Identifying an Efficacious Therapeutic for the Neurodevelopmental Disorder CTNNB1 Syndrome

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CTNNB1 syndrome- ***de novo*** **heterozygous loss-of-function pathogenic variants in *CTNNB1***

Insufficient β-catenin = core molecular pathology

CTNNB1 mutations-lead genetic cause in **misdiagnosed cerebral palsy**

CTNNB1/β-catenin- high confidence risk gene for intellectual disability, autism spectrum disorders

Several other ID and ASD-linked human genes are predicted to cause reduced β-catenin levels and/or functions

Treatment is lacking

limited knowledge of the underlying changes and few preclinical models

Generated two different preclinical models of CTNNB1 Syndrome-
***in vivo Ctnnb1* heterozygote mice and *in vitro CTNNB1* patient-derived cells**

- **Defining molecular and functional changes**
- **Identifying efficacious, safe therapeutic treatments**

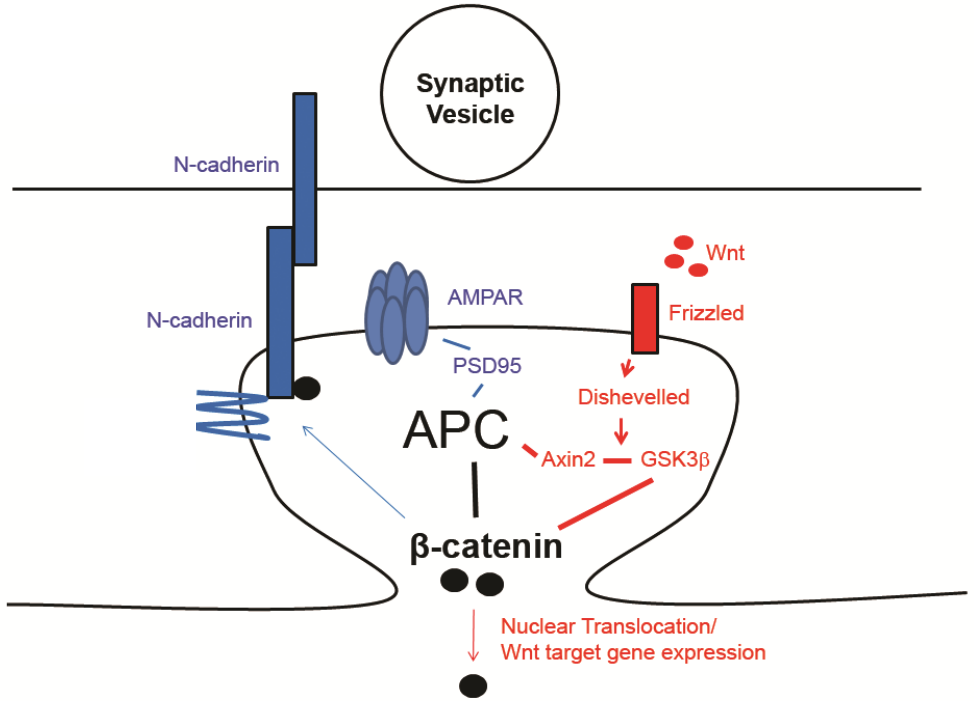
We have identified a small molecule efficacious therapeutic in preclinical mouse and patient cell models.

Tufts, with the Broad Institute, PCT patent application “Glycogen synthase kinase 3 (GSK3) inhibitors for treating CTNNB1 syndrome” filed September 20th, 2022 (WO2023056463A1).

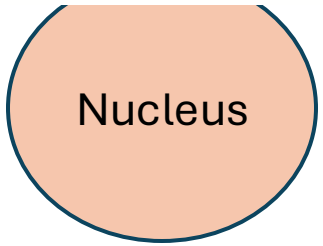
Insufficient β-catenin levels in CTNNB1 Syndrome

β-catenin plays key roles in two pathways important for brain development and function- Wnt signaling pathway and cadherin-based synaptic adhesion complexes.

