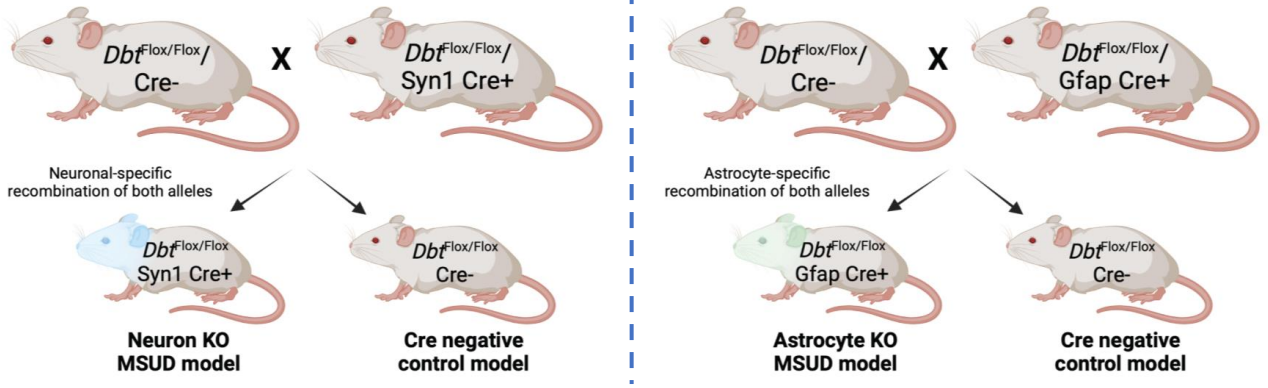


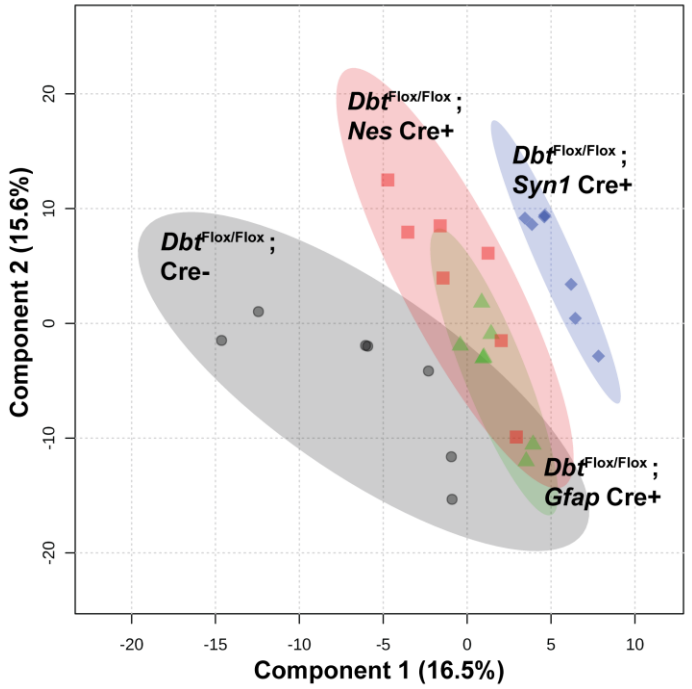


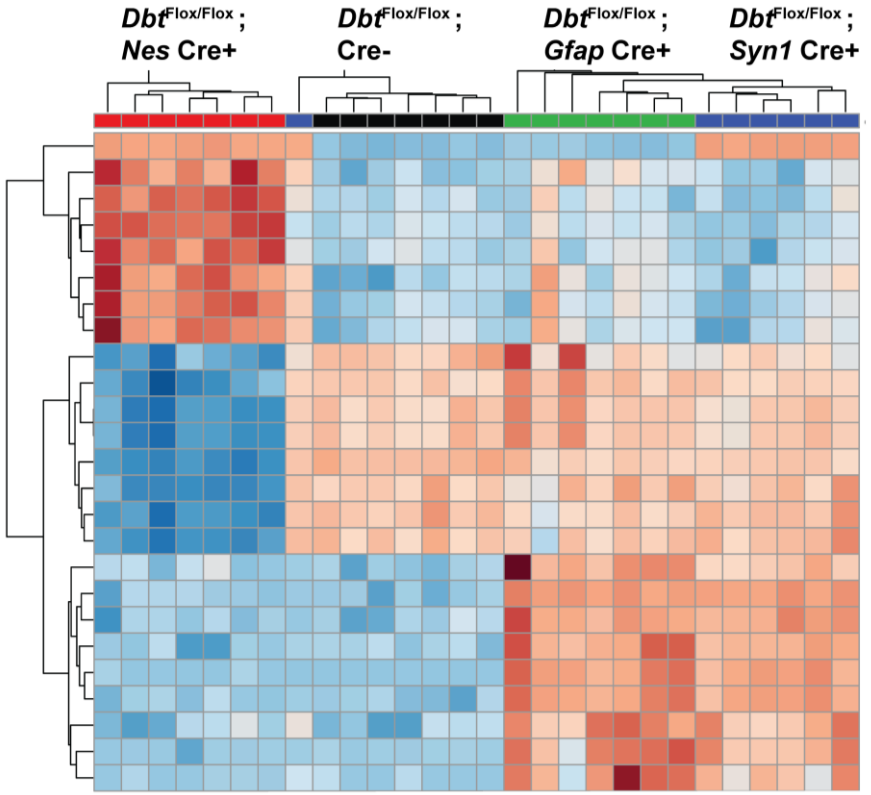




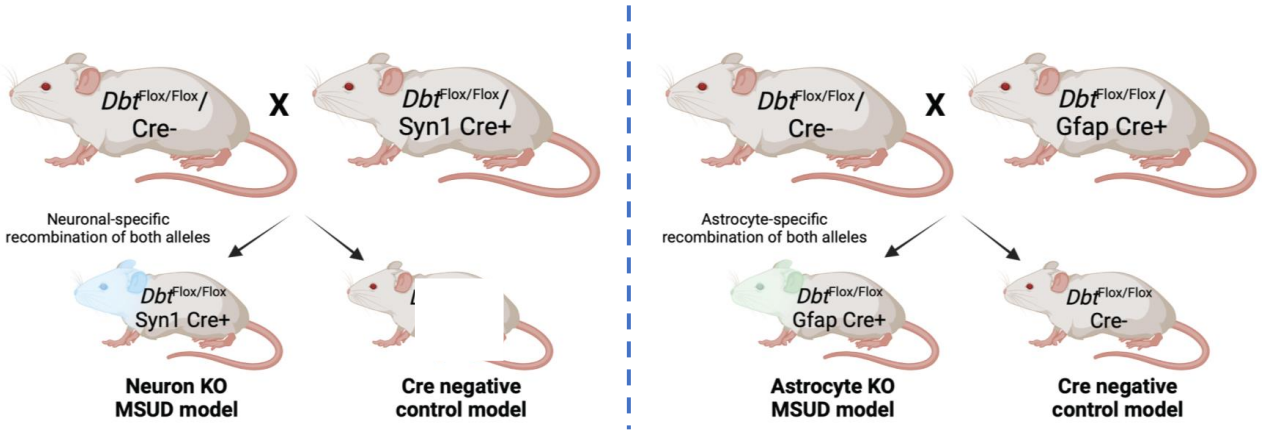
Loss of *Dbt* in either neurons or astrocytes alone alters global metabolic profiles

