Neuronal Mislocalization of Mutant DHHC9 in X-Linked Intellectual Disability

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Abstract

DHHC9 belongs to a family of protein acyl transferase (PAT) enzymes. PATs enzymatically add fatty acid palmitate to cysteine residues on specific protein substrates resulting in increased hydrophobicity and membrane association. This modification is reversible by the action of depalmitoylating thioesterases. There are over 300 candidate palmitoylated proteins identified in rat cortical neurons, suggesting that this dynamic process plays a key role in the spatiotemporal distribution of proteins within the neuron. Neurons are polarized cells with discrete protein domains and DHHC9 and other PATs facilitate proper trafficking of proteins to these domains. Recently, mutations in the *zDHHC9* gene have been identified in individuals with X-Linked Intellectual Disability (XLID). Here, we characterize a specific XLID nonsense mutation (*R298) in the *zDHHC9* gene that results in the expression of a C-terminal truncated protein, in the context of the mature hippocampal neuron.

Background

Intellectual Disability

What is intellectual disability?

Intellectual disability (ID) is an umbrella term for a clinical disorder reflecting limitations, deficits or abnormalities in:

- Cognitive abilities (IQ<70)
- 2 or more social/behavioral adaptive skills

What causes intellectual disability?

The etiology of the disease is due to:

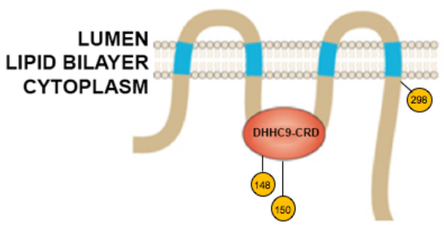
- Environmental causes and/or
- Genetic causes
  - De novo
  - Autosomal Recessive
  - **X-linked**

X-Linked Intellectual Disability

Protein-coding Genes Identified in X-Linked Intellectual Disability (XLID)[1]

- ~10% of protein-encoding genes on the X chromosome have been implicated in XLID
- XLID accounts for about 15% of all male ID cases
- The PAT gene DHHC9 maps to position Xq26.1

DHHC9 Mutations Associated with XLID



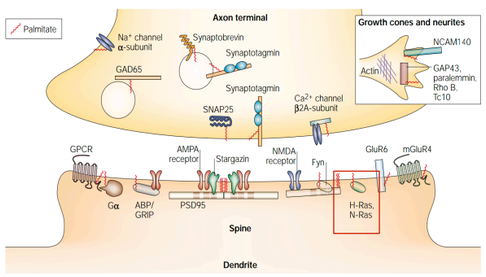
XLID DHHC9 Mutations[2]

- Missense Mutations (2):
  Occur within the catalytic cysteine-rich domain (CRD)
  - R148W
  - P150S
- Nonsense Mutation (1)
  Results in a catalytically active, C-terminal truncated enzyme
  - R298*

Protein Palmitoylation in the Neuron

Protein Palmitoylation Regulates Neuronal Structure and Function[3]

- PATs add fatty acid palmitate to substrate proteins , which acts as a membrane anchor
- Palmitoylation of proteins involved in trafficking of specific proteins to discrete neuronal domains
- DHHC9 identified protein substrates:
  - H-Ras
  - N-Ras



