Contribution to Science

A. **Abnormalities in Mitochondrial Function in Autism Spectrum Disorder**: Abnormalities in mitochondrial function appear to affect a significant number of children with ASD but the nature of these abnormalities is poorly understood. Through critical literature reviews, clinical research and basic research, my team and I have investigated the nature of mitochondrial abnormalities in ASD, specifically the connection between other metabolic abnormalities associated with ASD such as redox metabolism abnormalities and mitochondrial dysfunction. Our recent research suggests the nature of mitochondrial dysfunction in ASD is distinct from other forms of mitochondrial disease in its metabolic nature and with respect to molecular signaling pathways

1. Rossignol, D., ***Frye, R.E.*** **Mitochondrial Dysfunction in Autism Spectrum Disorders: A Systematic Review and Meta-Analysis**. Molecular Psychiatry, 2012 Mar;17(3):290-314. doi: 10.1038/mp.2010.136. PMID: 21263444,.
2. ***Frye, R.E.,*** Rossignol, D. **Mitochondrial dysfunction can connect the diverse medical symptoms associated with autism spectrum disorders.** Pediatric Research, 2011 May;69(5 Pt 2):41R-7R. PMID: 21289536,.
3. ***Frye, R.E.,*** DeLaTorre, R. Taylor, H. Slattery, J., Melnyk, S. James, S.J. **Redox Metabolism Abnormalities in Autistic Children Associated with Mitochondrial Disease**. Translational Psychiatry, 2013 3:e273. doi: 10.1038/tp.2013.51.
4. Rose, S., ***Frye, R.E.,*** Slattery, J., Melnyk, S., Wynne, R., Tippett, M., James, S.J. **Oxidative Stress Induces Mitochondrial Dysfunction in a Subset of Autism Lymphoblastoid Cell Lines in a Well-Matched Case Control Cohort**. PLOS One, 2014 Jan 8;9(1):e85436. doi: 10.1371/journal.pone.0085436
5. Rose, S., ***Frye, R.E.,*** Slattery, J., Melnyk, S., Wynne, R., Tippett, M., James, S.J. **Oxidative Stress Induces Mitochondrial Dysfunction in a Subset of Autism Lymphoblastoid Cell Lines**. Translational Psychiatry, 2014; 4:e377. doi: 10.1038/tp.2014.15.

B. Redox Metabolism in Autism Spectrum Disorder: Abnormalities in redox metabolism affects a significant number of children with ASD. Through critical literature reviews and clinical and basic research, my team, along with our colleagues Drs James and Melnyk, have investigated the nature of redox abnormalities in ASD, specifically with respect to treatment and their connection to abnormalities in mitochondrial function.

1. Rossignol, D., ***Frye, R.E.*** **A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures,** Molecular Psychiatry, 2012 Apr;17(4):389-401. doi: 10.1038/mp.2011.165. PMID: 22143005.
2. Rose, S., Melnyk, S., Pavliv, O., Bia, S., Nick, T., ***Frye, R.E***., James, J.S. **Evidence of oxidative damage and inflammation associated with low glutathione redox status and mitochondrial dysfunction in the autism brain**. Translational Psychiatry, 2012 Jul 10;2:e134. doi: 10.1038/tp.2012.61. PMID: 22781167,
3. ***Frye, R*.E.,** James, S.J. **Metabolic pathology of autism in relation to redox metabolism**. Biomarkers in Medicine, 2014; 8(3):321-30. doi: 10.2217/bmm.13.158. PMID: 24712422
4. ***Frye, R.E.***, Melnyk, S., Fuchs, G., Reid, T., Jernigan, S., Pavliv, O., Hubanks, A., Gaylor, D.W., Walters, L., James, S.J. **Effectiveness of methylcobalamin and folinic acid treatment on adaptive behavior in children with autistic disorder is related to glutathione redox status.** Autism Research and Treatment, 2013;2013:609705. doi: 10.1155/2013/609705. Epub 2013 Oct 12.

C. **Folate Receptor Alpha Autoantibodies in Autism Spectrum Disorder**. There are few medical treatments for children with autism spectrum disorder (ASD) that addresses either core symptoms or pathophysiological abnormalities associated with ASD. Our preliminary study in **Molecular Psychiatry** demonstrated that about 75% of children with ASD have autoantibodies that impede the ability of folate to enter the central nervous system. We also showed that children with ASD and these autoantibodies respond favorably to a special type of folate called folinic acid which can circumvent the blockage. Unpublished pilot data from a double blind placebo controlled study that confirms the open label findings of the **Molecular Psychiatry** report.

