Biomedical/Epigenetic Research for Assessment & Treatment of ASD

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Faculty Disclosure

- Robert L. Hendren, DO, is a member of Advisory Boards for BioMarin Pharmaceutical Inc., Forest Laboratories, Coronado Bioscience, BioZeus and Janssen and has received financial support for clinical trials from Autism Speaks Inc, BioMarin Pharmaceutical Inc., Curemark, Forest Laboratories, Roche, Vitamin D Council, and the National Institute of Mental Health.

Prevalence of Autism

- 25 years ago 1/10,000
- 10 years ago 1/500 – 1/1,000
- Current (2012) 1/88; maybe 1/50
- 5:1 boys:girls 1 in 54 boys
- 78% increase between 2002-2008
- There has been a > 600% increase in prevalence over the past 2 decades.[2]

[1]NICHD. *Autism Overview*. NIH; 2005. NICHD website. http://www.cdc.gov/ncbddd/autism/data.html. Accessed April 1, 2013.
[2]http://autisminnb.blogspot.com/2011/01/autism-speaks-cdc-autism-prevalence.html. Accessed April 1, 2013.

Prevalence of Autism (cont'd)

- Possible explanations include diagnostic expansion and substitution, better reporting, increased recognition, increasing acceptability, immigration for services, environmental toxins infectious and immune vulnerability and epigenetics.[1,2]

[1]Rutter M. *Acta Pediatric*. 2005;94:2-15.
[2] Centers for Disease Control and Prevention. *Autism Spectrum Disorders*. CDC website. http://www.cdc.gov/ncbddd/autism/. Accessed July 11, 2011.

ASD Genetic Etiology

- Multiple genes - 2q, 7q31-36, 15q11-13, 16p11.2, SHANK 3, NLGN ¾, PTEN
- Identical twins - 60% - 90%
- Fraternal twins – 0 - 36%; siblings 4% - 19%
- Clear genetic etiology account for 25% of autism cases
- Hundreds of genetic mutations, some de novo, lead to many ways to develop and treat autism.

Freitag CM, et al. *Eur Child Adolesc Psychiatry*. 2010;19:169-178.
Levy D, et al. *Neuron*. 2011;70:886-897.
Miles JH. *Genet Med*. 2011:13(4):278-294.
State; Eichler; Daly Nature

ASD and Environmental Risk

- Documented: Prenatal or early postnatal exposure to viral infections (rubella), valproic acid, thalidomide[1,2]
- Proposed: Influence of mercury, lead, environmental toxins, vaccines, lack of vitamin D[1,2]
- Parental age[3,4] in multiple generations [5,6]
- Maternal metabolic conditions[7]
- Influenza or fever during pregnancy[8]
- Genetic susceptibility[1,2]

[1]Herbert MR. *Curr Opin Neurol*. 2010:23:103-110.
[2]Landrigan PJ. *Curr Opin Pediatr*. 2010;22:219-225.
[3]Durkin MS, et al. *Am J Epidemiol*. 2008;168:1268-1276.
[4]Shelton JF, et al. *Autism Res*. 2010;3:30-39.
[5]Frans, EM et al. *JAMA Psychiatry*. 2013
[6]Silver, M.
[7]Krakowiak, 2012.
[8]Zerbo O, *JADD*, 2012.

ASD and Environmental Pollution

- Environmental mercury release, special education rates, and autism disorder: and ecological study of Texas[1]
- Children with autism more likely to be exposed to traffic related air pollution during gestation (AOR, 1.98) and during thte first year of life (AOR, 3.10)[2]
- Maternal residence near agricultural pesticide applications and ASD in California central valley[3]
- Children of migrant parents were at increased risk of low-functioning autism (AOR, 1.5, 95% CI 1.3-1.7)[4]

[1]Palmer RF et al. Health Place. 2006;12:203-209.
[2]Volk HE et al. JAMA Psychiatry 2013; 70:71-77
[3]Roberts EM et al. Environ Health Perspect. 2007;115:1482-1489.
[4]Magnusson C, Br J Psychiatry. 2012; 201:109-15

