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.

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.

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.

ASD and Environmental Risk

- Documented: Prenatal or early postnatal exposure to viral infections (rubella), valproic acid, thalidomide
- Proposed: Influence of mercury, lead, environmental toxins, vaccines, lack of vitamin D
- Parental age in multiple generations
- Maternal metabolic conditions
- Influenza or fever during pregnancy
- Genetic susceptibility
ASD and Environmental Pollution
- Environmental mercury release, special education rates, and autism disorder: an 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 the 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

2Volk HE et al. JAMA Psychiatry 2013; 70:71-77

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

Translating from “Terroir” Model
- Epigenetics encompasses all layers of genetic control
- Epigenetic changes may become permanent and get passed to future generations
- Suggest targets for intervention

Gene-Enviroment (G-E) Interaction and Endophenotype
Level 2
- Immune abnormalities/Inflammation (Gomes P, Van de Water J, Curr Opin Neurol, 2010;23:111-117.)
- 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.)
- Excitotary/Inhibitory imbalance (Rubenstein JL, Curr Opin Neurol, 2010;23(2):118-123.)
- "Hormonal effects" (Hollander EJ, et al. JAMA, 2012;307:79-89.)

Moving Treatment Targets
Translating Levels of Assessment

Level 4
- 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

  - Genetics
    - Comparative genome hybridization (CGH) array
    - Gene expression (mRNA, Transcriptome)
  - Oxidative Stress
    - Nitro Tyrosine
    - Urine porphyrins
    - Transferin/total iron
    - Glutathione (GSH)/glutathione disulfide (GSSG)
    - Cysteine/Cystine (oxidized)
    - 8-OHdG, 8-OHG

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

  - 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

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

  - Allergy
    - IgG, IgE food antibodies if indicated


Level 3
- Neurodevelopmental Biomedical Assessment
  - Genetics
  - Oxidative Stress
  - Mitochondrial Dysfunction
  - Immune/Inflammation
  - Gastrointestinal (GI) Function
  - Hormones
  - Allergy

Level 2
- Neurodevelopmental Biomedical Assessment
  - Genetics
  - Oxidative Stress
  - Mitochondrial Dysfunction
  - Immune/Inflammation
  - Gastrointestinal (GI) Function
  - Hormones
  - Allergy

Level 1
- Neurodevelopmental Biomedical Assessment
  - Genetics
  - Oxidative Stress
  - Mitochondrial Dysfunction
  - Immune/Inflammation
  - Gastrointestinal (GI) Function
  - Hormones
  - Allergy

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)
- Zoe sleep monitor (if already doing overnight)
- Wearable monitors (e.g. for Autonomic Nervous System (ANS))
### Targeting Treatments

<table>
<thead>
<tr>
<th>Symptom Group</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Cognition</td>
<td>Melatonin</td>
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<tr>
<td>Brain Structure/Function</td>
<td>IV/IG</td>
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<tr>
<td>Neurodevelopment</td>
<td>Corticosteroids</td>
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<tr>
<td>Physiological Processes</td>
<td>NSAIDs - e.g.) Celecoxib (Asadabadi, Psychopharmacology, 2012</td>
</tr>
<tr>
<td>Cell Modulation</td>
<td>Methylation</td>
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<tr>
<td>mRNA</td>
<td>Folic/folinic Acid – Suren, JAMA, 2013, 309:570-77</td>
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<tr>
<td>DNA</td>
<td>Mitochondrial Function</td>
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<tr>
<td></td>
<td>Carnitine</td>
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<td></td>
<td>Coenzyme Q 10</td>
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<td>Vitamin C</td>
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<td>Lipoic acid</td>
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<td>Pantothenate</td>
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<td>Vitamin E</td>
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</tbody>
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### Biomedical Therapeutic Strategies

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

#### Neurotransmitter Production
- Tetrahydrobiopterin (Kuroi) – (Frye R, Transl Psychiatry, 2013, 3:e2217)
- 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

### 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

- 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 affect 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.

**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 and treatments are identified as having potential efficacy, the network can be used to conduct large, randomized-controlled trials of specific interventions.

**Outcome Measures**

- Diagnostic - Social Communication Questionnaire (parent-completed); DSM-IV Checklist (clinician-completed); IQ-testing (research assistant completed).
- Overall assessment – Clinician CGI-S and CGI-I and 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.

**Biomedical Treatment Studies**

- UCSF & MIND:
  - 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)

**Methyl B12 Study**

- 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.

**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)
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

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

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. ($F = 6.80; p < .001; d = .96$)

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.

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 ($p=0.009$).
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