1. ***Frye, R.E.,*** Sequeira, J.M., Quadros, E.V., James, S.J., Rossignol, D. **Cerebral folate receptor autoantibodies in autism spectrum disorder,** Molecular Psychiatry, 2013 Mar;18(3):369-81. doi: 10.1038/mp.2011.175. PMID: 22230883.

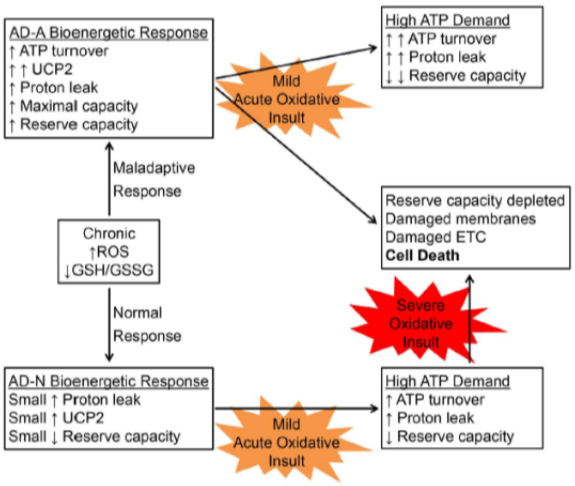
D. **Autism Treatments:** The evidence for many medical treatments for children with ASD is preliminary and not well documented in many cases. I have worked with colleagues and working groups to lead systematic reviews of the literature with regards to the evidence base for treatment for ASD so clinicians can have better guidance for prescribing agents to improve core and associated ASD symptoms. In addition, I have recently completed an open-label trial examining the metabolic and behavioral effects of tetrahydrobiopterin, a treatment that is hypothesized to treat both core symptoms of ASD and metabolic abnormalities associated with ASD (neurotransmitter deficits, redox and nitric oxide abnormalities).

1. ***Frye, R.E.***, DeLaTorre, R., Taylor, H.B, Slattery, J., Melnyk, S., James, S.J. **Metabolic Effects of Sapropterin Treatment in Autism Spectrum Disorder: A Preliminary Study.** Translational Psychiatry. 2013 Mar 5; 3: e237. doi: 10.1038/tp.2013.14. PMID: 23462988.
2. ***Frye R.E.,*** Rossignol, D.A. **Treatments for biomedical abnormalities associated with autism spectrum disorder.** Front Pediatr 2014; 2:66. doi: 10.3389/fped.2014.00066. PMID: 25019065.
3. Rossignol, D.A., ***Frye, R.E.*** **The use of medications approved for Alzheimer's disease in autism spectrum disorder: a systematic review.** Front Pediatr. 2014; 2:87. doi: 10.3389/fped.2014.00087. PMID:25202686.
4. Rossignol, D., ***Frye, R.E.*** **Melatonin in Autism Spectrum Disorders: A Systematic Review and Meta-analysis.** Developmental Medicine and Child Neurology, 2011 Sep;53(9):783-92. doi: 10.1111/j.1469-8749.2011.03980.x. PMID: 21518346.
5. ***Frye, R.E.,*** Rossignol, D., Casanova, M.F., Martin, V., Brown, G., Edelson, S.M., Coben, R., Lewine, J.D., Slattery, J.C., Lau, C., Hardy, P., Fatemi, S.H., Folsom, T.D., MacFabe, D.F., Adams, J. **A review of traditional and novel treatments for seizures in autism spectrum disorder: Findings from a systematic review and expert panel.** Frontiers in Public Health, 2013 1:31. doi: 10.3389/fpubh.2013.00031. **Featured Article**

E. Microbiome: Increasing evidence suggests that the enteric microbiome plays a critical role in the etiology and symptomatology of ASD, at least in some cases. To better understand this connection, in June 2014 my staff and I organized and hosted the ***1st International Symposium on the Microbiome in Health and Disease with a Special Focus on Autism*** followed by a workshop the next day focusing on approaches to studying and manipulating the enteric microbiome to improve autism symptoms. From this meeting a special issue of ***Microbial Ecology in Health and Disease*** was published. We have also investigated the clinical and basic research aspects of enteric microbiome metabolites on mitochondrial function.