Model for Autism Etiology

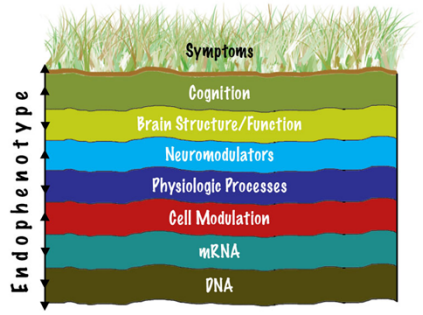
- First hit - Genetic neurodevelopmental vulnerability
- Second hit - environmental "stressor" and interaction between the two (Hallmayer J, Risch. *N. Arch Gen Psychiatry*. 2011 [Epub].)
- Third hit - Restricted development

Endophenotypes

- Phenotypes represent the full picture of the expression of an individual's genes given the environment
- Endophenotypes are partial "internal phenotypes" or collections of subclinical traits that are illuminated by a more fine-grained examination of a phenotype
- They are closer to the site of the primary causative agent than diagnostic categories
- Epigenetics encompasses all layers of genetic control
- Epigenetic changes may become permanent and get passed to future generations
- Suggest targets for intervention

Gottesman II, Gould TD. Am J Psychiatry. 2003;160:636-645. Skipper MK. Nature. 2010.

Translating from "Terroir" Model



mRNA: messenger ribonucleic acid
DNA:deoxyribonucleic acid

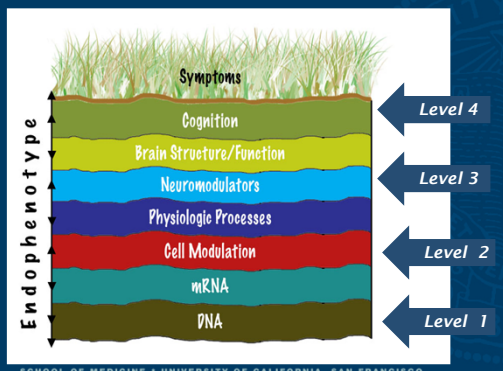
Gene-Enviroment (G-E) Interaction and Endophenotype

Level 2

- Immune abnormalities/Inflammation (Goines P, Van de Water J. *Curr Opin Neurol.* 2010;23:111-117.)
- Oxidative stress (James SJ. *Am J Clin Nutr.* 2009;89:425-430.)
- Disturbed methylation (James SJ. *Am J Clin Nutr.* 2009;89:425-430.)
- Mitochondrial dysfunction (Frye RE, Rossignol DA. *Pediatr Res.* 2011;69(5(2)):41R-7R.)
- Free fatty acid metabolism (Bell JG, et al. *Br J Nutr.* 2010;103:1160-1167.)
- Excitotory/inhibitory imbalance (Rubenstein JL. *Curr Opin Neurol.* 2010;23(2):118-123.)
- Hormonal effects (Harony H, Wagner S. *Neurosignals.* 2010;18:82-97.)

Moving Treatment Targets

Translating Levels of Assessment



Neurodevelopmental Biomedical Assessment

LABS

- Metabolic Panel – glucose and liver function tests (LFTs)
- Complete blood count (CBC), Differential and Sedimentation
- Magnesium (red blood count (RBC))
- Selenium
- Zinc/copper (RBC)
- Vitamin C
- Vitamin D3 (look at both 1,25 (OH) and 25 (OH)
- Fat soluble vitamins
- Ferritin, Total Iron (Fe), total iron binding capacity (TIBC), % Fe sat
- Lead screening
- Serum amino and urine organic acid if indicated
- Cholesterol - Lipid panel if indicated
- RBC Folate, B12
- Ceruloplasmin

Neurodevelopmental Biomedical Assessment

GENETICS

- Comparative genome hybridization (CGH) array
- Gene expression (mRNA, Transcriptome)

OXIDATIVE STRESS

- Nitro Tyrosine
- Urine porphyrins
- Transferrin/total iron
- Glutathione (GSH)/glutathione disulfide (GSSG)
- Cysteine/Cystine (oxidized)
- 8-OHdG, 8-OHG

