Functional alteration in gating behavior of IP3R channel mediating Calcium Signaling as common biomarker in Autism Spectrum Disorder

GALINA SCHMUNK, RACHEL L. NGUYEN, DAVID FERGUSON, YULIA MEDVEDEVA, IAN PARKER, J.JAY GARGUS.

[1]*Department of Physiology and Biophysics, School of Medicine, University of California, Irvine, Irvine, CA*
[2]*Center for Autism Research and Translation, University of California, Irvine, CA*

Background:
To date, only clinical measures can be used to diagnose Autism Spectrum Disorder (ASD), a developmental disorder that ranges widely in severity but shares core characteristics of deficits in social interaction, verbal and non-verbal communication, and repetitive interests and behaviors. However, even at its most refined, ASD remains a group that is defined by behavioral qualities, not by its pathogenesis. Studies on the genetic architecture of ASD have brought light to the central role of calcium signaling pathways implicated in ASD pathogenesis. For this reason, we dissected functional components of calcium signaling in ASD and used a super-resolution STORM system to obtain molecular-resolution optical patch clamp analysis on monogenic ASD models, resolving a shared functional state-change in IP3R channel gating. There was no change in channel conductance or latency in opening, but the model ASD cell channels shared a common "flicker" kinetic component of opening, in which the elementary event duration follows a single exponential, with a dramatically reduced t½ (15 msec) when compared with controls (32 msec). Then using a high throughput FLIPR assay, we were able to capture a highly reproducible diagnostic that is able to detect this differences in IP3-mediated calcium signaling between fibroblasts derived from typical "sporadic" autistic patients and healthy control patients. Our results show an appreciable deficit in IP3-induced calcium signaling in skin cell lines derived from autistic patients when compared to paired control cell lines, which continues to be resolved in their IPSc- derived lines, all in the absence of mutations in the IP3Rs. This may be a compelling biomarker in fibroblasts as a measure for early detection and in future studies as a tractable therapeutic target.

Objective: We propose that depressed function in IP3R mediated calcium release channels in the ER may be a reliable diagnostic biological marker for monogenic and 'sporadic' forms of ASD.

Methods: Functional components of calcium signaling were dissected in ASD and a super-resolution STORM system was used to obtain molecular-resolution optical patch clamp analysis on monogenic ASD models. Fibroblasts derived from skin biopsies of healthy, unaffected individuals and patients with rare monogenic forms or 'sporadic' autism were cultured and monitored for agonist-evoked calcium signals using a high throughput FLIPR assay. Human induced pluripotent stem cells (hiPSCs) were generated from primary fibroblasts using the Thermo-Fisher Sendai virus protocol. hiPSCs were differentiated into neuronal progenitors, and measured with UV-activated caged iP3.

Results: Local IP3-mediated calcium signaling was decreased in fibroblasts derived from patients with monogenic forms of ASD when compared to those derived from healthy, control patients. Likewise, IP3-mediated calcium signaling was repeatedly decreased in fibroblasts derived from patients with monogenic or 'sporadic' forms of ASD when compared to healthy, control patients. iPSC- derived neuronal precursors from patient fibroblasts also share this signaling defect.

Conclusion: Our results strongly implicate deregulated calcium signaling in the pathogenesis of ASD and supports iP3-mediated calcium signaling as a diagnostic biological marker for ASD. Furthermore, a high-throughput FLIPR assay may be used as a highly reproducible diagnostic that is able to capture differences in IP3-mediated calcium signaling.