1. ***Frye, R.E.,*** Slattery, J., MacFabe, D.F., Allen-Vercoe, E., Parker, W., Rodakis, J., Adams, J.B., Krajmalnik-Brown, R., Bolte, E., Cerniglia, C.E., Kahler, S., Jennings, J., James, S.J., Midtvedt, T. **Approaches to studying and manipulating the enteric microbiome to improve autism symptoms**. Microbial Ecology in Health and Disease, 2015 May 7;26:26878.
2. ***Frye, R.E.,*** MacFabe, D.F. **Gastrointestinal dysfunction in autism spectrum disorder: The role of the mitochondria and the enteric microbiome**. Microbial Ecology in Health and Disease, 2015 May 7;26:27458.
3. ***Frye, R.E.,*** Melnyk, S. MacFabe, D. **Unique acyl-carnitine profiles are potential biomarkers for acquired mitochondrial disease in autism spectrum disorder**. Translational Psychiatry, 2013 Jan 22;3:e220. doi: 10.1038/tp.2012.143.

Basic Research Laboratory



Dr Frye's laboratory aims to define the unique abnormalities in mitochondrial function associated with autism spectrum disorder (ASD). The field of mitochondrial medicine is still in the early stages of development, making it an excellent field for pioneering research. Over the past decade, many sources of data point to abnormalities in mitochondrial function associated with ASD but some of the data appears inconsistent if one views it from the perspective of classic mitochondrial disease. Classic mitochondrial disease is viewed as a failure of the mitochondria to produce energy. However, in our studies we have found that a subset of lymphoblastiod cell lines from children with ASD have a unique type of mitochondrial dysfunction where the mitochondrial is overactive, producing almost twice the amount of energy typically produced in a control cell. We find that when the mitochondria is in this state it is very sensitive to metabolic stresses such that a metabolic stress that might be mild to a normal cell would be deadly to the ASD cell such that a small stressor could cause cellular dysfunction and death. We initially reported this discovery in two papers, one in *PLOS ONE* and one in *Translational Psychiatry*.

Since that time we have taken several paths to validate our findings, discover the cause of these changes in mitochondrial function and investigate potential therapeutic treatments that could be investigated further in clinical trials. First we have demonstrated that these unique

changes in mitochondrial occur in patients, not just cell line models. We have confirmed our findings in fresh immune cells derived from over 150 children with ASD, muscle and skin biopsies from over 50 children with ASD and buccal cells obtained from over 100 children with ASD. In addition, in collaboration with Harvard Medical School / Massachusetts General Hospital, we have demonstrated this unique change in mitochondrial function in biopsies obtained from the gastrointestinal tract of children with ASD. This latter finding is important and groundbreaking. This explains the reason behind why gastrointestinal disorders in children with ASD are poorly understood and difficult to treat.

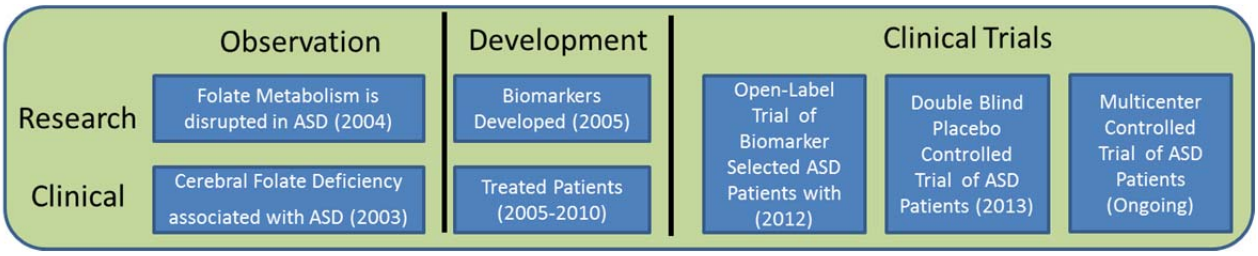
Next we have been investigating the causes of these changes in mitochondrial function. Since the mitochondria is particularly susceptible to environmental influences we sought to understand who these changes in mitochondrial function are differentially affected by environmental influences and whether particular detrimental environmental influences linked to ASD could induce these unique mitochondrial changes. We have demonstrated that certain common environmental toxins seem to be able to induce the unique pattern of mitochondrial function in control cell lines, suggesting that it is possible that some environmental agents may have a role in producing these unique abnormalities in mitochondrial function seen in children with ASD. To this end Dr Rose, the post-doctoral fellow in the Autism Program has received the Marion B. Lyon New Scientist Development Award to investigate other potential environmental agents that might cause similar changes to mitochondrial function. We are also collaborating with the University of Texas at San Antonio to analyze baby teeth of environmental exposures and correlate changes in mitochondrial function with specific exposures on a case-by-case basis in children with ASD. The previous R01 we submitted was well received but not funded and will be resubmitting the application in the near future.

