Effects of an Autism-related mutation in Trio's spectrin repeat domain

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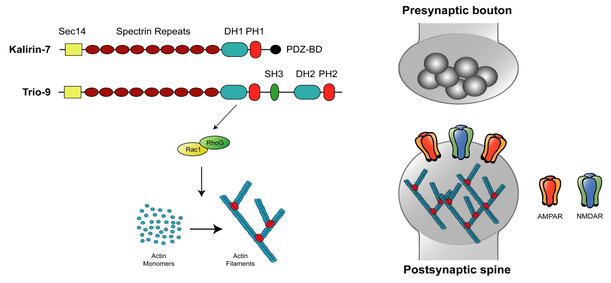
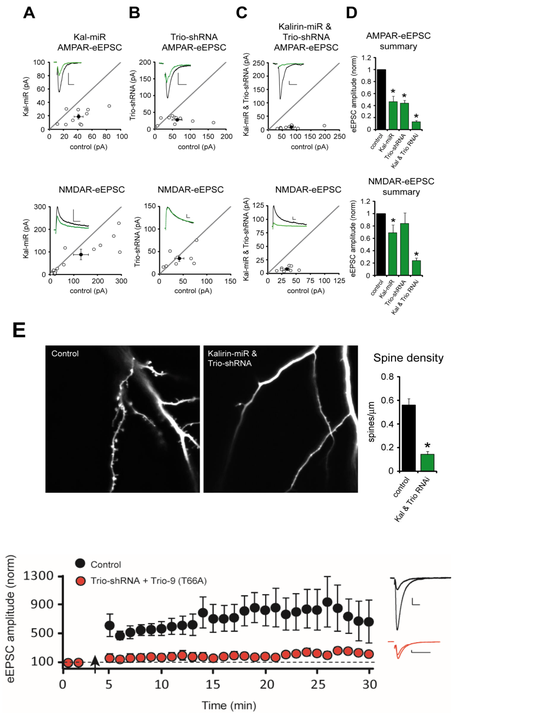
Introduction

Our lab has recently discovered that a large number of patients with Autism Spectrum-like disorders (ASD) have de novo mutations in a synaptic protein called Trio. Many of these mutations are clustered in Trio's GEF1 domain. We have been studying such ASD-related mutations in the GEF1 domain of this protein, which is the domain that activates another protein called Rac1. It is through Trio's ability to activate Rac1 that it is able to affect the size and strength of synapses through actin polymerization. We have found that various mutations in the GEF1 domain of Trio affect synapse, or "spine", structure- a phenotype that is observed in ASD.

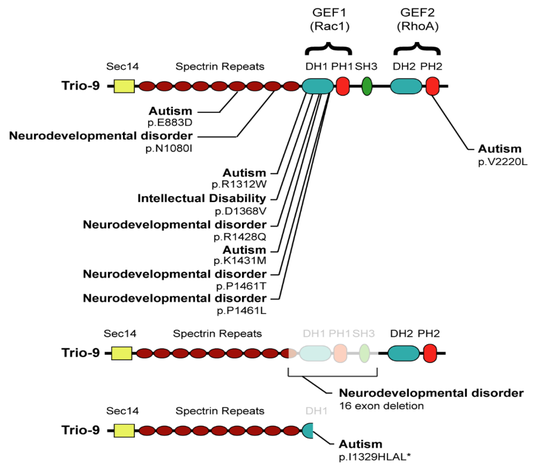
The goal of this project is to look at mutations in a spectrin repeat of Trio, characterize its effects on glutamatergic synapses, and attempt to draw a conclusion about the function of this region in Trio's ability to promote actin polymerization.

Background



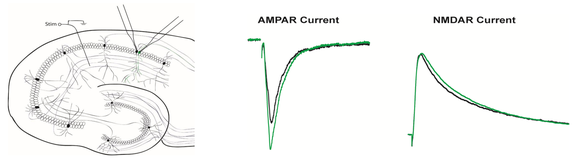


ASD-related de novo mutations in Trio



Methods

Dual whole-cell patch clamping of CA1 pyramidal neurons



Super-resolution structured illumination microscopy

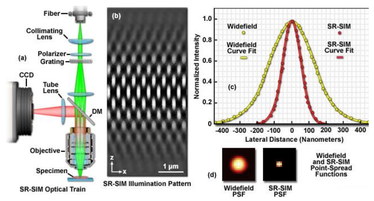
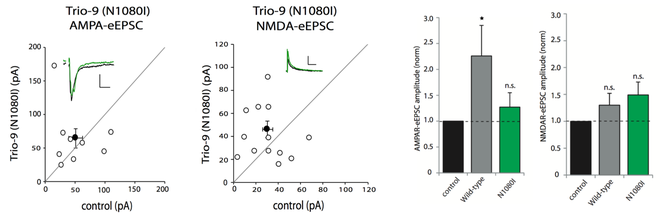


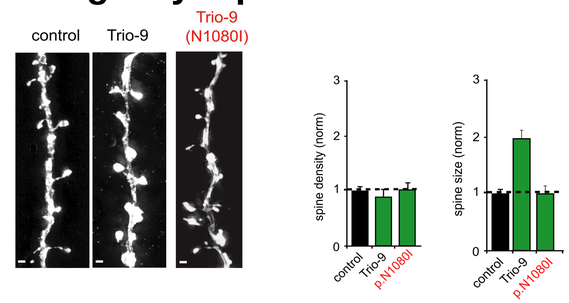
Image from Zeiss.com

Trio-9 p.N1080I inhibits Trio's impact on glutamatergic synapse function



Electrophysiology shows Trio-9 N1080I mutation prevents Trio-9 from increasing synaptic AMPA-receptor expression.

Trio-9 p.N1080I inhibits Trio's impact on glutamatergic synapse structure



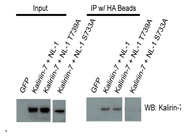
N1080I mutation prevents Trio-9 overexpression from increasing synapse density or size.

Conclusions

- A de novo mutation in a spectrin repeat of Trio that has been observed in a patient with an ASD-relate disease and prevents Trio's impact on glutamatergic synapse structure and function.
- This mutation is inhibiting Trio's ability to promote actin polymerization, likely by inhibiting Trio's interaction with another regulatory protein.

Future directions

Now that we have confirmed that this mutation does in fact affect Trio's impact on synaptic structure and function, we next would like to identify proteins that interact with Trio at this particular spectrin repeat. Such a protein likely facilitates Trio's ability to activate Rac1, and thus whose interaction with Trio may be inhibited by this mutation. One such possible protein is Neuroligin-1, which has been shown to interact with Trio's paralog, Kalirin, as shown in the Western blot below.



We would also like to characterize additional synaptic functions that are compromised by this mutation, including synaptic plasticity and long term potentiation as well as synaptic maturation. Identifying these affected processes and proteins can aid in developing therapies that ensure that the signaling cascade that Trio is involved in remains fully functional for normal synaptic transmission.

References and Acknowledgements

Herring BE and Nicoll RA. Kalirin and Trio proteins serve critical roles in excitatory synaptic transmission and LTP. *Proceedings of the National Academy of Sciences* **113**, 2264-2269 (2016).

Sadybekov et al. "Discovery of a ASD-related de novo mutation "hotspot" in Trio's GEF1 domain." *Under revision.*

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