MITOCHONDRIAL DYSFUNCTION

- Lactate/Pyruvate
- Carnitine/acetylcarnitine
- Creatine kinase
- Ubiquinone
- Ammonia

Neurodevelopmental Biomedical Assessment

IMMUNE/INFLAMMATION

- Antinuclear antibody test (ANA), erythrocyte sedimentation rate (ESR)
- Anti-casein, gluten, soy, immunoglobulin M (IgG)
- Activated T&B cell subsets
- IgG, IgM, IgA, IgE
- C-Reactive Protein

GASTROINTESTINAL (GI) FUNCTION

- Comprehensive digestive studies: Bristol Stool sample
- GI questionnaires
- Calprotectin

Neurodevelopmental Biomedical Assessment

HORMONES

- Thyroid function: FT3, FT4, TSH
- Cortisol: saliva
- Oxytocin/ vasopressin

ALLERGY

- IgG, IgE food antibodies if indicated

TOXICS

- Urine prophyrins

CEREBRAL SPINAL FLUID (CSF)

- Brain-derived neurotrophic factor (BDNF)
- Folate

Neurodevelopmental Biomedical Assessment

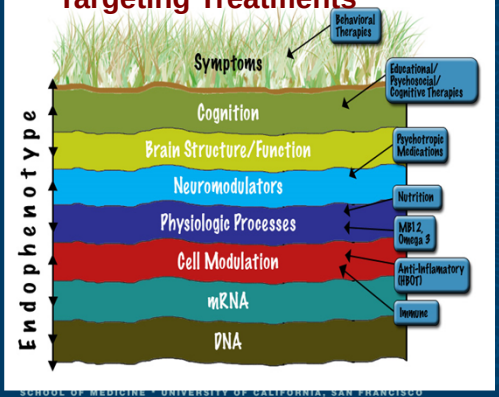
ROUTINE

- Electroencephalogram (EEG) during regression
- Extended EEG for seizure activity

RESEARCH

- Structural T1 magnetic resonance image (MRI) (volmetric)
- Quantified EEG (QEEG)
- Near-infrared reflectance spectroscopy (NIRS)
- Zeo sleep monitor (if already doing overnight)
- Wearable monitors (e.g. for Autonomic Nervous System (ANS))

Targeting Treatments



Biomedical Therapeutic Strategies

Immune/Inflammation
- Melatonin
- IV/IG
- Corticosteroids
- NSAIDS - e.g.) Celecoxib (Asadabadi, *Psychopharmacology*, 2012.)

Methylation
- Folic/folinic Acid – Suren P, JAMA, 2013, 309:570-77

Mitochondrial Function (Kelly RI)
- *Carnitine*
- *Coenzyme Q 10*
- *Vitamin C*
- *Lipoic acid*
- *Pantothenate*
- *Vitamin E*

Biomedical Therapeutic Strategies

Oxidative Stress
- Glutathione
- Methyl B12
- Curcumin anti-inflammatory and antioxidant activity

Neurotransmitter Production
- Tetrahydrobiopterin (Kuvan) – (Frye RE, Transl Psychiatry, 2013, 5:e237)
- Rivastigmine (Exelon) parasympathomimetic or cholinergic agent
- Galantamine acetylcholinesterase inhibitor

GABA
- Arbaclofen (STX209)
- Bumetanide – is a diuretic
- tiagabine (Gabitril)

Glutamate
- Riluzole - used to treat amyotrophic lateral sclerosis (ALS)
- D-cycloserine - partial agonist of the neuronal NMDA receptor

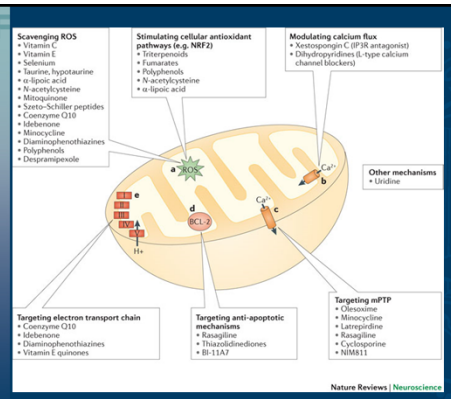


FIGURE | Investigative, pharmaceutical and nutritional compounds that target various aspects of mitochondrial function. FROM THE FOLLOWING ARTICLE:
Hosseini, Manji, Tadafumi Kato, Nicholas A. Di Prospero, Seth Ness, M. Flint Beal, Michael Krams & Guang Chen. Targeted mitochondrial function in psychiatric disorders (May 2012) Nature Reviews Neuroscience 13, 293-307.