Next we have investigated the molecular mechanisms that have resulted in these unique changes in mitochondrial function in the ASD cell lines. By systematically evaluating several molecular pathways we have found that PGC 1α appears to be a key regulator in these changes but that other key downstream pathways do not respond to PGC 1α as expected. Specifically, the mTOR pathway, a pathway important for neurodevelopment that has been implicated in ASD, appears to be dysregulated. In collaboration with Dr Laura James' laboratory we have found that specific microRNAs may be abnormally regulating mTOR such that mTOR is tonically inhibited but hyper-reactive to PGC 1α signals resulting in wide variation in mitochondrial function. We are conducting further experiences to investigate these changes on systematically manipulated cell lines.

We are investigating how to improve mitochondrial function and protect mitochondrial against environmental stressors. These preclinical studies are being conducted with a goal of developing potentially life changing therapies for children with ASD. In our published experiments we have demonstrated that N-acetyl-cysteine can protect atypically function mitochondrial from oxidative insults. We have recently completed a contract with Mitosynergy, LLC (Kaukauna, WI) to examine the therapeutic effect of a special form of copper, Cunermuspir, that should improve Complex IV function in the electron transport chain of the mitochondria. We now have contracts with Reata Pharmaceuticals, Inc, (Irving TX) and GW Pharmaceuticals (Cambridge, UK) to investigate novel compounds for modulating mitochondrial function.

Translational Research Laboratory

The Translational Research Program has been engaged in developing novel treatments and biomarkers for children with ASD.



Dr Frye has developed the first medical treatment that targets abnormal physiological processes underlying ASD. This safe and effective treatment for children with ASD significantly improves verbal communication within 12 weeks. The treatment is a special form of folate known as folinic acid (leucovorin calcium) that bypasses blocks in folate metabolism that are associated with ASD. This discovery arose from careful research and clinical observations combined with the discovery of biomarkers for folate abnormalities. These observations were used to develop and conduct a preliminary open-label trial conducted by Dr Frye which showed that children with ASD and folate pathway abnormalities had significant improvement in verbal communication within a few months of treatment initiation. Dr Frye's has now completed a single-site double-blind placebo controlled trial at Arkansas Children's Research Institute that objectively confirms these preliminary observations. The study also demonstrates that the folate receptor alpha autoantibody, a biomarker of folate metabolism abnormalities, can predict which children with respond to the treatment. The Number Needed to Treat for children ASD and language impairment who are positive for the folate autoantibody is less than two which means that one child will response significantly for each two treated. This demonstrates the extraordinary effectiveness of the treatment and the biomarker being studied. A multicenter trial is planned to launch soon to confirm the effectiveness of this treatment in ASD, leading this treatment to become widely used and potentially improve the lives of millions of children with ASD.

Industry Supported Clinical Trials

Dr Frye has been involved in some of the most cutting edge industry sponsored clinical trials in autism research. We were a site for one of the largest international studies on autism which investigated the effect of memantine on core autism symptoms. We were one of a few select sites to study a highly novel therapy, Trichuris suis ova, for core autism symptoms. Currently we a site of a novel treatments for core autism symptoms that targets abnormalities in protein digestion that has been linked to autism and we are one of a few select sites involved in a pioneering study examining metabolites signatures specific to autism in order to develop a test for early diagnosis and screening for autism. In the near future we will be involved in a highly novel clinical trial which transplants a health microbiome into the guts on children with autism in order to improve gastrointestinal and core autism symptoms.