Synaptic adhesion complex



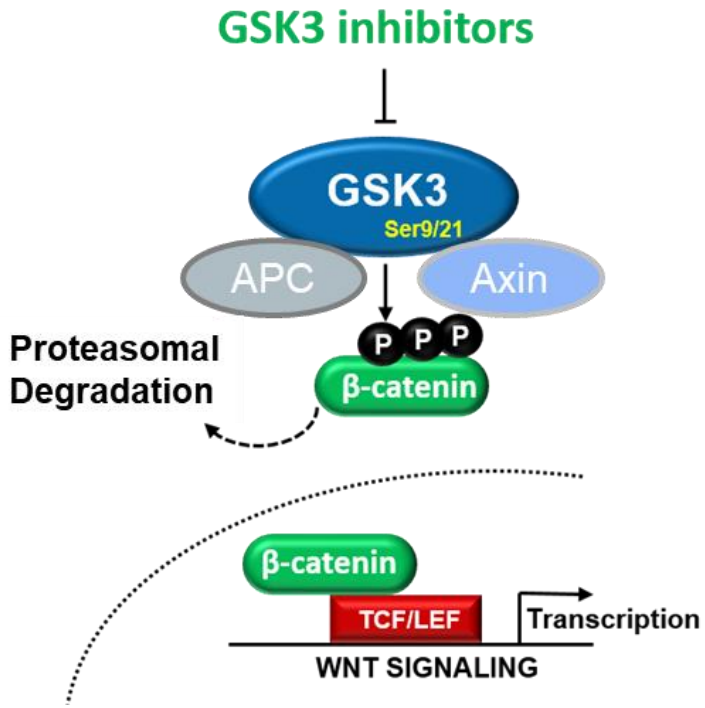
Nucleus, Wnt stimulated gene expression



Michel Weïwer

BROAD INSTITUTE | CENTER FOR THE DEVELOPMENT OF THERAPEUTICS

Drug treatment goal: increase β-cat levels to resemble the normal baseline range of wildtype littermates



Target is GSK3 (glycogen synthase kinase)

endogenous negative regulator of β-catenin levels

well-defined mechanism of action

validated druggable target in the β-catenin/Wnt signaling pathway

GSK3 α,β dual paralog inhibitors

high kinome selectivity, potency, brain permeability, metabolic stability

F. Wagner *et al.*, *ACS Chem Bio* **2016**, 1952; F. Wagner *et al.*, *Sci. Transl. Med.* **2018**, eaam8460.

Jon Alexander

Leeanne Vazquez Ramirez

Crystal Lin

EMBO Molecular Medicine

2024

Inhibition of GSK3α,β rescues cognitive phenotypes in a preclinical mouse model of CTNNB1 syndrome

Jonathan M Alexander [1], Leeanne Vazquez-Ramirez [1], Crystal Lin[1], Pantelis Antonoudiou[1], Jamie Maguire[1], Florence Wagner[2,3] & Michele H Jacob [1]✉

Ctnnb1 **germline heterozygote mouse line,**
Displays phenotypes resembling key features of human CTNNB1 syndrome

Small molecule, high selectivity inhibitor of GSK3 significantly normalizes β-cat levels and phenotypes in the *in vivo* mouse model.

CTNNB1 Syndrome mouse model

50% decreases in β-cat protein levels in brain, spinal cord and skeletal muscle- tissue types relevant to key phenotypes in patients