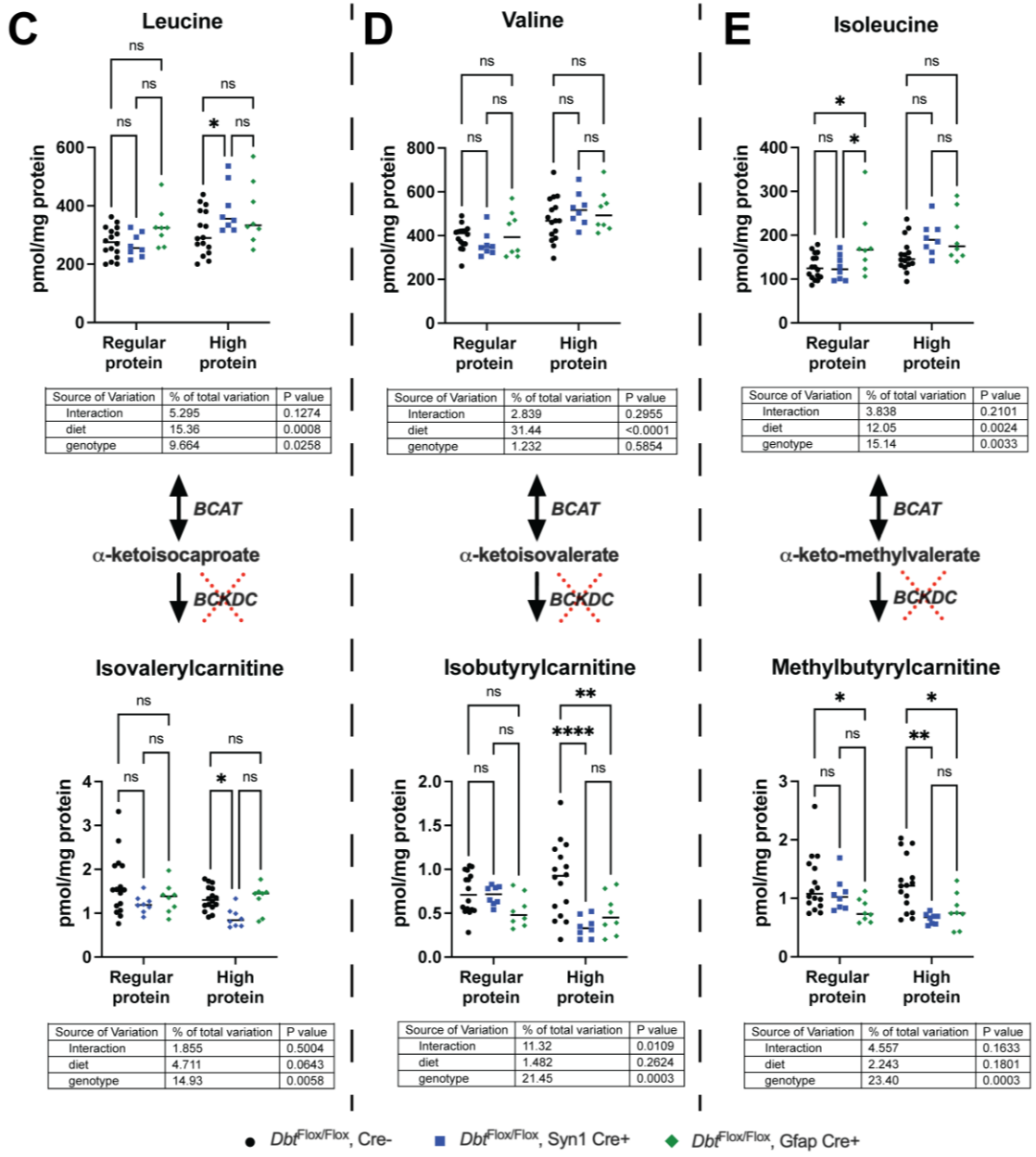




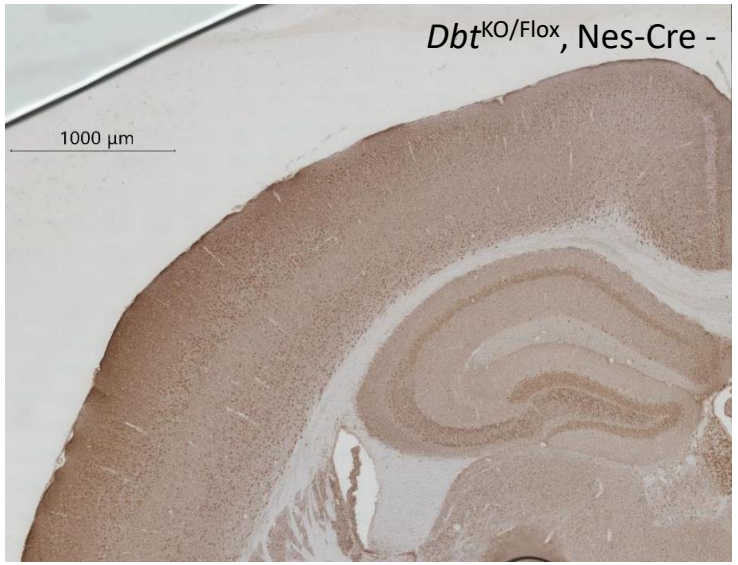


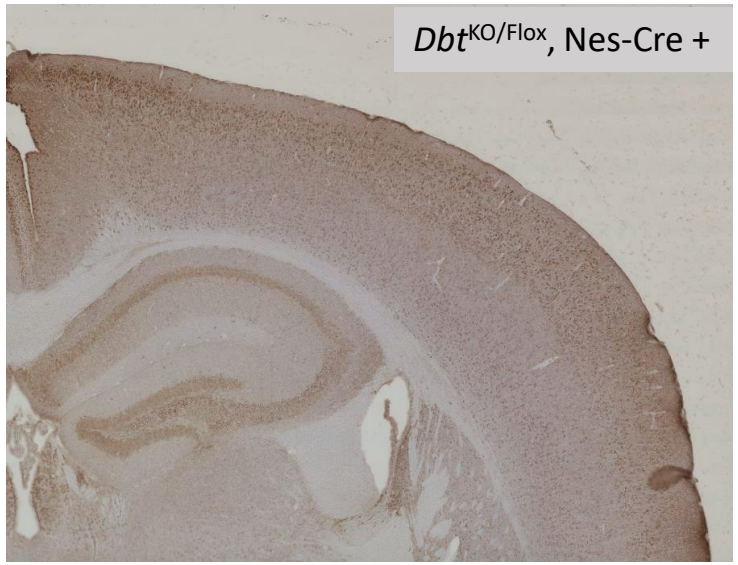
High protein diet in neuronal or astrocyte MSUD models exacerbates BCAA levels in brain

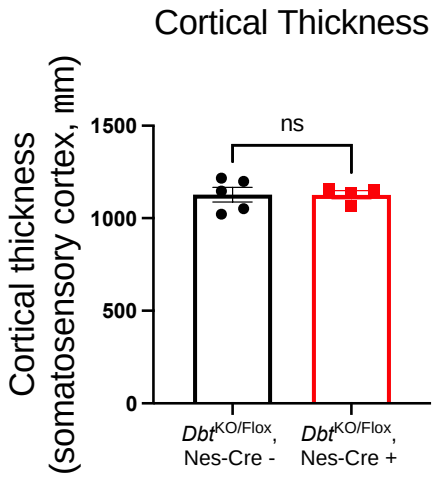


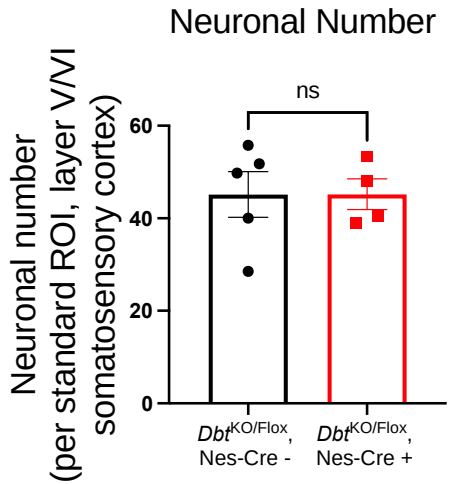


Peripheral carrier, brain KO MSUD mice have no significant changes in neuron number



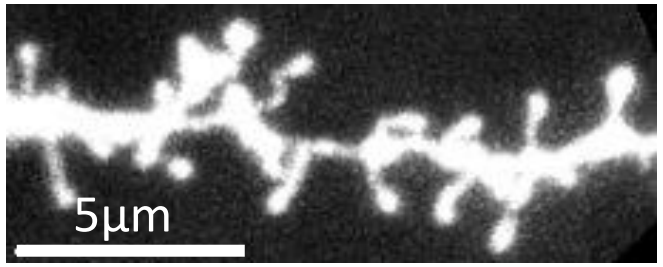




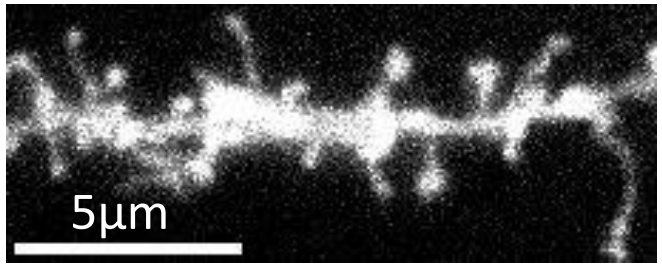


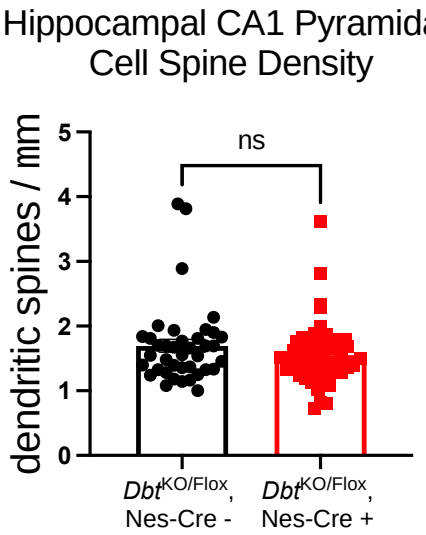
Peripheral carrier, brain KO MSUD mice have normal dendritic spines in the hippocampus

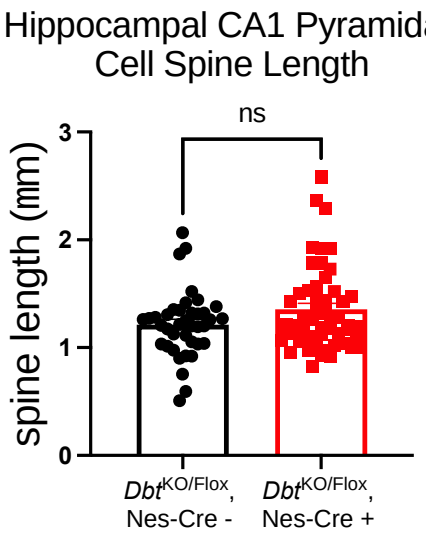
Dbt$^{KO/Flox}$, Nes-Cre -



Dbt$^{KO/Flox}$, Nes-Cre +







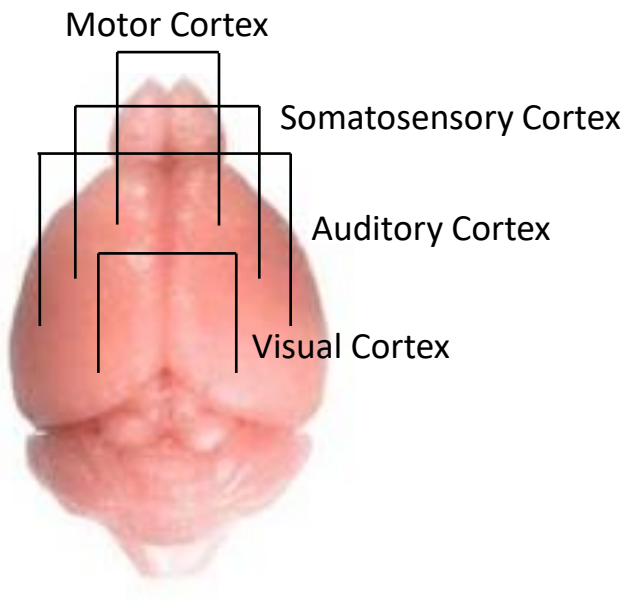
Does the brain-specific MSUD model have abnormalities on EEG?