Results

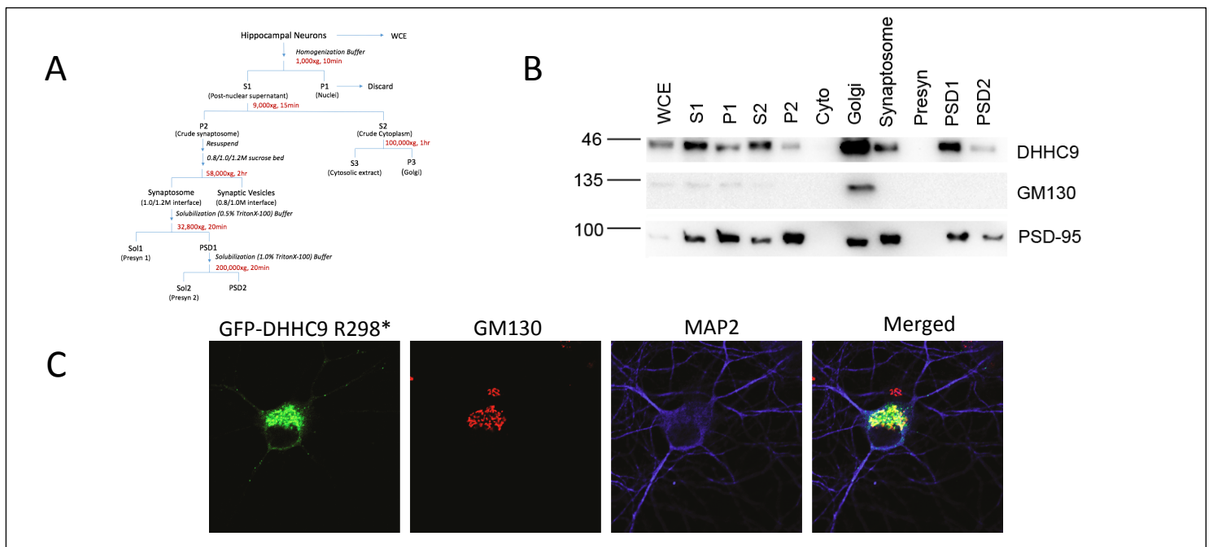


Figure 1. Endogenous DHHC9 Enriched in Golgi and Post-synaptic Density Neuronal Fractions. (A) *Subcellular Fractionation*: Cultured primary hippocampal neurons were homogenized and differentially centrifuged to yield insoluble and soluble fractions. The synaptosomal fraction was layered on a sucrose gradient and the synaptosomal interface was collected. (B) Protein from soluble and insoluble fractions were analyzed by *Western Blot*. Briefly, subcellular fractions were run on a 12% Tris-glycine gel and transferred to nitrocellulose membrane. The membrane was probed with DHHC9 rabbit polyclonal, GM130 mouse monoclonal (Golgi marker) and PSD-95 mouse monoclonal (Post-synaptic density marker) antibodies. Endogenous DHHC9 was enriched in the Golgi and Post-synaptic (PSD1 and PSD2) densities. (C) *Plasmid Construction*: GFP-tagged zDHHC9 plasmids were constructed with the GFP gene attached in frame to the 5' end of the zDHHC9 allele. Lipofectamine 2000 was used to transfect plasmid into primary hippocampal neurons. I*mmunostaining*: Transfected neruons were immunostained using GM130 mouse monoclonal (Golgi Marker) and MAP2 rabbit polyclonal (neuronal marker) antibodies. Alexafluor 594 and 350 conjugated secondary antibodies were used, respectively. A TCS SP5 II confocal microscope (Leica) was used and images were taken using 3D stacks under 63X magnification.

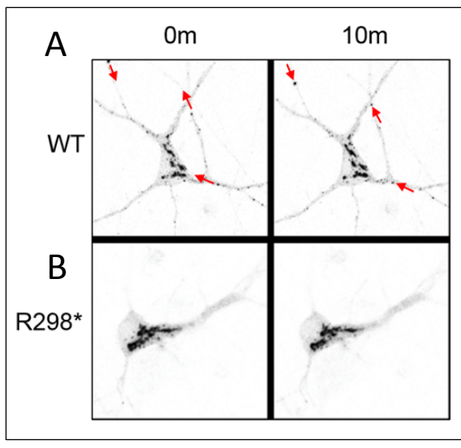


Figure 2. The C-terminus of DHHC9 is Necessary for Protein Trafficking. (A) and (B) *Live Cell Imaging*: Neurons were transfected with (A) GFP-WT DHHC9 or (B) GFP-DHHC9 R298* constructs using Lipofectamine 2000. Time lapse imaging was performed using a TCS SP5 11 confocal microscope (Leica) at 63X magnification. 3D image stacks were taken and projected to 2D images. (A) GFP-WT DHHC9 localization is localized to the cell body and trafficked (indicated by red arrows) over time to the dendrites and axons. (B) GFP-DHHC9 R298* is restricted to the cell body. Images represent a 10 minute time-span.