Challenges of Biomedical Research

- Sample size for effect size
- Heterogeneity of ASD
- Duration of trial
- Biomarker for inclusion
- Holding other treatments constant
- Blinding
- Formulation variability
- IRB issues
- Ethical issues
- Funding

Autism Translating To Treatment (AT3) Project

Project Leader: Robert L. Hendren, D.O.
Data Coordination Center Leader: Stephen Bent, MD
(Jane Botsford Johnson Foundation)

- To develop an integrated, comprehensive, science-based, assessment of biomedical mechanisms involved in the etiology autism, which will be critical…
- To evaluate the efficacy of targeted treatments for autism and related neurodevelopmental disorders.

Background

- Epigenetic explanation and ASD etiology - changes in gene expression or cellular phenotype, caused by mechanisms beyond changes in the underlying DNA sequence
- Benefits of biomedical treatment that ultimately effect gene expression and improve resilience
- Potentially helpful biomedical treatments not being widely used
- Difficulties in doing Randomized Controlled Trials with biomedical treatment
- Potential role of biomarkers
- Potential role of personalized "precision" medicine and relational outcomes database

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Design and Rational for Practice-Based, Non-Randomized, No Placebo Control

- The study proposed here is a practice-based, longitudinal cohort study intended to obtain the preliminary, "proof of concept" data to prepare for a larger cohort study with greater power to examine associations between biomedical tests, treatments, and clinical responses.
- This proposal aims to initially establish a multi-site network to clarify the viability and efficacy of biomarker profiles.
- Once specific biomarkers & treatments are identified as having potential efficacy, the network can be used to conduct large, randomized-controlled trials of specific interventions.

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Outcome Measures

(baseline, 3, 6 months)

- Diagnostic - Social Communication Questionnaire (parent-completed); DSM-IV Checklist (clinician-completed); IQ-testing (research assistant completed)
- Overall assessment – Clinician CGI-S and CGI-Iand Parent CGI-S and CGI-I
- Behavior – Aberrant Behavior Checklist (ABC)
- Social – Social Responsiveness Scale (SRS)
- Language – Brief language questionnaire
- Sensory Survey
- GI Symptom Questionnaire
- Pediatric Quality of Life

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Biomedical Treatment Studies

UCSF & MIND

- Vitamin D – immune system (Becker KG. *Acta Psychiatr Scand.* 2011;124:74; Hossein-Nezhad A; PLoS One, 2013;8(3):e5872; Patrick & Ames. FASEB J. 2014, Feb 20.
- HBOT – inflammation (Jepson B, et al. *J Autism Dev Disord.* 2011;41:575-588. Rossignol DA, *BMC Pediatr.* 2007;16(7):36.)
- Methyl B12 – oxidative stress (Bertoglio K…Hendren RL. *J Altern Complement Med.* 2010;16:555-560.)
- Omega-3 free fatty acids (FFA) metabolism (Bent S…Hendren RL. *J Autism Dev Disord.* 2011;41(5):545-554.)
- Double-blind placebo controlled study of memantine (Namenda) – excitotoxicity and stimulation of synapse formation
- Pancreatic Digestive Enzymes (Curemark)

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Methyl B12 Study

(MIND & Autism Speaks)

- 30 subjects completed the 12-week, double-blind study.
- No statistically significant mean differences in behavior tests or in glutathione status between active and placebo groups.
- 9 subjects (30%) demonstrated clinically significant improvement on the Clinical Global Impression Scale (CGI) and at least two additional behavioral measures.
- Responders exhibited significantly increased plasma concentrations of GSH and GSH/GSSG.
- 55 new subjects have completed a study at UCSF funded by Autism Speaks. Data in analysis