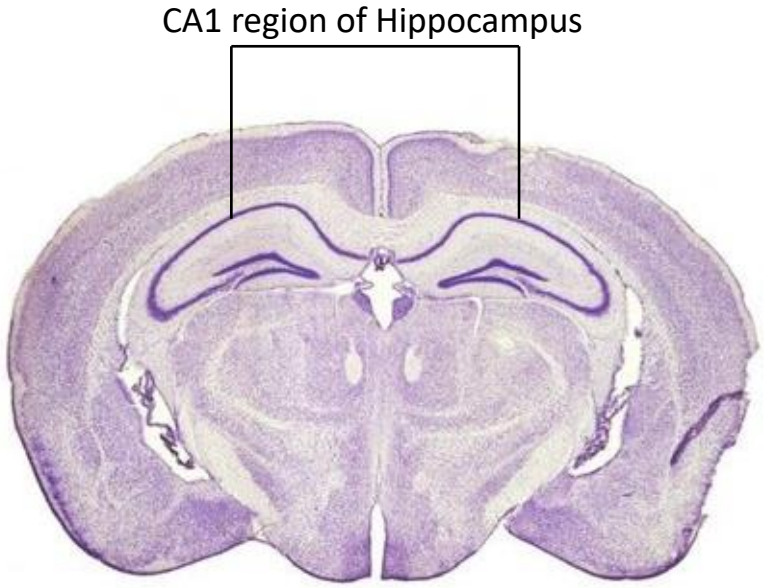
Day 0
Implant electrodes in p60 mice

Day 2
Begin recording on regular (20% protein) diet

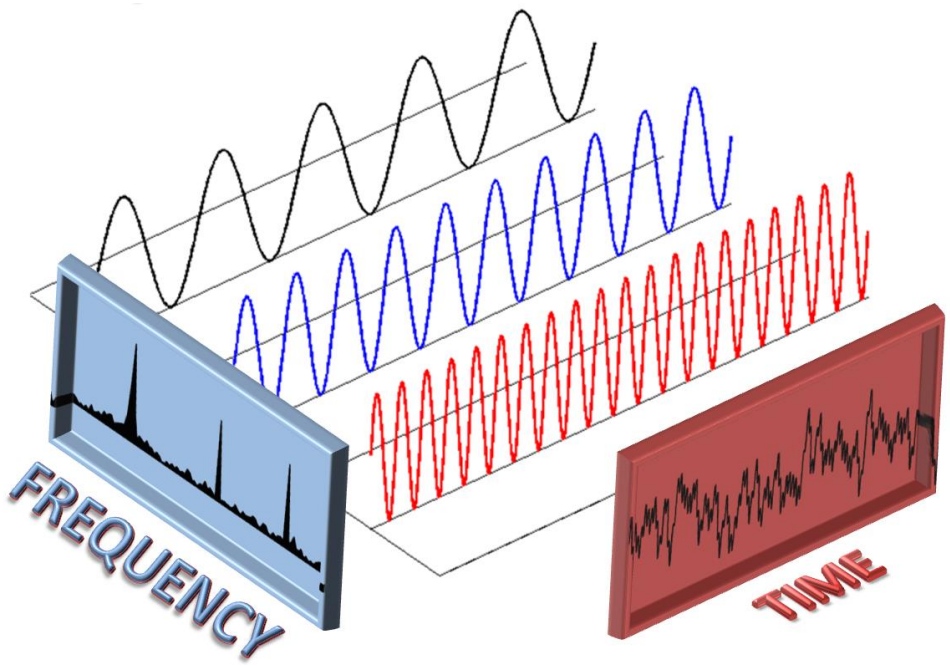
Day 4-5
Transition to 40% protein diet

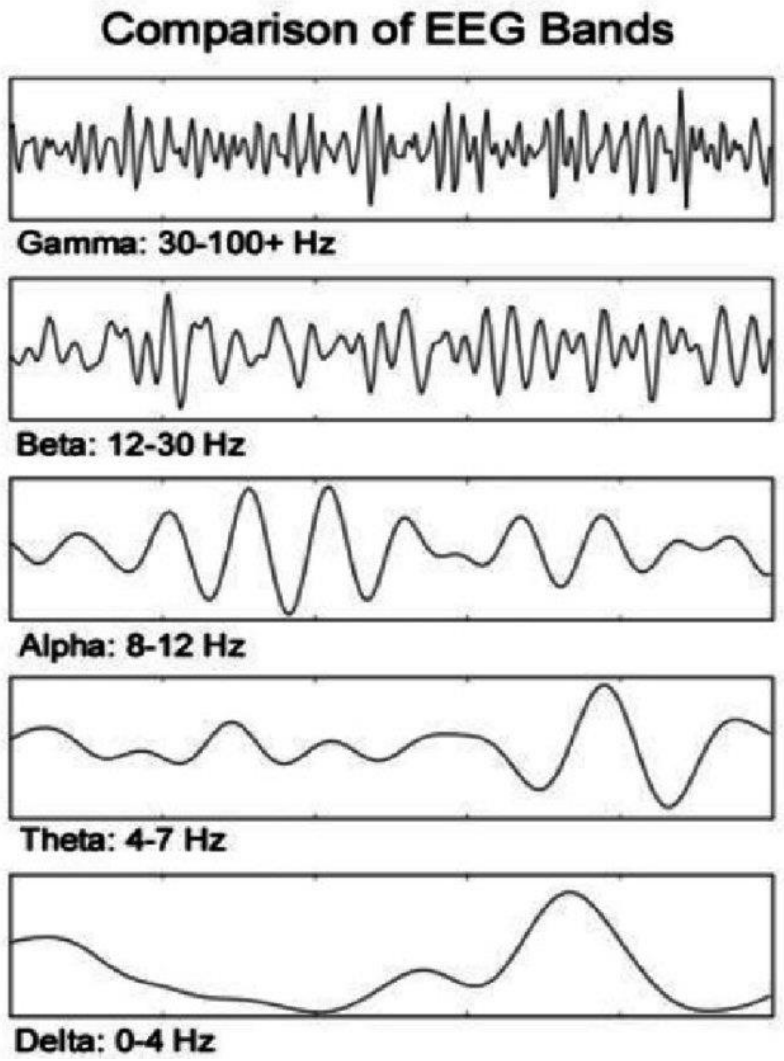
Day 6-7
Collect tissue for histology



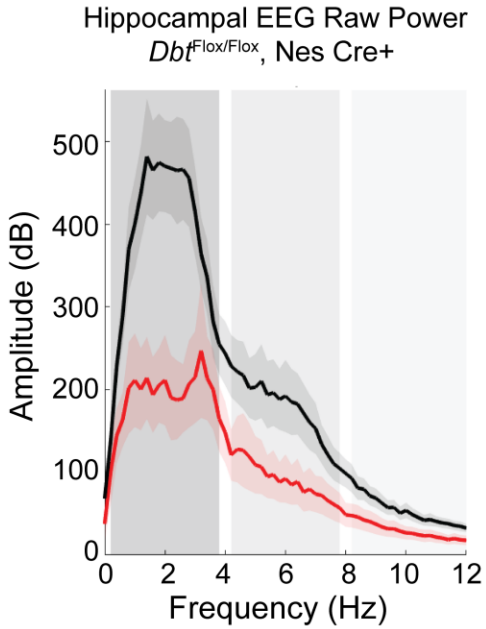


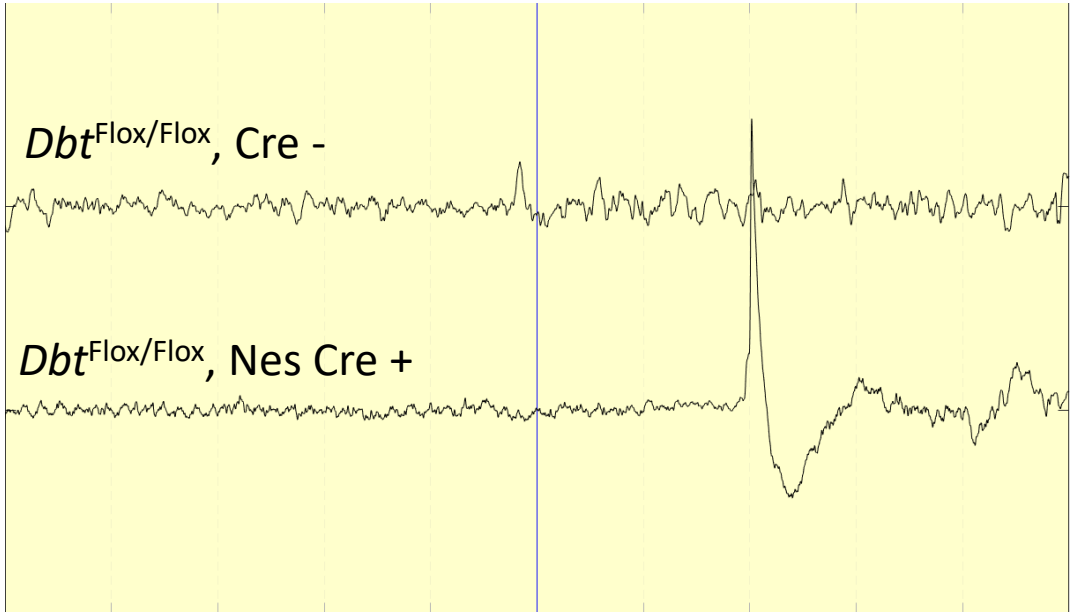
Analyzing background frequency shifts in EEG



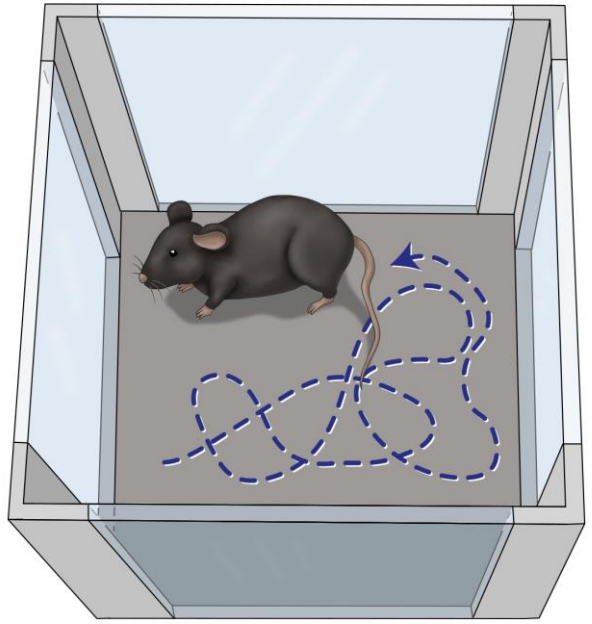


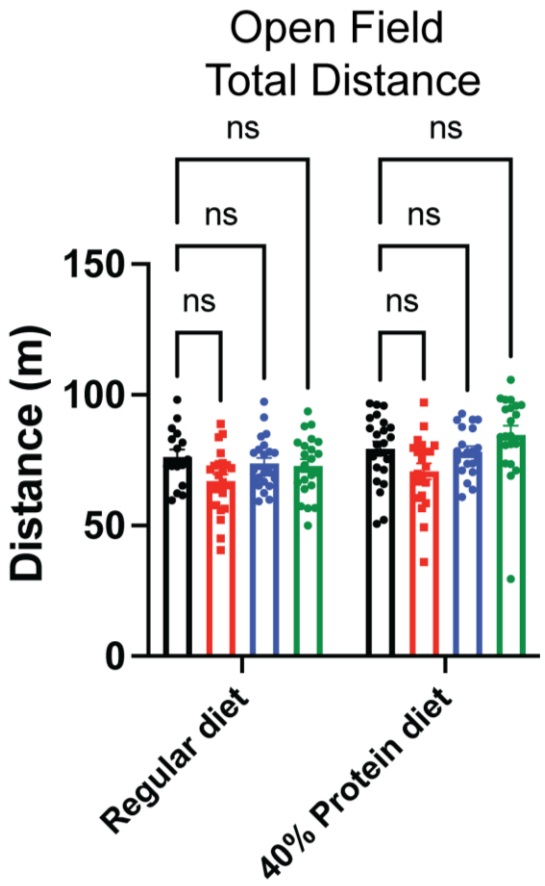
Brain-specific MSUD mice demonstrate abnormal hippocampal activity

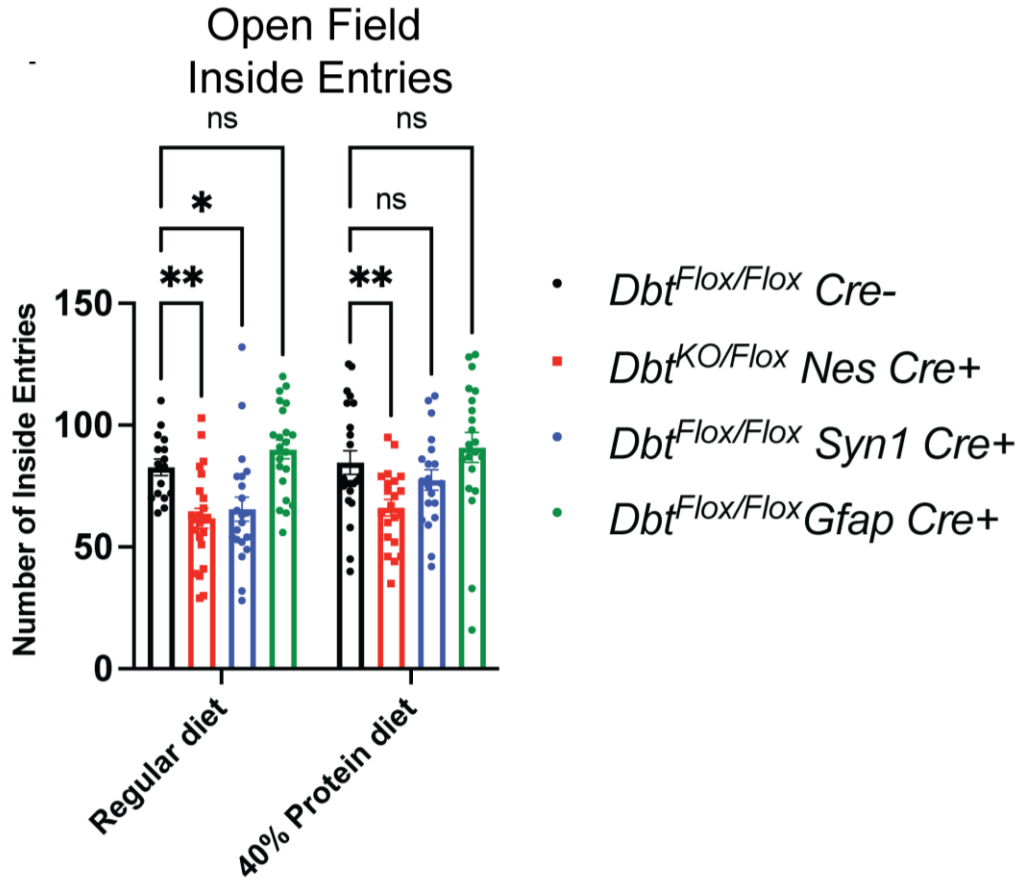




Brain-specific MSUD mice demonstrate subtle anxiety phenotypes on behavioral assays