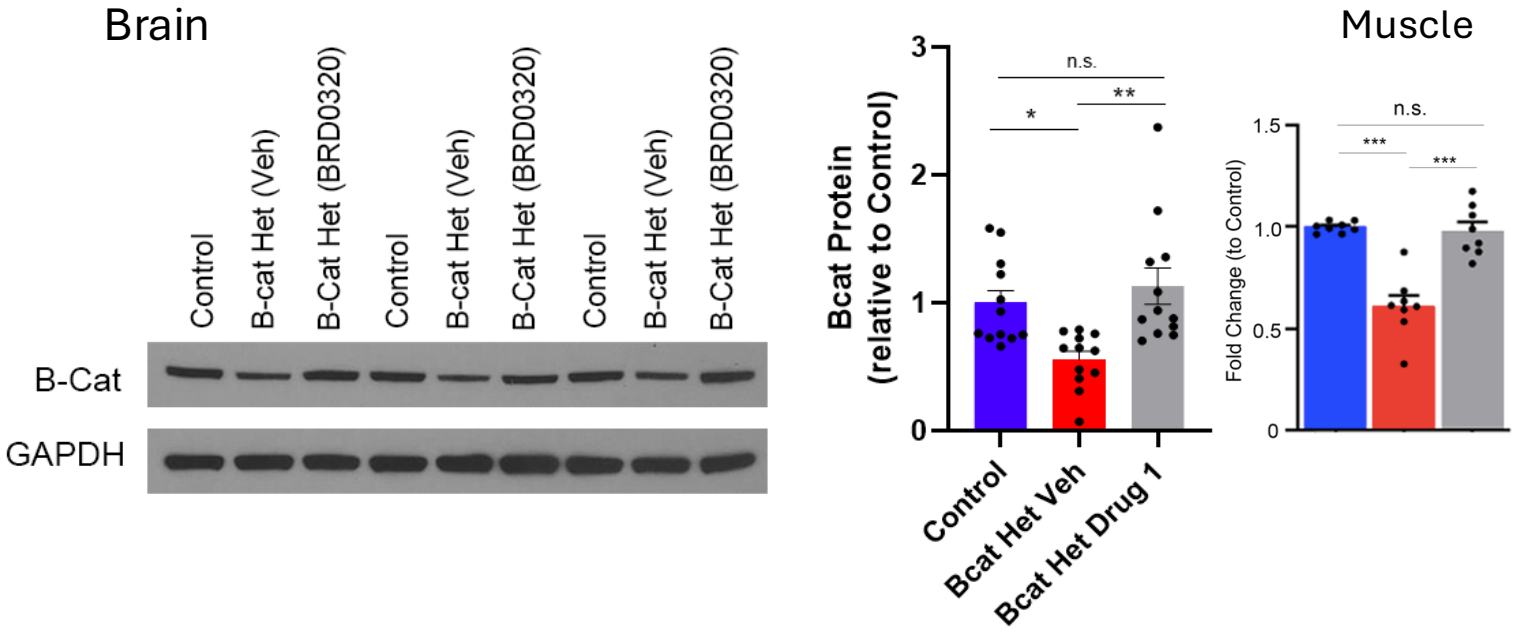
Identified novel molecular and functional property changes that impact excitability of neurons

Reductions in associative learning, motor learning and coordination; muscle weakness (diagnostic behavioral assessment tasks designed for mice)

***Ctnnb1* het mice display phenotypes resembling key features of human CTNNB1 syndrome**

GSK3 α,β inhibitor normalizes β-catenin levels *in vivo* in *Ctnnb1* heterozygote mice

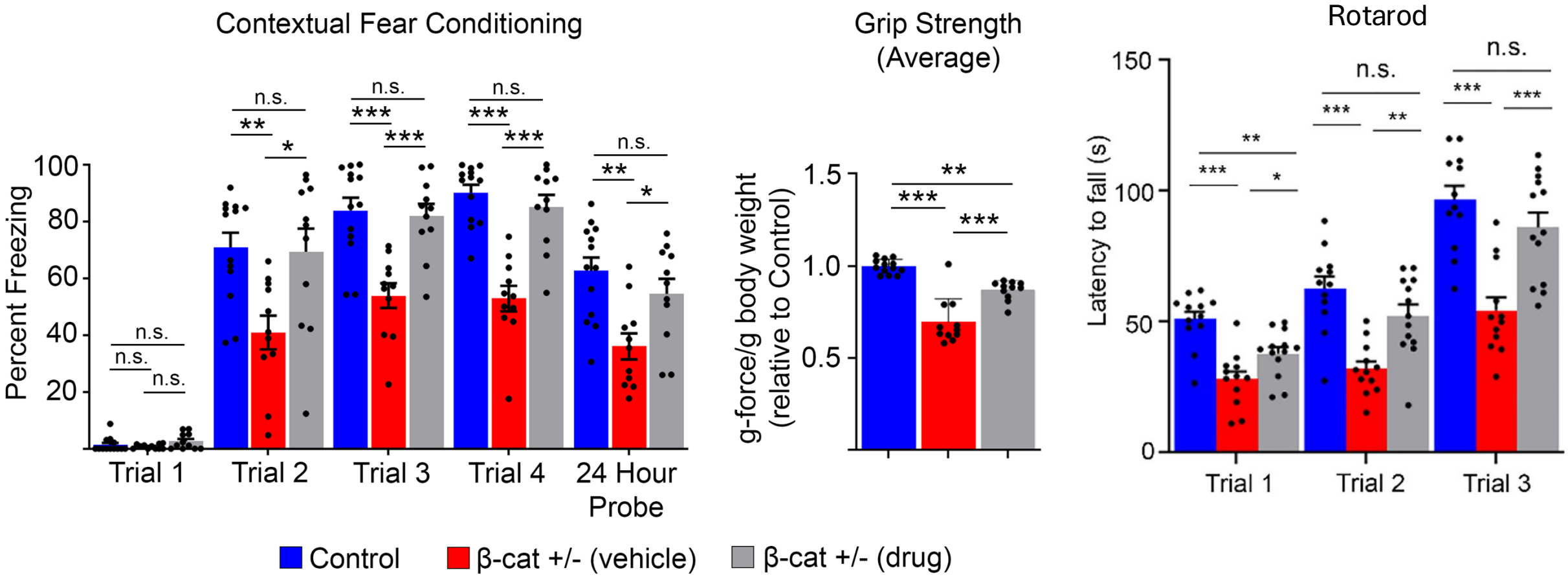
GSK3 inhibitor brings the reduced β-cat levels to within, and not above, normal baseline range of wild-type littermates.



