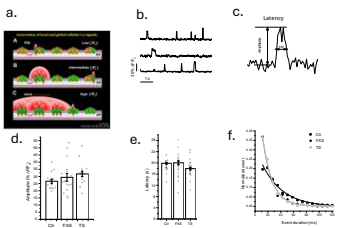


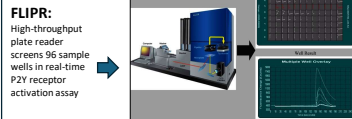
Introduction

- Autism spectrum disorder (ASD) has no defined biomarkers for diagnostics or novel drug discovery.
- A major cluster of ASD-associated loci encode calcium signaling proteins.
- Functionalizing these signals in fibroblasts showed only intracellular Ca²⁺ release altered in rare forms of monogenic ASD syndromes (associated with *FMR1*, *TSC1*, *TSC2*).
- A molecular defect in IP₃ channel gating was resolved with single-channel optical patch clamp showing all forms have a reduced mean open-time (an unstable flicker opening) and therefore low flux, while channel abundance, selectivity, conductance, and latency are unchanged, similar to kinetic changes seen in genetic channelopathies.¹

Figure 1: Single Channel Gating is Altered in ASD¹



- A high-throughput FLIPR screen was used to image global Ca²⁺ signals from the channel, revealed as depressed signals from P2Y purinergic stimuli in syndromic and non-syndromic ASD.
- Other monogenic brain disorders signaled like neurotypical controls.²



Methods & Results

Figure 2: Representative Ca²⁺ responses to extracellular application of purinergic agonists and ionomycin in absence of extracellular Ca²⁺ in fibroblasts from ASD subjects & neurotypical controls³

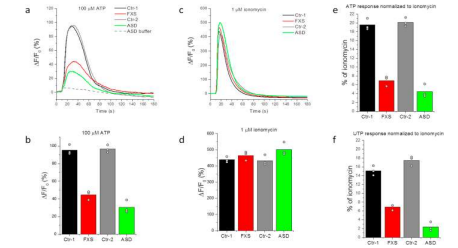
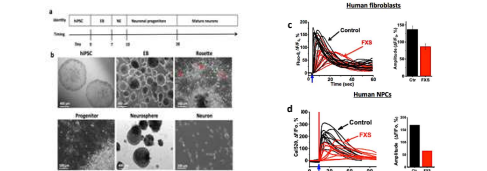


Figure 3: Signaling in iPSC derived neurons share ASD calcium signaling deficiency.³ iPSC-derived gaba neuronal precursors, generated along protocol in A and shown in B, from FMR1 ASD patient fibroblasts share the signaling defect seen in fibroblasts, shown in C and D.



Receiver operating characteristic (ROC) curves can distinguish at 84% AUC high sensitivity and specificity, between syndromic and sporadic ASD samples, which signal with a similar small, slow wave in this assay, while neurotypicals have a fast, large signal (Figure 4,5).³ Therefore, IP₃R signaling appears to be at a node in a signaling pathway at which many forms of ASD are unified into a shared defective output that limits intracellular Ca²⁺ release.

Figure 4: ATP Response Normalized to IM

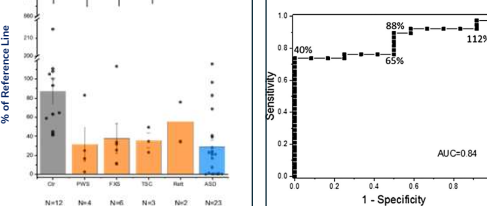
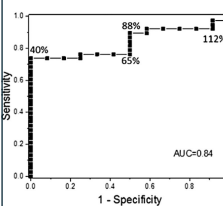
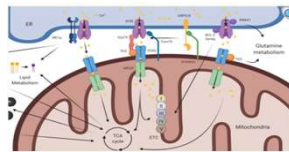


Figure 5: ROC curves for ATP-evoked Ca²⁺ signaling in sporadic and syndromic ASD combined vs neurotypical controls.



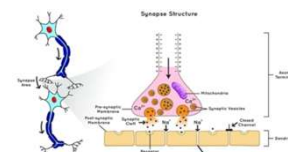
Cell Organelles and CNS Disease

It is well established that IP₃R Ca²⁺ release flux is required to maintain proper mitochondrial functioning, setting the rate of ATP production.⁴ Defects in mitochondrial bioenergetics are a phenotype long associated with ASD^{5,6} and abnormalities in mitochondrial energetics have recently been discovered via cortical brain imaging in areas responsible for social/facial stimuli.^{7,8} IP₃R signaling impacts the full ASD phenotype including GI and immune dysfunctions and the excitation/inhibition ratio as the final step in axonal synaptic neurotransmitter release. The ER is also a key stress sensor, perhaps contributing to environmental inputs to ASD.