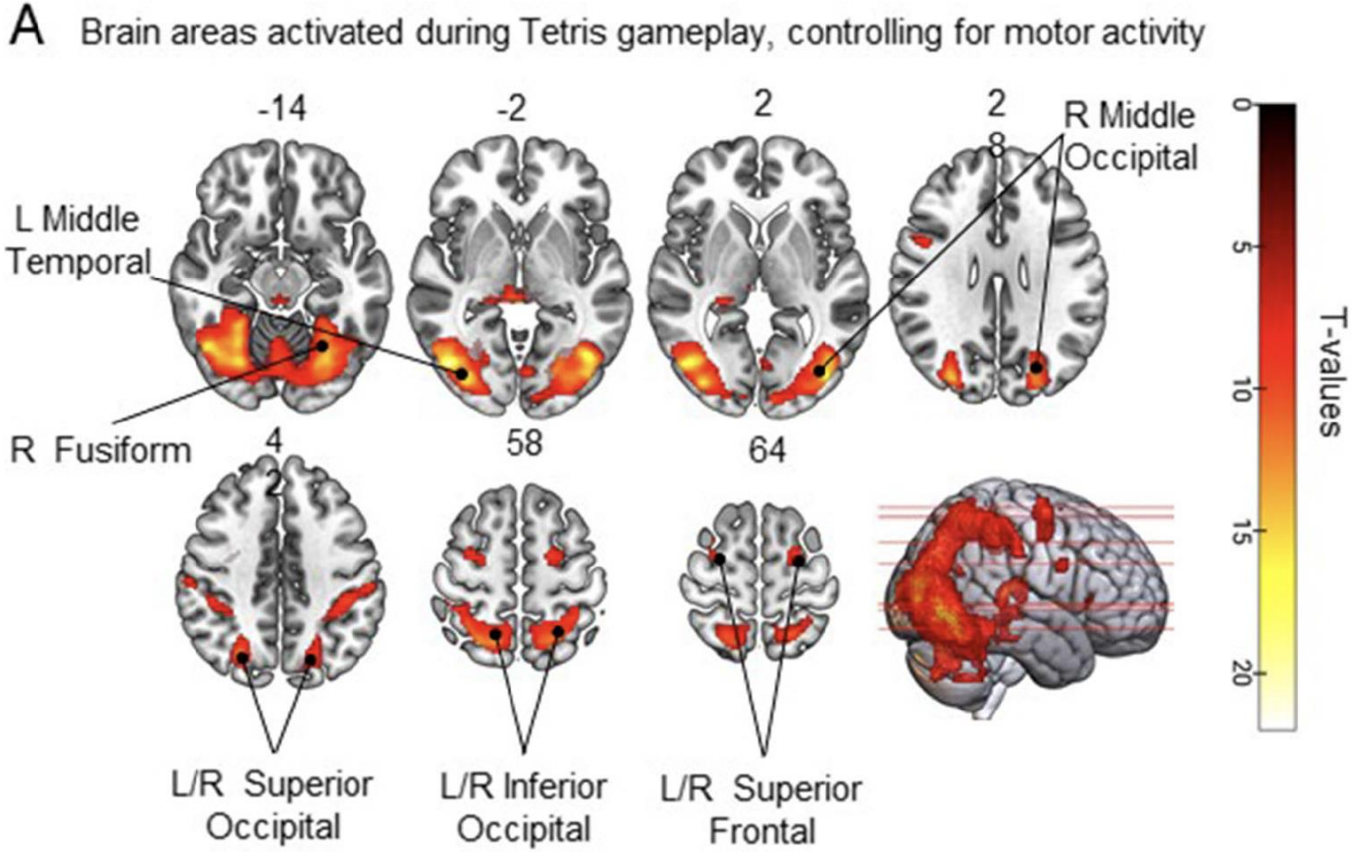






Is there a better marker of neurologic dysfunction in brain-specific MSUD mice?

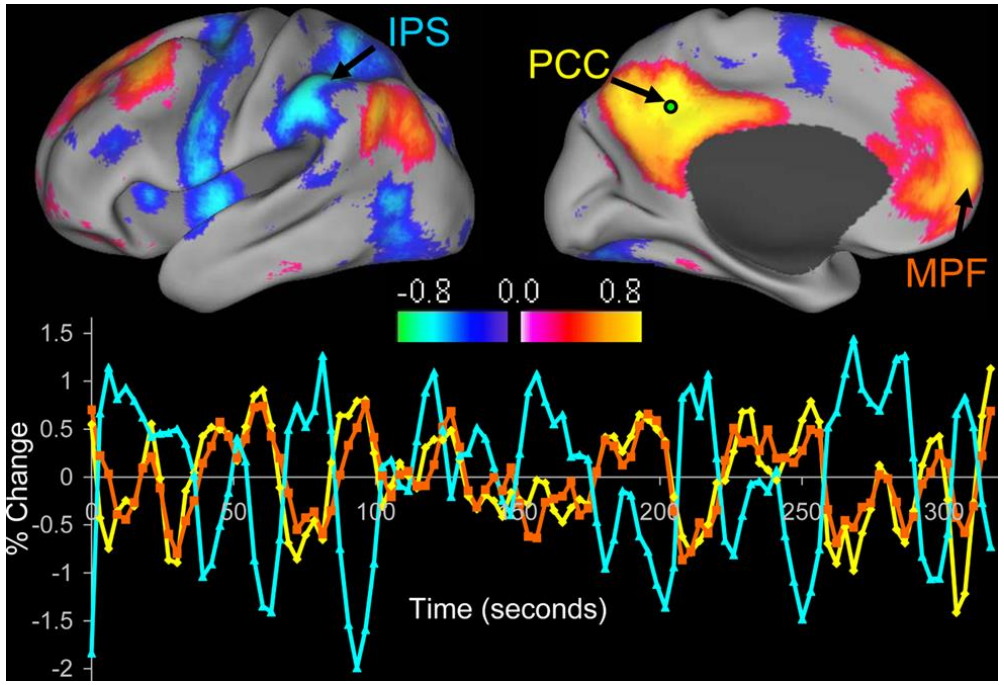
Could functional neuroimaging detect neurologic deficits?

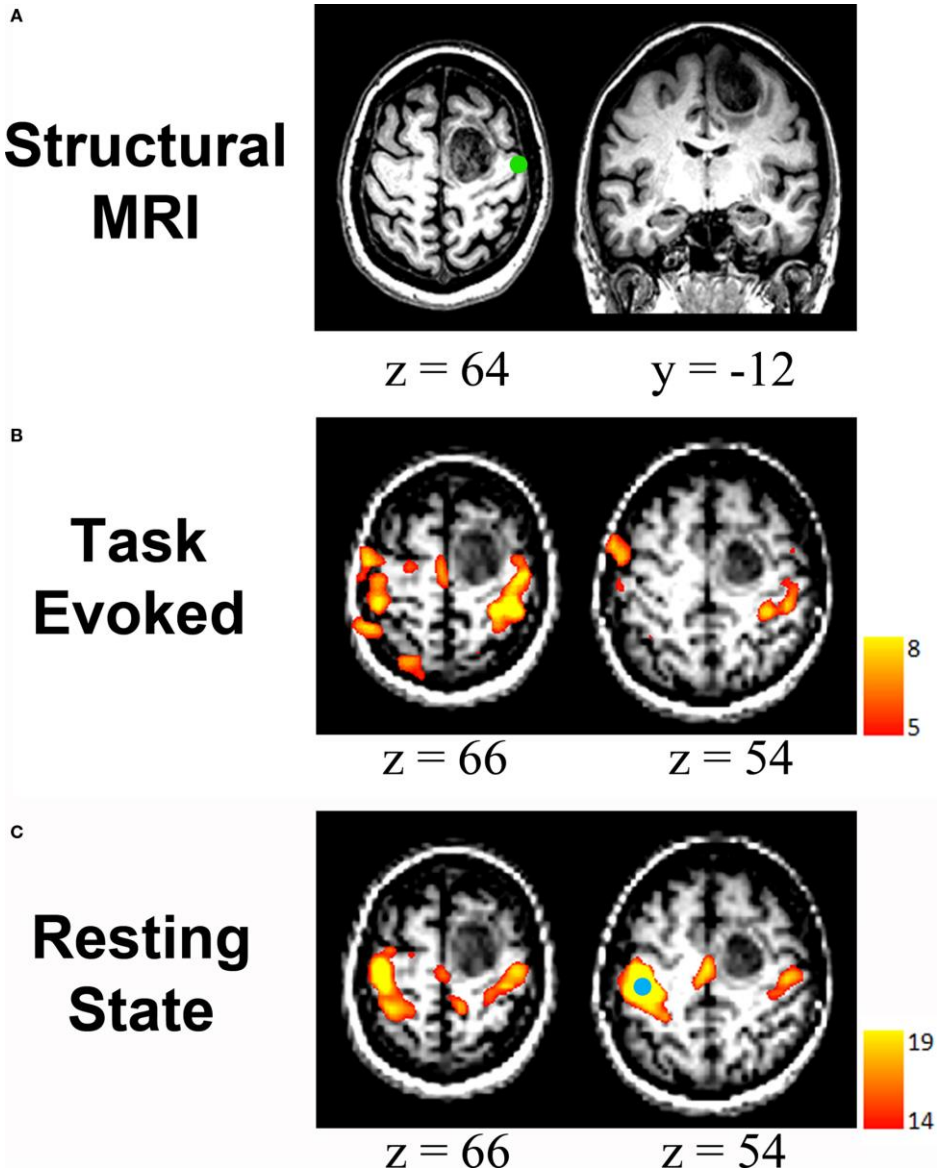


Curr Psychol (2023) 42:8156–8163

Can functional neuroimaging be helpful when someone can't perform a task?

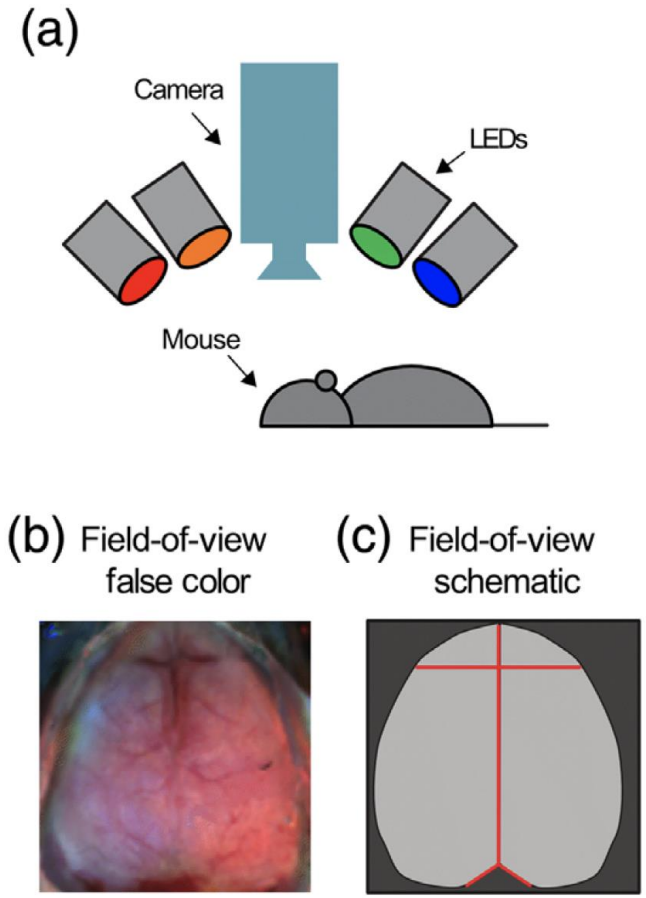
Resting state functional connectivity analysis measures correlation of activity in different brain regions at rest.