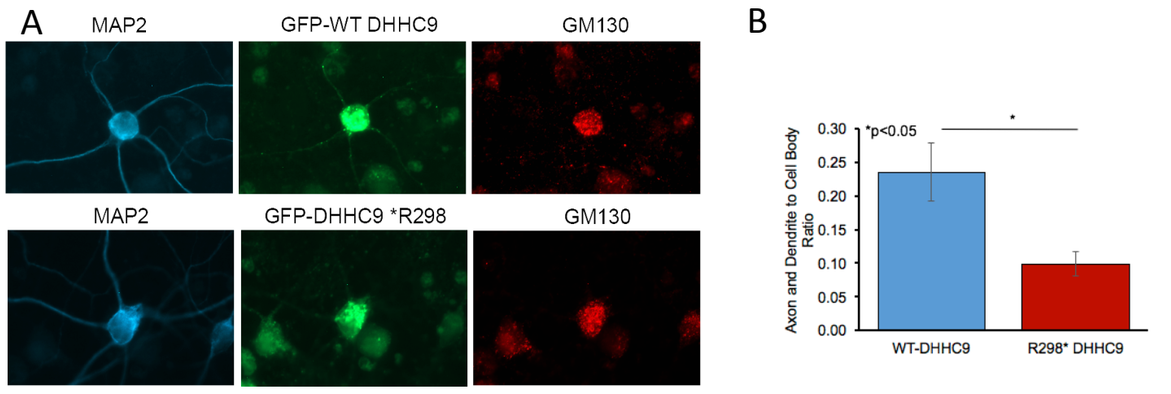
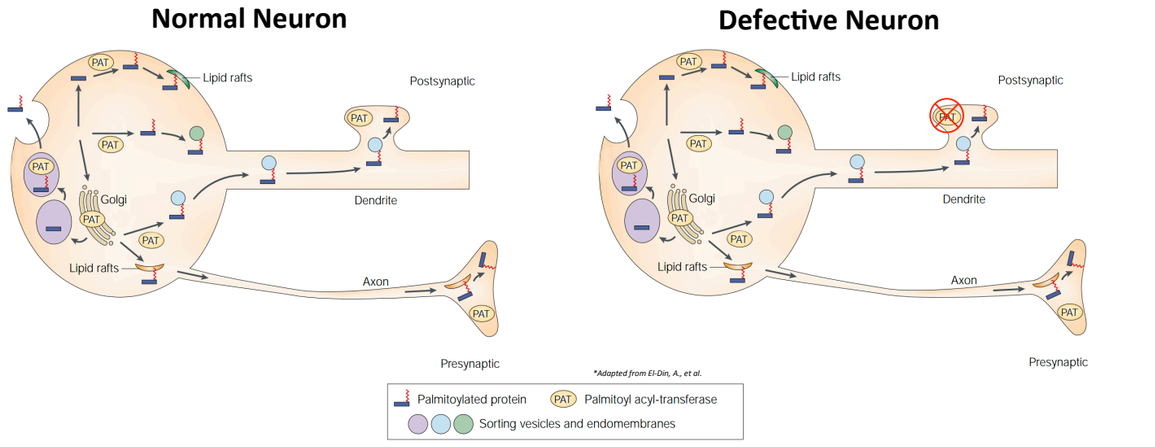


Figure 3. Mutant R298* Exhibits Limited Axon/Dendrite Trafficking and is Retained in the Golgi. (A) Primary hippocampal rat neurons were transfected with with GFP-WT DHHC9 or GFP-DHHC9 R298* plasmids using Lipofectamine 2000. Neurons were immunostained using GM130 mouse monoclonal (Golgi Marker) and MAP2 rabbit polyclonal (neuronal marker) antibodies. Alexafluor 594 and 350 conjugated secondary antibodies were used, respectively. A TCS SP5 II microscope and images were taken under 63X magnification. (B) The histogram reflects a of total corrected fluorescence in axons and dendrites taken to total corrected fluorescence of cell body, n=30 neurons quantified per treatment. Statistical analysis performed from 3 independent experiments with Student's T-Test, *p<0.05.

Conclusions



References

1. Piton, A., et al. **XLID-Causing Mutations and Associated Genes Challenged in Light of Data From Large-Scale Human Exome Sequencing**. (2013) *The American Journal of Human Genetics* 64(2)
2. Fukata, Y. and Fukata, M. **DHHC-type palmitoylating enzyme family and identified enzyme-substrate pairs.** (2010) *Nature Reviews Neuroscience* 11
3. El-Din, A., et al. **Palmitoylation: a regulator of neuronal development and function.** (2002) *Nature Reviews Neuroscience* 3

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