Intersection of Epigenetic Regulation and Mitochondrial Function in Autism

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Autism is a common neurodevelopmental disorder with US incidence of 1 in 68 individuals. Autism presents as a spectrum of symptoms that include difficulties with social interactions, verbal and nonverbal communication, and restricted/repetitive behaviors. The overall objective of this project is to determine if genetic modulation of mitochondrial function and metabolism represents a unifying mechanism in autism spectrum disorders [ASD]. We aim to use the intersections of epigenetic ASD susceptibility, metabolic dysfunction as an indicator of ASD pathophysiology, and molecular phenotype for sex differences in ASD susceptibility as a foundation upon which to build a collaborative research program across institutions in South Carolina. We are leveraging the expertise of our investigators plus research capability-enabling strategies to test the hypothesis that neural systems are uniquely sensitive to metabolic disturbances and transcriptional dysregulating agents converge upon mitochondria to disrupt neuronal development and/or function. Preliminary studies from Boccuto and Champaigne have shown alterations in mitochondrial metabolism in human ASD cells and work from Lizarraga points to alterations in nuclear-encoded mitochondrial protein expression in human neurons exposed to epigenetic modifiers associated with increased ASD risk. Drs. Champaigne, Lizarraga and Freeman will present initial studies that bring together a combination metabolic analyses in cell lines from of human ASD individuals, molecular and cellular analyses of human ASD model neurons derived from human induced pluripotent stem cells, and behavioral and tissue-based analyses in a mouse model of ASD using *in utero* exposure to valproic acid. ASD shows a remarkable preponderance of males compared to females. Thus, parallel studies in the Twiss and Bagasra labs focus on sex differences in neuroblastoma cell lines and primary mouse neurons exposed to ASD susceptibility agents, particularly effects on gene expression and mitochondrial function [Work supported by the SC EPSCoR Stimulus Research Program Grant, 18-SR04].

Altered Tryptophan Utilization in Autism Spectrum Disorder

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Previous studies at GGC have shown abnormalities in energy production by lymphoblastoid cell lines (LCLs) from individuals with autism spectrum disorder (ASD) when exposed to the amino acid tryptophan or certain ionic species, suggesting a link between mitochondrial metabolism and ASD. In order to investigate this, we used Biolog technology to test energy production by LCLs from typically developing controls (TDs) in the presence of tryptophan and ions after the exposure to valproic acid (VPA) and its analogue valpromide. VPA is an anti-convulsant, and in utero exposure to VPA has been linked to increased susceptibility for ASD. VPA affects ion transport through cell membranes and inhibits histone deacetylation to alter gene expression. Valpromide replicates VPA's effects on ions but not on histone deacetylation. Therefore, we designed our experiments to test if exposing TD cells to

VPA can replicate the abnormal metabolic activity noted in ASD cells and to assess if VPA's effects are through altered ion transport or epigenetic dysregulation. Preliminary findings on 10 LCLs show no effect of either VPA or valpromide with tryptophan exposure. However, a dose-dependent effect was observed on ion responses (Biolog PM-M5 plate). Almost no differences were detected in cells exposed for 24 hours to either VPA or valpromide, but longer exposure to valpromide generated the similar metabolic abnormalities as observed in ASD cells. Exposure to VPA generated similar but less significant trends. These initial results confirm that alteration of ion transport at the cell membrane can affect the mitochondrial activity and shed new light on VPA susceptibility mechanisms [Work supported by the SC EPSCoR Stimulus Research Program Grant, 18-SR04].

Epigenetic Modulation of Neurotrophin Signaling in Autism Spectrum Disorders

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Chromatin remodeling genes are one of the most mutated gene classes in autism spectrum disorders (ASD), suggesting that widespread genetic changes can alter neuronal development. Recent studies have provided compelling evidence that mutations in the histone methyl-transferase ASH1L are associated with an autism subtype that is associated with postnatal microcephaly, seizures and intellectual disability. However, the molecular mechanisms by which the chromatin modifying factor ASH1L functions in normal development and how it goes awry in ASD has remained elusive. Exposing human neurons to valproic acid (VPA) alters their expression of ASH1L. We find that ASH1L modulates neuronal arborization and it is a regulator of the neurodevelopmentally important BDNF/TrkB signaling pathway. Activation of TrkB by the neurotrophin BDNF regulates neuronal morphogenesis and development. Further, TrkB activation has been shown to rescue neuronal circuitry defects in animal models of syndromic autism. Therefore, our work provides a potential link between epigenetic regulators, the BDNF-TrkB signaling pathway, and the structural development of neuronal connectivity [Work supported by the SC EPSCoR Stimulus Research Program Grant, 18-SR04].

Evaluating Nicotinamide Riboside Supplementation in a Mouse Model of Autism

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Previous research has shown that autism spectrum disorders (ASD) may be characterized by altered mitochondrial respiration through defective tryptophan utilization. The aim of our study is to test the possibility that a mouse ASD phenotype can be rescued by targeting tryptophan metabolic pathways. Tryptophan is an important precursor in the kynurenine pathway to generate nicotinamide adenine dinucleotide (NAD+). We are using a well-characterized *in utero* exposure to valproic acid (VPA) as our mouse model. Post-natal treatment with the nutraceutical nicotinamide riboside (NR) was used to determine if increasing cellular NAD+ levels would reverse or improve ASD-linked behaviors. NR is precursor of NAD+, thus it brings potential to elevate NAD+ and increase mitochondrial function. We have evaluated an initial cohort of animals on a behavioral battery including: the open field test, three-chamber sociability test, marble burying, and the elevated plus maze. We are currently expanding the study to include more animals. [Work supported by the SC EPSCoR Stimulus Research Program Grant, 18-SR04].