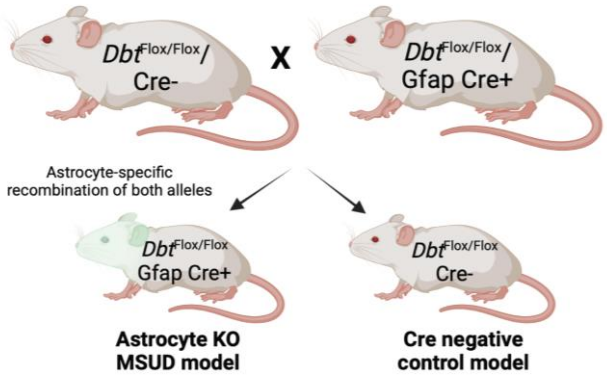
Front. Syst. Neurosci., 17 June 2010

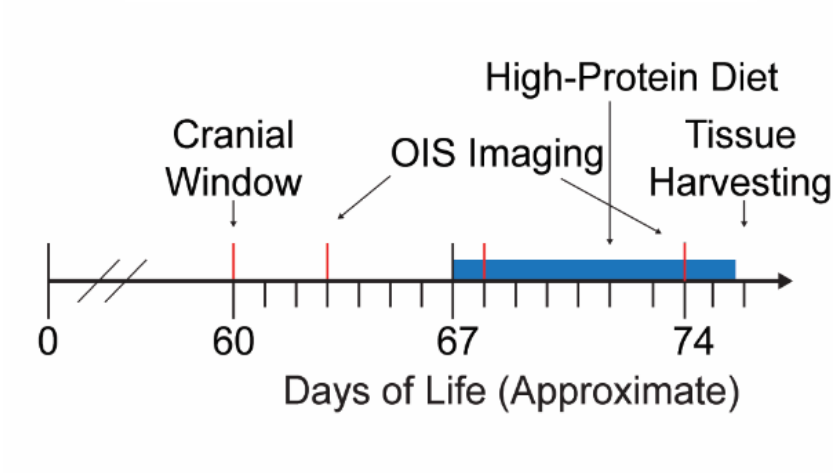
Resting state functional connectivity neuroimaging in mice using optical intrinsic signals

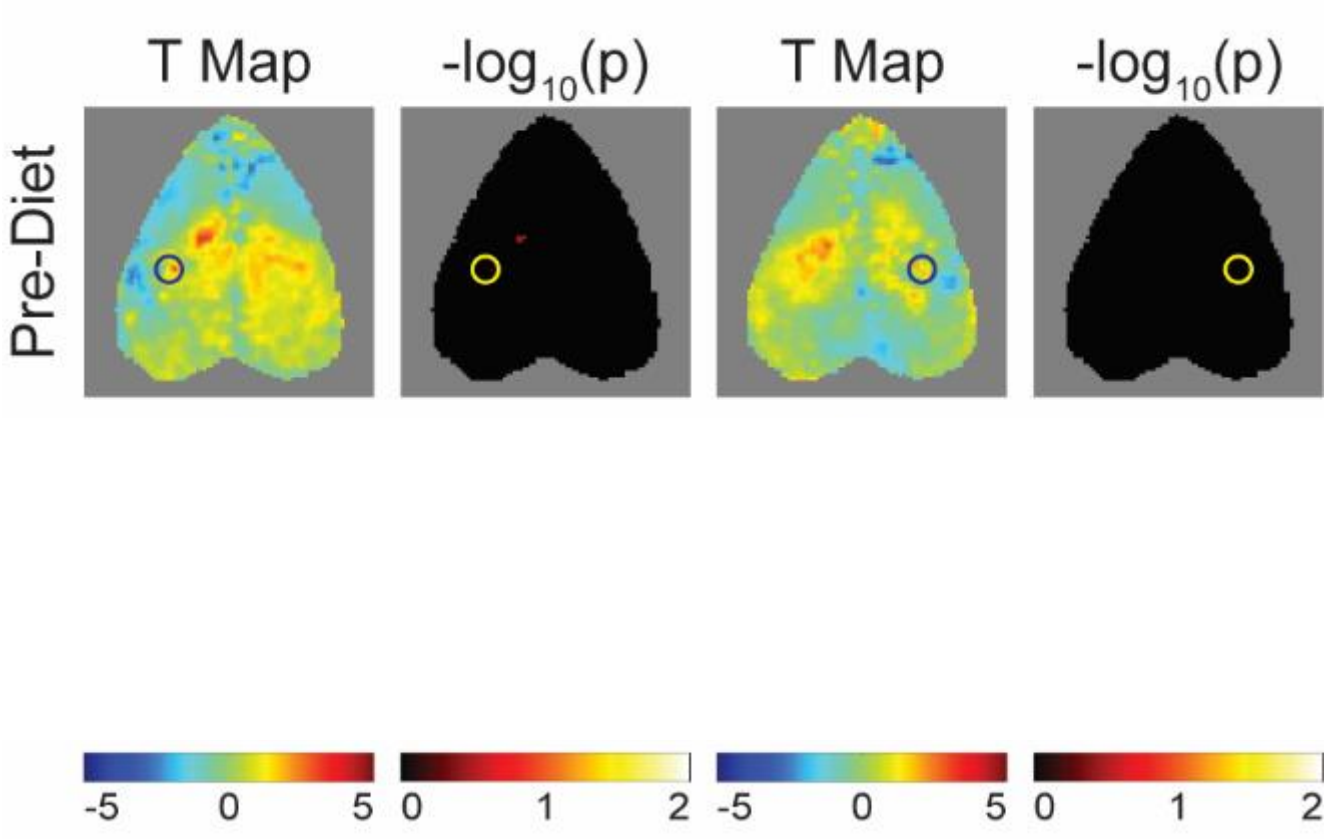


Brian White, MD, PhD

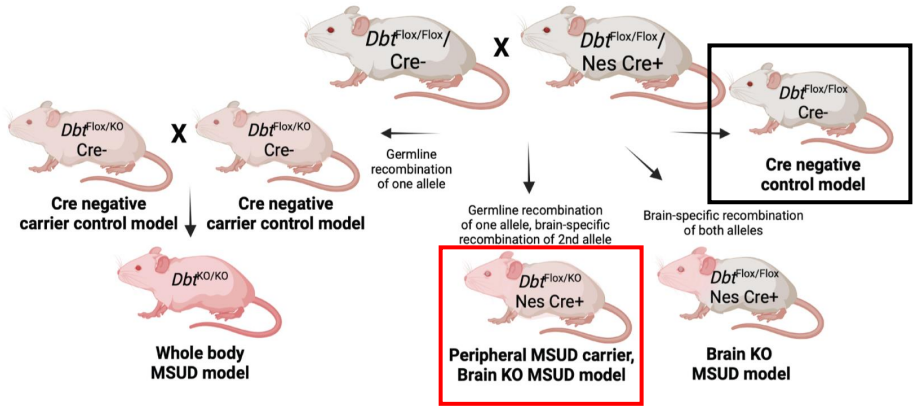
Astrocyte-specific MSUD mice on a high protein diet have increased resting state functional connectivity in posterior somatosensory cortex

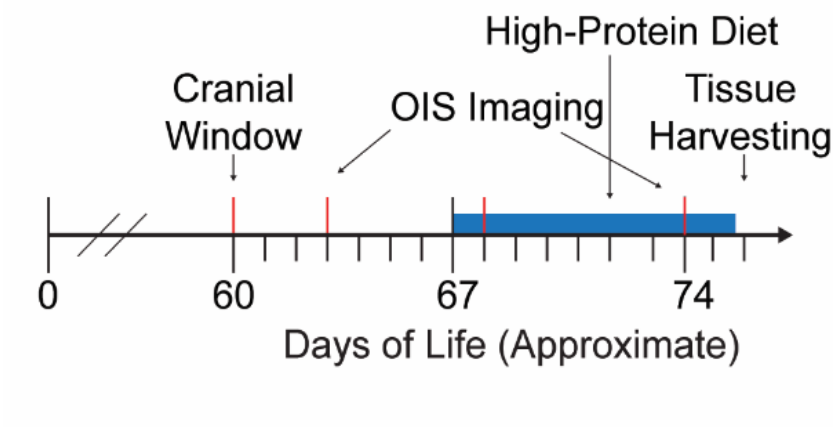


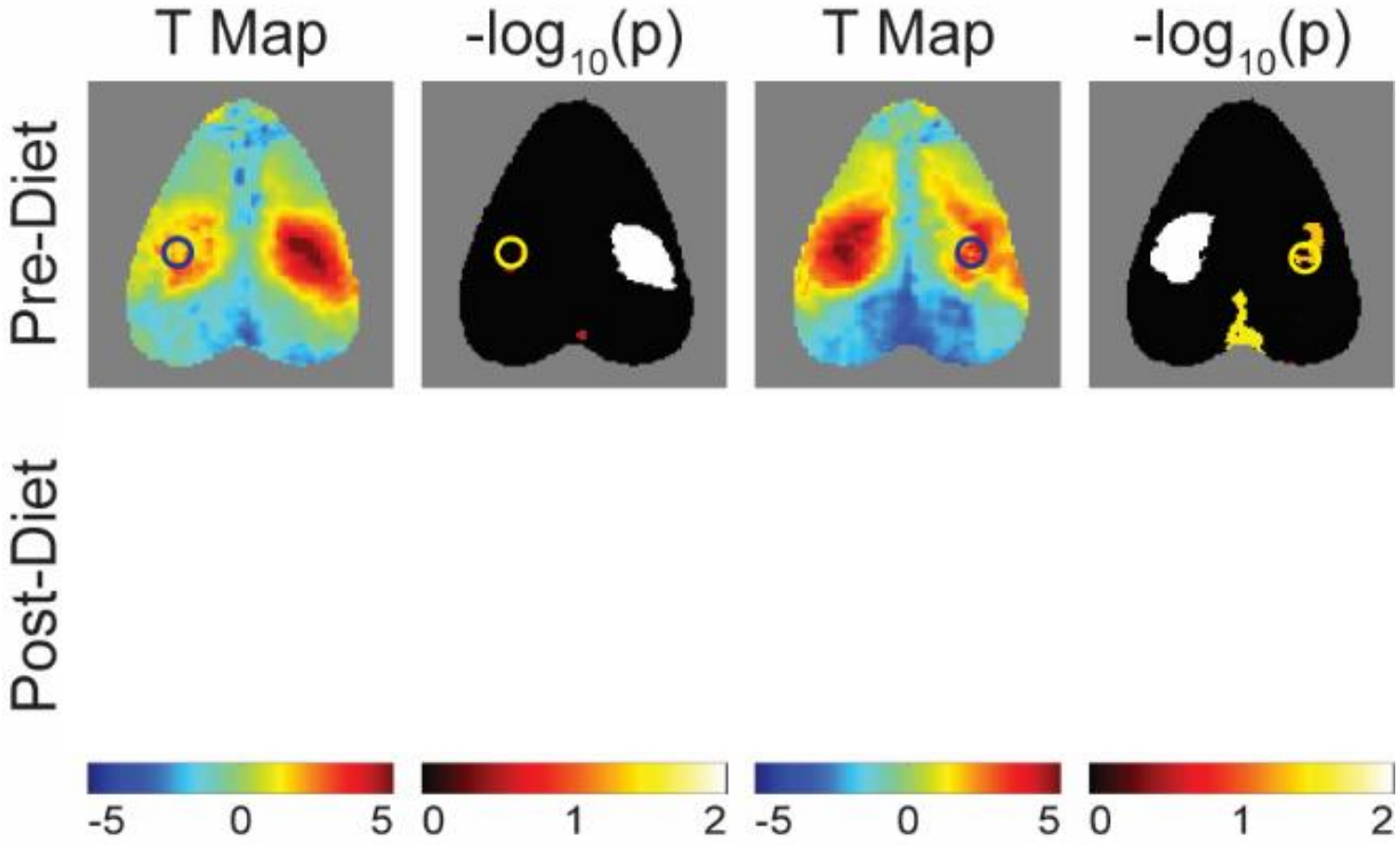




Brain-specific MSUD mice have increased resting state functional connectivity in posterior somatosensory cortex on regular diet that worsens on a high protein diet







Conclusions:

- Loss of BCKDH activity in brain alone is enough to increase branched chain amino acid (BCAA) levels in brain
- Metabolic differences extend beyond just BCAAs
- High protein diet worsens BCAA levels in brain knock-out mice, including changes in key neurotransmitters in the brain
- Mice lacking BCKDH activity in neurons or astrocytes alone also have subtle metabolic differences, suggesting both cell types play an important role in brain BCAA homeostasis
- Loss of BCKDH activity in brain does not change neuronal numbers or dendritic complexity
- Brain knockout MSUD mice have decreased power and increased spiking activity on EEG analysis
- Brain knockout mice also have very subtle anxiety phenotypes on behavioral testing
- Resting-state functional connectivity analysis may be a more sensitive measure of network-level neurologic deficits in MSUD mouse models

MSUD prevalence estimates from publicly available datasets

Rebecca Ahrens-Nicklas, MD, PhD
Division of Human Genetics, Section of Metabolism
The Children's Hospital of Philadelphia

Children's Hospital of Philadelphia®

How common is MSUD?