GSK3 inhibitor significantly improves molecular and functional phenotypes in β-cat heterozygote mice, relative to WT littermates.

GSK3 α,β inhibitor treated young adult Ctnnb1/β-cat het mice show significant improvements in learning and motor skills

The beneficial outcomes were achieved with treatment initiated in symptomatic late adolescent/ young adult mice.



Takeaways from preclinical mouse studies

Identified an efficacious therapeutic treatment for this unmet medical need

small molecule high specificity GSK3 inhibitor *in vivo* mouse model

- Significantly improved learning and motor capabilities in symptomatic β-catenin het mice treated at late adolescent age
- Normalized core molecular pathology- β-catenin levels *in vivo*
- Corrected phenotypes- molecular, functional, behavioral
- No adverse effects in wild-type mice for length of treatment- no significant change in learning or β-catenin levels

Assess GSK3 inhibitor therapeutic efficacy in
CTNNB1 syndrome patient derived cells
carrying distinct *CTNNB1* pathogenic variants spanning the gene.

[Confidential slides with unpublished patient data have been removed from this public version]

These essential preclinical patient derived cell and *in vivo* mouse studies are providing a critical opportunity to develop an efficacious and safe small molecule drug candidate for clinical trials.

Funding for the CTNNB1 studies

Advancing CTNNB1 Cures and Treatments, Inc. PI: Michele Jacob 10/2019-4/1/2026
(currently CTNNB1 Connect and Cure)
Identify molecular and functional changes caused by CTNNB1 disruptive mutations and test treatment strategies for correcting them

NIH/NINDS R21NS131841 PI: Michele Jacob 9/2023-8/2025
Defining the potential of gene therapy to correct motor disabilities of CTNNB1 syndrome using in vivo mouse and in vitro human cell models

NIH NCATS TDB PI: Michele Jacob Co-I: Michel Weiwer (Broad Institute of MIT and Harvard) 2024- 2026
(National Center for Advancing Translational Science, Therapeutic Development Branch) Corrective Treatment for CTNNB1 Syndrome

Oxford-Harrington Rare Disease Scholar Award PI: Michele Jacob 2024-2026
Identifying the lead compound for efficacious treatment of CTNNB1 syndrome Shortlisted, pending approval.

Recently Completed
NIH NINDS R21 NS119958 PI: Michele Jacob
Investigating molecular mechanisms and treatments for CTNNB1 Syndrome using mouse and human models

NIH NIMH R01MH106623 PI: Michele Jacob
Molecular causes of cognitive and autistic disabilities.

NIH NINDS R01 NS100706 C. Dulla, PI, M.H. Jacob, Co-I
The role of beta-catenin in the pathophysiology of infantile spasms.