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Memantine (Namenda)

- Aberrant functioning of N-methyl-D-aspartate (NMDA) receptor and/or altered glutamate may play a role in autism.
- Reports of case series demonstrating significant improvement in language and socialization in children with autism. (Chez, 2006.)
- Well tolerated in children. Some experience fatigue, modest increase in LFTs.
- Multi-site RCT completed (Forest)
- Memantine + risperidone 10 wk RCT -> greater reduction in irritability, stereotypic behavior and hyperactivity (Ghaleiha, Int J Neuropsychopharmacology. 2012, 24:1-7)

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Pancreatic Digestive Enzymes

- Enzyme deficiencies in children with autism result in an inability to digest protein.
- The inability to digest protein affects the production of amino acids, essential for brain function.
- RCT completed (Curemark)
- Biomarker – fecal chymotrypsin

Recent Biomedical Studies and Ideas

- Cerebral Folate Deficiency (Frye, 2013)
- NAC - oxidative stress (Hardan, 2012.)
- Melatonin (Rossignol, 2011)
- Micronutrients (Adams, 2011)
- Oxytocin – trust/socialization (Gregory, et al., 2009.; Anagnostou et al., 2014; Zheng et al., 2014)

Cerebral Folate Deficiency

- High prevalence (75%) of folate receptor-α autoantibodies (FRAs), an autoantibody that prevents folic acid from entering the brain, in children with ASD
- Improvement in ASD symptoms with high-dose folinic acid (2mg/kg/day; max 50mg; in two divided doses
- 12-week treatment with high-dose folinic acid in children with ASD improves mitochondrial function, specifically the ability of the mitochondrial to be resilient against oxidative stress

Frye RE et al: Mol Psychiatry. 2012; Ramaekers VT, Mol Psychiatry. 2012

N-Acetylcysteine (NAC) in Children with Autism

- NAC is an glutamatergic modulator and an antioxidant.
- 12-week, double-blind, randomized, placebo-controlled study of NAC in children with autistic disorder.
- NAC was initiated at 900 mg daily for 4 weeks, then 900 mg twice daily for 4 weeks and 900 mg three times daily for 4 weeks.
- Thirty-three subjects (31 male subjects, 2 female subjects; aged 3.2-10.7 years) were randomized.
- Oral NAC was well tolerated with limited side effects.
- Compared with placebo, NAC resulted in significant improvements on ABC irritability subscale. (F = 6.80; p < .001; d = .96)

Hardan A, *Biol Psychiatry*. 2012;956-961.

Melatonin

- Endogenous neurohormone causes drowsiness, and sets the body's sleep clock.
- Review and meta-analysis of 35 studies reported that of 18 treatment studies, there were 5 randomized controlled trials (RCTs) (N = 61, 2-10 mg/day) where sleep duration (44 min, ES=0.93) was increased, sleep onset latency was decreased (39 min, ES = 1.28) but nighttime awakenings were unchanged. Side effects were minimal to none.

Rossignol DA, Frye RE. Dev Med Child Neurol. 2011;53(9):783-92.

Vitamin/Mineral Supplement and ASD

- RCT of oral vitamin/mineral supplement for 3 months with 141 children and adults with ASD.
- Improved the nutritional and metabolic status of children with autism, including improvements in methylation, glutathione, oxidative stress, sulfation, ATP, NADH, and NADPH.
- The supplement group had significantly greater improvements than did the placebo group on the Parental Global Impression-R Average Change (p=0.008), Hyperactivity (p=0.003) and tantruming (p=0.009).

Adams JB, *BMC Pediatrics*, 111, 2011.

Integrated Approach to Autism Treatment

- Medical – genetic, neurology, GI, other medical symptoms
- Ancillary – Speech, Occupational Therapy (OT)
- Behavioral
- Treat Associated Symptoms – Pharmacology
- Biomedical Treatments – melatonin, omega 3, vitamin D3, probiotics, digestive enzymes

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