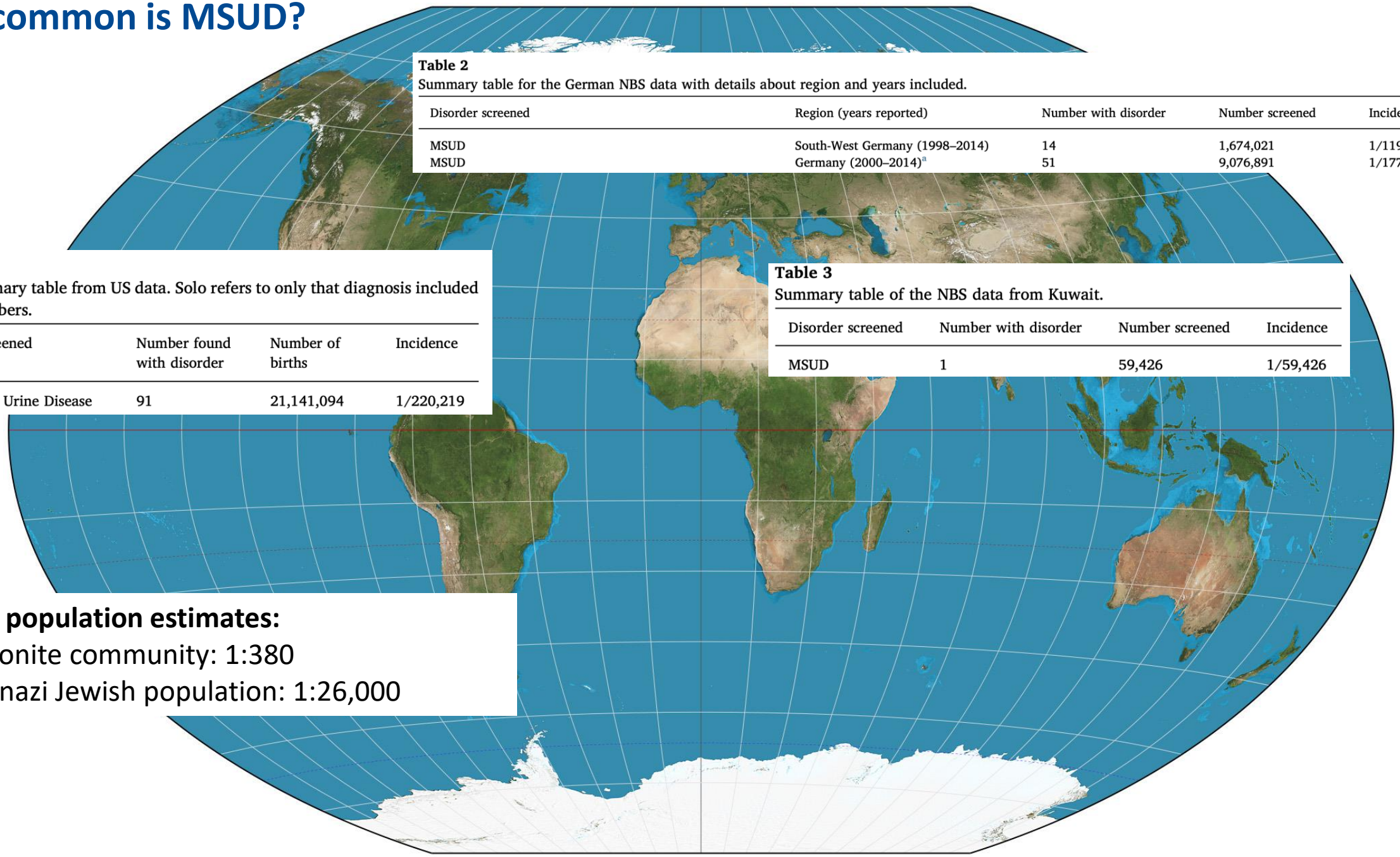


Table 2
Summary table for the German NBS data with details about region and years included.

Disorder screened	Region (years reported)	Number with disorder	Number screened	Incidence
MSUD	South-West Germany (1998–2014)	14	1,674,021	1/119,573
MSUD	Germany (2000–2014)[a]	51	9,076,891	1/177,978

Table 1
Overall summary table from US data. Solo refers to only that diagnosis included in these numbers.

Disorder screened	Number found with disorder	Number of births	Incidence
Maple Syrup Urine Disease	91	21,141,094	1/220,219

Table 3
Summary table of the NBS data from Kuwait.

Disorder screened	Number with disorder	Number screened	Incidence
MSUD	1	59,426	1/59,426

Other population estimates:
Mennonite community: 1:380
Ashkenazi Jewish population: 1:26,000

Chapman, et al. MGM Reports 15 (2018) 106–109

Why is it important to know incidence? Orphan drug designation

Orphan drug designation qualifies sponsors for incentives including:

- 7-year marketing exclusivity to sponsors of approved orphan products

- 25% federal tax credit for expenses incurred in conducting clinical research within the United States
  - Tax credits may be applied to prior year or applied over as many as 20 years to future taxes

- Waiver of Prescription Drug User Fee Act (PDUFA) fees for orphan drugs
  - A value of approximately $2.9 million in 2021

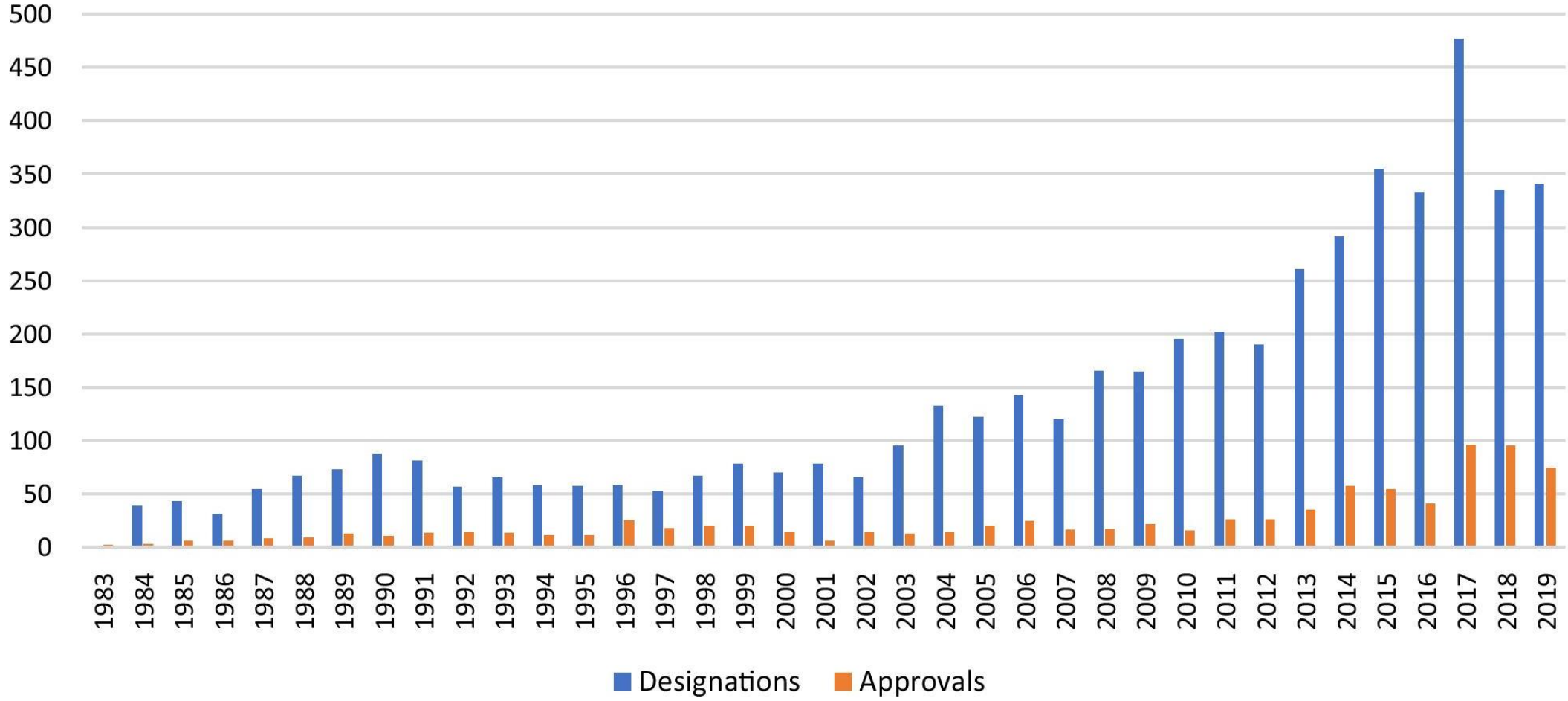
- Ability to qualify to compete for research grants from the Office of Orphan Products Development (OOPD) to support clinical studies for orphan drugs

- Eligibility to receive regulatory assistance and guidance from the FDA in the design of an overall drug development plan

Why is it important to know incidence? Orphan drug designation

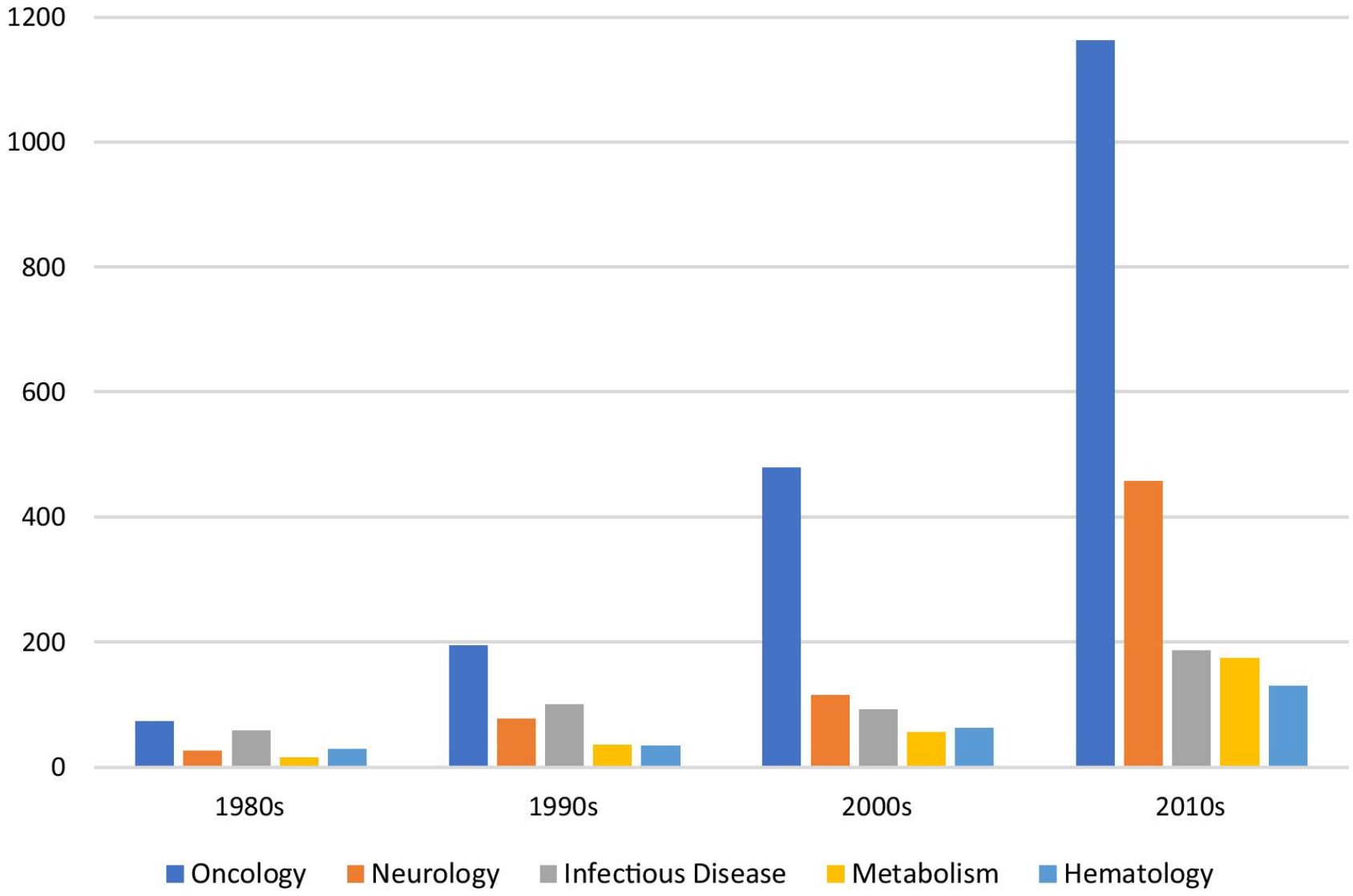
The Orphan Drug Act defines a rare disease as a disease or condition that affects less than 200,000 people in the United States.