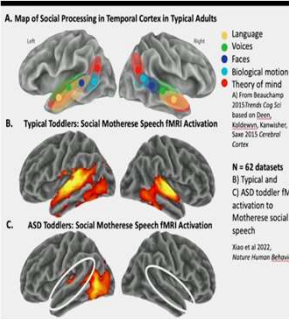
Mitochondrial energy-deficient phenotype

IP₃R Ca²⁺ signaling in neuronal cells impacts, via MAMs (structure shown above) all mitochondrial ATP production and energetics via control of the three calcium-sensitive TCA dehydrogenases.



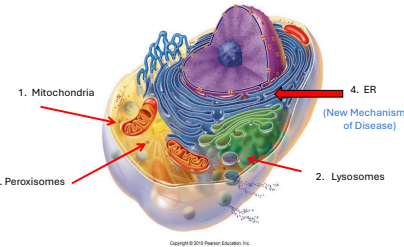
Voltage-activated calcium channel flux is amplified by ER calcium release which triggers fusion of docked synaptic vesicles at axon terminus, releasing presynaptic neurotransmitter into synapse to activate post-synaptic dendrite.

Mitochondrial energy-deficiency in social signals in ASD temporal lobe^{7,8}



Mitochondrial energy-deficient signal seen in right temporal lobe "social" areas in fMRI in ASD. The same signal is detected in EEG gamma frequency waves, and at much higher resolution is detected with near infrared spectroscopy of the neuronal mitochondrial cytochrome oxidase copper center (7,8).

Defects in all organelles cause CNS diseases and... All are diagnosed with a functional assay on skin biopsy



IP₃R functional defect is suggestive that ASD is yet another organellar disease, along with those of mitochondria, lysosomes and peroxisomes, and similarly diagnosable in fibroblasts.

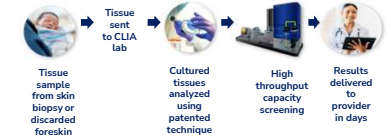
Conclusions

- In monogenic ASD syndromes, a molecular defect in the ER organelle IP₃ channel gating is seen
 - All forms have a short flicker open time, similar to that seen in genetic seizure, arrhythmia, and muscle channelopathies. It is post-translational, perhaps conveyed by lipid raft, and they have no mutation. It is a new form of organelle disease, an organelle channelopathy
 - A high-throughput screen was developed to capture this defect in the monogenic and typical ASD.
 - Patent issued and is licensed by NeuroQure, Inc.
 - iPSC-derived neuronal precursors from patient fibroblasts share this signaling defect.
 - ROC curves can distinguish ASD samples and neurotypical controls which give a robust rapid signal.
 - The assay captures 75 – 85% of ASD with minimized false positives, none below cut-off.
 - IP₃R signaling appears to be at a node in a signaling pathway, at which many forms of ASD are unified into a shared defective output that limits intracellular Ca²⁺ release.
 - A) It controls mitochondrial ATP production
 - ...Explaining mitochondrial energy-deficiency biochemical phenotype & excess ROS.
 - B) EU-AIMS & MIT mitochondrial energy deficiency in right temporal lobe signals,
 - ...particularly in response to social/facial/voice stimuli in high-risk/low-risk infant cohorts
 - by EEG, fMRI, eye tracking, and best by mitochondrial NIRS
 - C) IP₃R release is final step in axonal synaptic neurotransmitter release...E/I imbalance
 - D) GI and immune dysfunction in ASD...both influenced by IP₃R function
 - ASD is not just a brain disorder
 - E) Ca²⁺ homeostasis of the endoplasmic reticulum is the fundamental cellular stress sensor
 - heat-shock, unfolded protein, etc.
- This IP₃R functional biomarker is useful as an autism risk detection test at any age, a clinical trial surrogate outcome measure or entry criterion, and potentially in a screen for novel therapeutics for ASD and for environmental stress risks for ASD.

Take Home Messages

- ASD has no defined biomarkers for diagnostics or novel drug discovery.
- IP₃R signaling appears to be at a node in a signaling pathway at which many forms of ASD are unified into a shared defective output.
- This highly specific clinical assay assesses autism risk at any age, with 75-85% of syndromic and non-syndromic ASD detected.
- Early intervention with currently available therapy could begin as early as possible providing the greatest possible achievement for people with ASD.

Diagnosis as Early as Birth



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