Why is it important to know incidence? Orphan drug designation



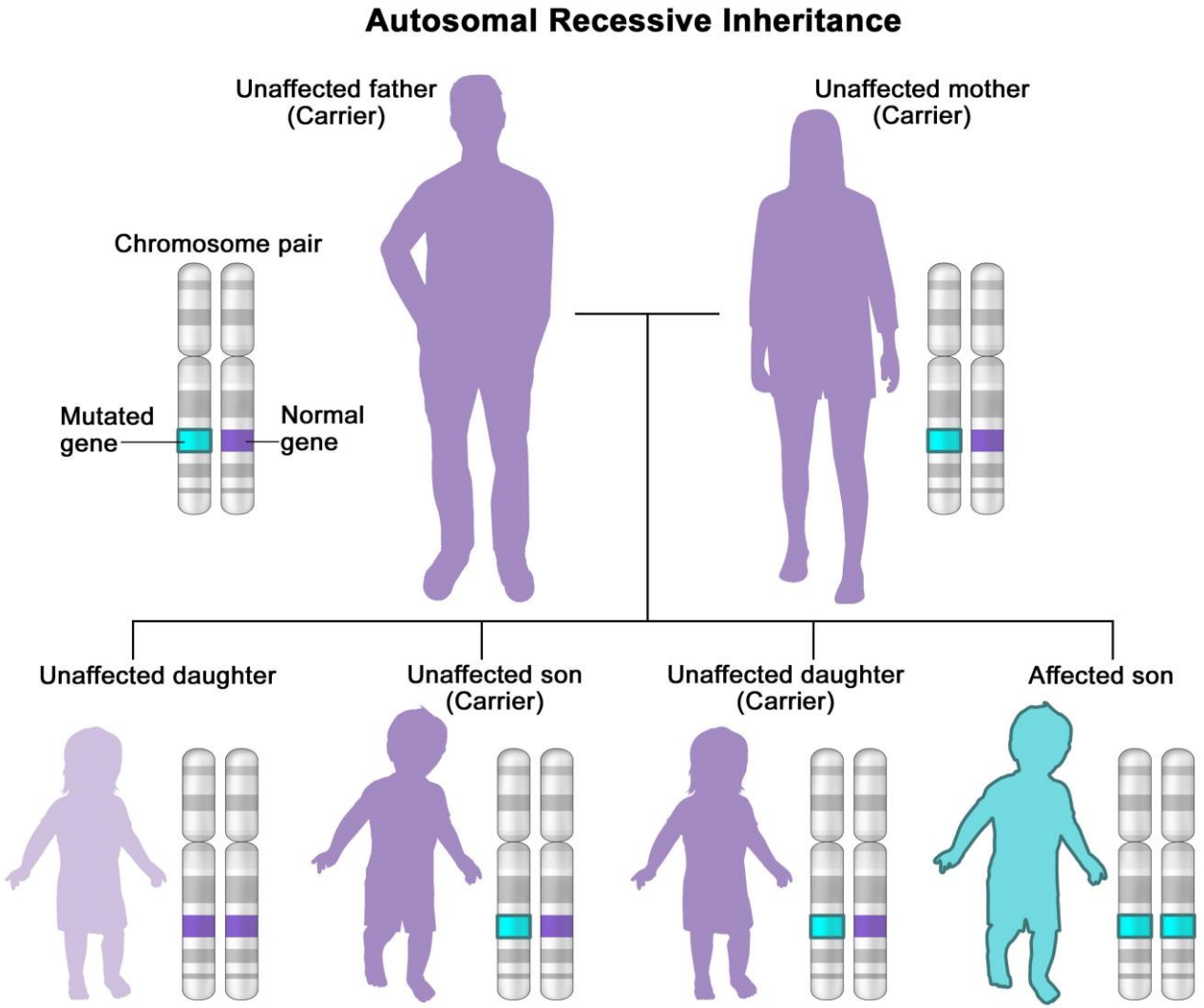
Miller et al. *Orphanet J Rare Dis* **16**, 265 (2021).

Why is it important to know incidence? Orphan drug designation

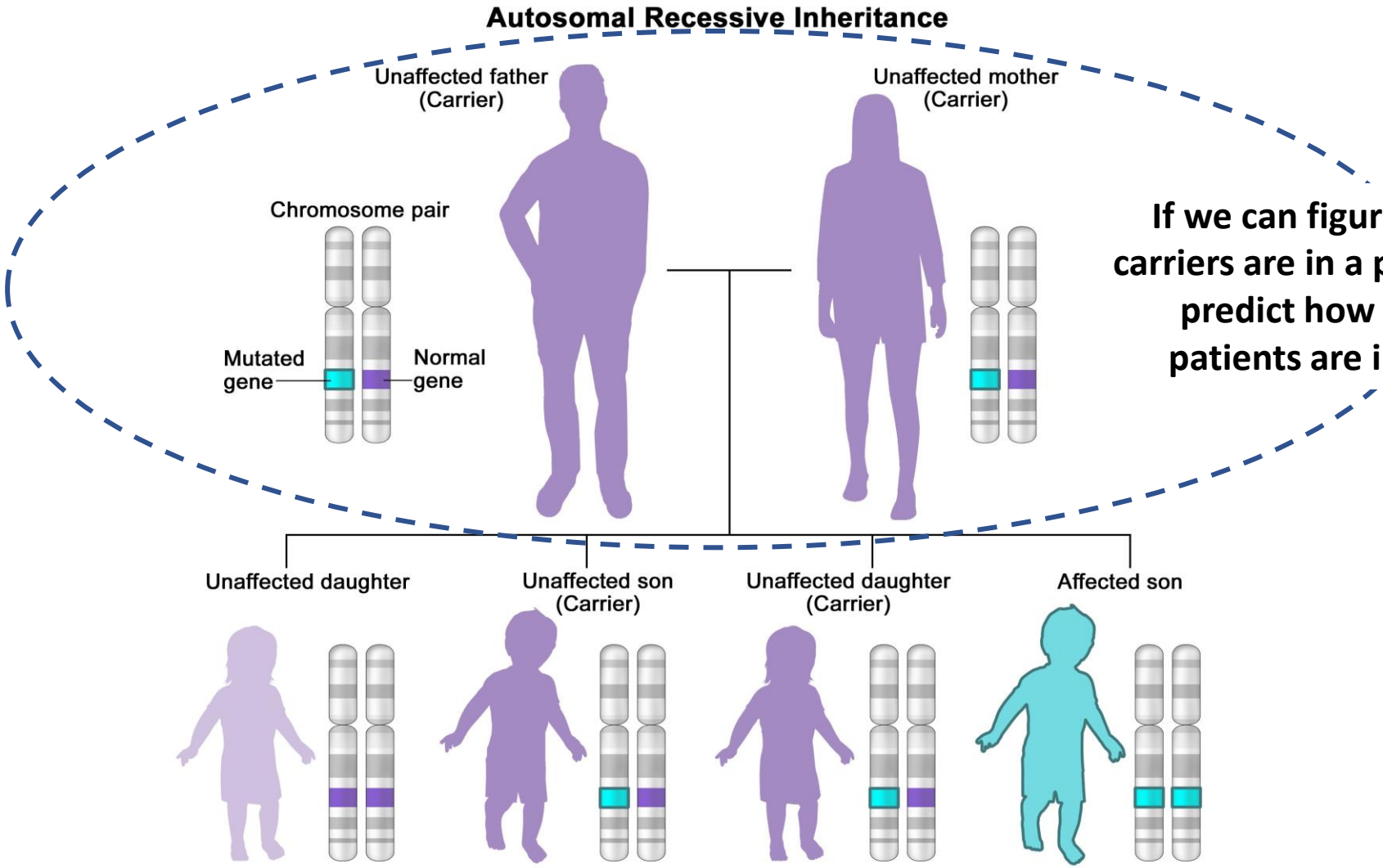


Miller et al. *Orphanet J Rare Dis* **16**, 265 (2021).

Is there another way to estimate incidence? Can we use basic genetic principles?

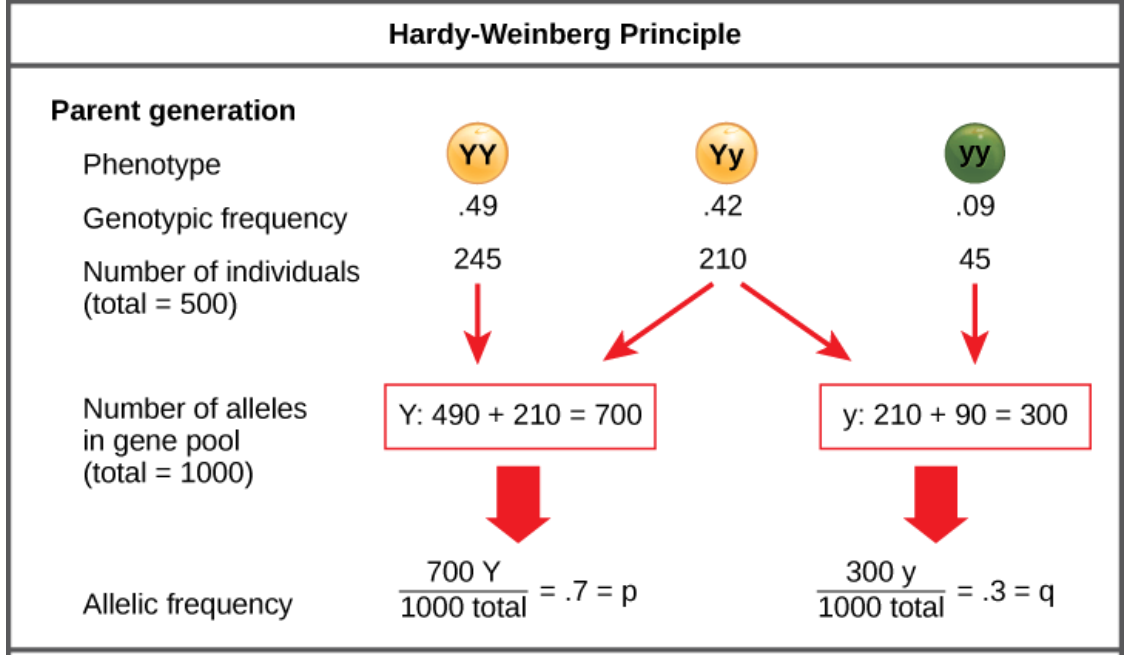


Is there another way to estimate incidence? Can we use basic genetic principles?



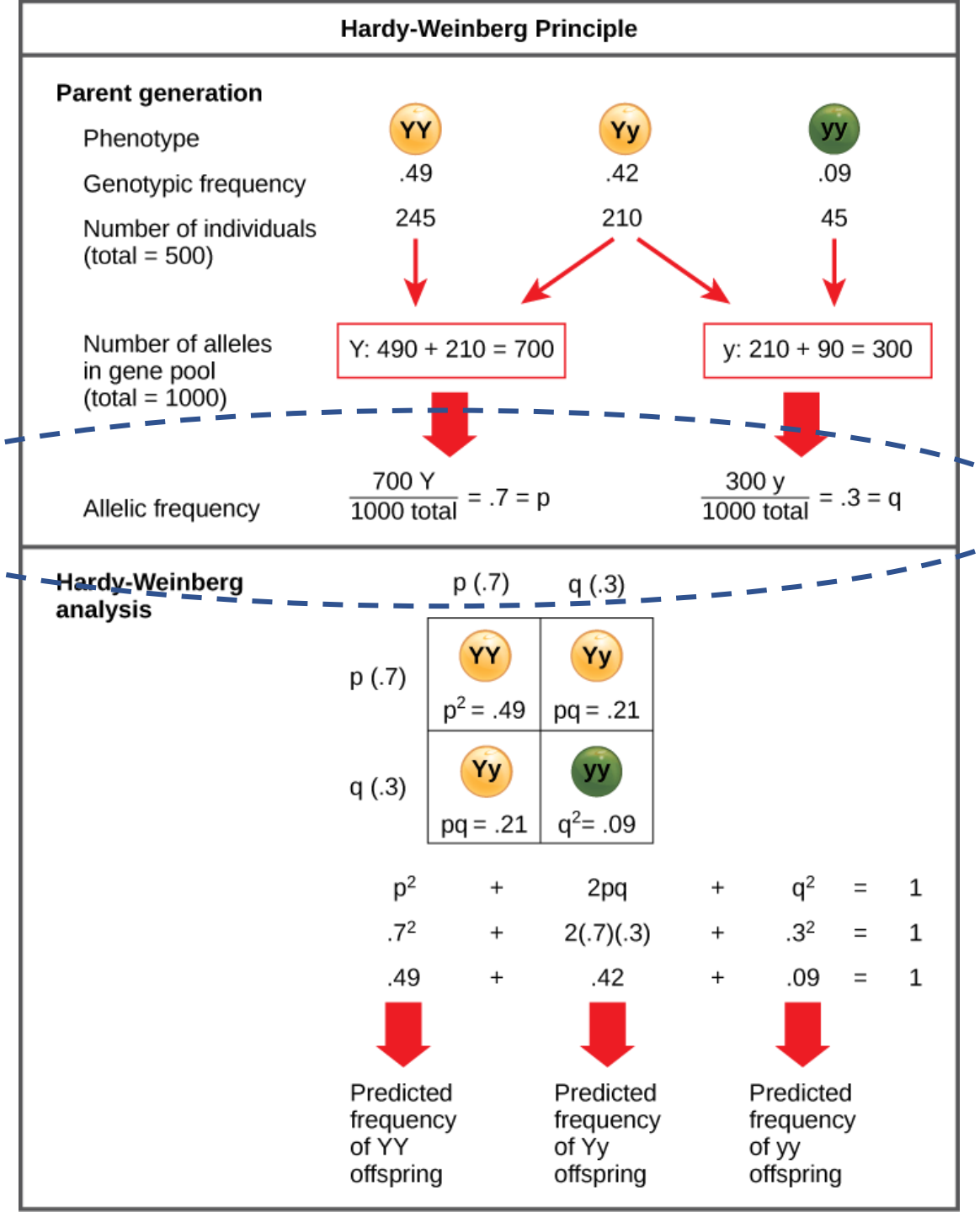
If we can figure out how many carriers are in a population, we can predict how many affected patients are in a population.

Is there another way to estimate incidence?
Can we use basic genetic principles?

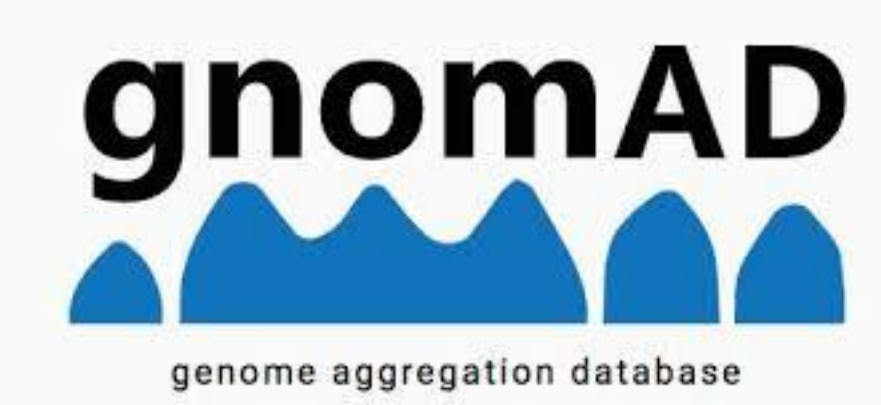


Is there another way to estimate incidence?
Can we use basic genetic principles?

Large genome sequencing datasets can help us figure out allele frequencies



Large databases of genomic data



Population	Description	Genomes
afr	African/African American	21,042
ami	Amish	450
amr	Latino/Admixed American	6,835
asj	Ashkenazi Jewish	1,662
eas	East Asian	1,567
fin	Finnish	5,244
nfe	Non-Finnish European	32,299
sas	South Asian	1,526
oth	Other (population not assigned)	1,077
Total		**71,702**

https://gnomad.broadinstitute.org

Examples of *DBT* variants in gnomAD

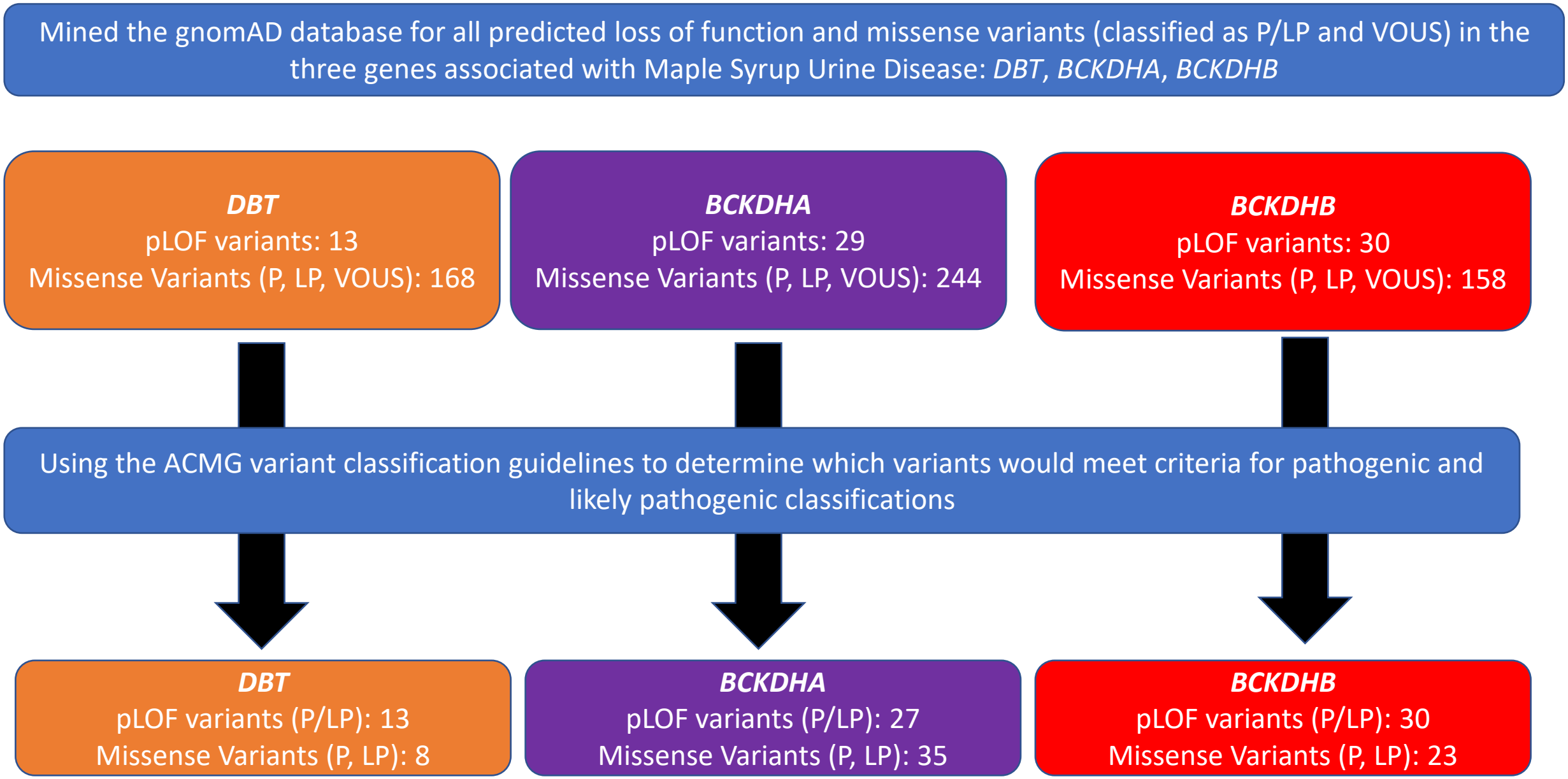
Variant ID	Clinical Significance	Allele Count	Allele Number	Allele Frequency	VEP Annotation
1-100196389-A-G		2	136152	1.47e-5	missense
1-100196395-C-G		1	125028	8.00e-6	missense
1-100196395-C-T	Conflicting interpretations of pathog...	2	125028	1.60e-5	missense
1-100196405-G-C		1	110086	9.08e-6	missense
1-100196410-A-G		1	130224	7.68e-6	missense
1-100196413-G-A	Pathogenic/Likely pathogenic	2	106800	1.87e-5	stop gained
1-100196425-AGAAAT-A		5	59838	8.36e-5	splice region

The next step is to determine which variants are truly disease-causing

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Diego Quintero, MS CGC

Kierstin Keller, MS CGC


Mined the gnomAD database for all predicted loss of function and missense variants (classified as P/LP and VOUS) in the three genes associated with Maple Syrup Urine Disease: *DBT*, *BCKDHA*, *BCKDHB*
DBT
pLOF variants: 13
Missense Variants (P, LP, VOUS): 168
BCKDHA
pLOF variants: 29
Missense Variants (P, LP, VOUS): 244
BCKDHB
pLOF variants: 30
Missense Variants (P, LP, VOUS): 158
Using the ACMG variant classification guidelines to determine which variants would meet criteria for pathogenic and likely pathogenic classifications
DBT
pLOF variants (P/LP): 13
Missense Variants (P, LP): 8
BCKDHA
pLOF variants (P/LP): 27
Missense Variants (P, LP): 35
BCKDHB
pLOF variants (P/LP): 30
Missense Variants (P, LP): 23

Determined variant carrier rate (VCR) of each likely pathogenic and pathogenic variant

VCR = (allele count – homozygote count) / (0.5 * allele number)

DBT variants: 21

BCKDHA variants: 62

BCKDHB variants: 53

Sum VCR to determine Gene Carrier Rate (GCR)

DBT **carrier rate**: 0.00191775

BCKDHA **carrier rate** : 0.001919908

BCKDHB **carrier rate** : 0.00288119

Calculate gene specific incidence using The Hardy-Weinberg Equation

DBT **Incidence**: 1 in 271,905

BCKDHA **Incidence:** 1 in 271,294

BCKDHB **Incidence:** 1 in 120,464

Combine to determine MSUD incidence

1 in 63, 837

AHRENS-NICKLAS LAB

A special thank you to all of the families, patients, and foundations that support our work!

Key Collaborators:

Brian White
Laura Adang
Lars Schlotawa
Kiran Musunuru
Bill Peranteau
Xiao Wang
Mohamad-Gabriel Alameh
Lindsey George
Eric Marsh
Beverly Davidson
Luis Tecedor
Elizabeth Bhoj
Stefano Rivella
Lucas Tricoli
Adeline Vanderver

MSUD FAMILY SUPPORT GROUP

NIH National Institute of Neurological Disorders and Stroke

Chan Zuckerberg Initiative

CGE Somatic Cell Genome Editing

the orphan disease center

BGTC AMP® Bespoke Gene Therapy Consortium FNIH

Children's Hospital of Philadelphia RESEARCH INSTITUTE | Intellectual and Developmental Disabilities Research Center

NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development

MSD ACTION FOUNDATION

HRB Health Research Board