Autism, Metabolic Diseases, and Immunizations

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What is Autism?
Impairments in social interaction
Impairments in communication
Repetitive and stereotyped behavior and activities

Metabolic Disorders in Autism Spectrum Disorder
• Untreated PKU
• Lysosomal storage disorders (e.g. MPS III)
• Disorders of purine and pyrimidine metabolism
  – Adenylosuccinate lyase deficiency
  – PRPP synthetase superactivity
  – Dihydropyrimidine dehydrogenase deficiency
• Creatine disorders
  – GAMT deficiency
  – Creatine transporter deficiency
• GABA metabolism
  – Succinic semialdehyde dehydrogenase deficiency
• Unknown sulfation defect
  – Urine S-sulfocysteine
• Mitochondrial disorders

The Mitochondrion
NUCLEAR MITOCHONDRIAL MUTATIONS

- SURF-1
- SCO1, SCO2, COX 10
- BCS1L
- NDUFS2, NDUFS4, NDUFS7, NDUFS8, NDUFV1
- SDH Fp subunit
- frataxin - Freidreich ataxia
- paraplegin - hereditary spastic paraplegia
- ABC7 - XLSA/A
- mtDNA depletion syndrome - POLG, DUOK, TK2, MPV17
- Translation defects - PUS1, MRPS16, EFG1, LRPPRC

Mitochondrial Disease in Autism Spectrum Disorder

- Population-based study of 120 children with autism
- Epilepsy present in 19
- 14/69 had ↑ lactate (2.5 to 6.9 mmol/L, mean 3.5, SD 1.3)
- Plasma and urine amino acids and urine organic acids were normal
- 11/14 underwent muscle biopsy (normal histology, except for sarcoplasm lipid droplets in 3 patients)
- Complex I, IV, V abnormalities in 6 patients
  - 5/11 diagnosed with definite and 1/11 with probable mitochondrial respiratory chain disorder
  - 5/69 = 7.2%

Dev Med Child Neurol 2005;47:185-9
Mitochondrial Disease in Autism Spectrum Disorder

- 25 patients with primary diagnosis of ASD
- 24/25 had >1 major clinical abnormality uncommon in idiopathic autism
  - 21 significant non-neurological problems
  - 19 constitutional symptoms (fatigability)
  - 15 abnormal neurological findings
  - Unusual developmental phenotypes
    - Marked delay in gross motor milestones (32%)
    - Unusual patterns of regression (40%)

↑Biochemical analytes
- Lactate (76%)
- ALT and/or AST (52%)
- Plasma alanine (36%)

Most common disorders
- Complex I deficiency (64%)
- Complex III deficiency (20%)

Presynaptic Terminal

Hannah Poling

- For 3 months, irritable, ↓ speech - all expressive language lost by 22 months
- Chronic watery diarrhea for 6 months
- Autistic behaviors
  - Spinning
  - Gaze avoidance
  - Disrupted sleep/wake cycle
  - Perseveration

J Child Neurol 21:170-172, 2006

Hannah Poling

- 19-month-old girl
- Normal development
- Developed fever within 48h of DTP, MMR, Hib, Polio, Varicella vaccines
  - Crying, irritability, lethargy, refuses to walk
- Night awakenings, opisthotonus, crawling up stairs 4d after immunizations
- Low-grade fever for 12 days
- Rash 10 days after immunizations
- Diagnosed with vaccine-induced varicella

J Child Neurol 21:170-172, 2006

Hannah Poling

- Mild persistent lactic acidosis
- ↑ CK, AST
- Muscle biopsy performed
  - Type 1 fiber atrophy
  - ↑ lipid
  - ↓ COX activity histochemically
  - mtDNA sequencing normal

J Child Neurol 21:170-172, 2006
Muscle Biopsy Enzymology

Table 1
Skeletal Muscle Oxidative Phosphorylation Enzymology Results

<table>
<thead>
<tr>
<th>Complex Assay</th>
<th>Primer Result</th>
<th>Mean</th>
<th>SD</th>
<th>P44 Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I (o-dec octanoyl)</td>
<td>0</td>
<td>185</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>Complex I (octanoyl)</td>
<td>0</td>
<td>200</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Complex II</td>
<td>0</td>
<td>383</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>Complex III</td>
<td>250</td>
<td>175</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Complex IV</td>
<td>170</td>
<td>150</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Complex V</td>
<td>130</td>
<td>120</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Complex VI</td>
<td>100</td>
<td>90</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Complex VII</td>
<td>70</td>
<td>60</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Complex VIII</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>8</td>
</tr>
</tbody>
</table>

*Units of enzyme activity as expressed in nanomoles of substrates oxidized per hour.*

J Child Neurol 21:170-172, 2006

Legal Action

- Sued Department of health and Human Services under the Vaccine Injury Compensation Program (VICP)
- Won
  - “The results in this case may well signify a landmark decision with children developing autism following vaccinations.”
    - Jon Poling March 6, 2008

Offit Editorial

“Let me be very clear that the government has made absolutely no statement... indicating that vaccines are a cause of autism.”
- Julie Gerberding, Director CDC

Perspective

Vaccines and Autism Revisited — The Hannah Poling Case
Paul A. Offit, M.D.

“Going forward, the VICP should more rigorously define the criteria by which it determines that a vaccine has caused harm. Otherwise, the message that the program inadvertently sends to the public will further erode confidence in vaccines and hurt those whom it is charged with protecting.”

=Paul A. Offit, Chief of ID at CHOP

NEJM 358:2089-2091, 2008
United Mitochondrial Disease Foundation Statement

“The UMDF and its Scientific and Medical Advisory Board strongly recommended that parents continue to have their children vaccinated, using the schedule recommended by the American Academy of Pediatrics. Overwhelming evidence demonstrates that this course of action is both safe and in the best interest of the child.”

http://www.medicalnewstoday.com/articles/99808.php

Autism has a high genetic component

Autism is multigenic

Concordance between MZ twins is 70-90%

Concordance between siblings is 5%

The Autistic Child

- Various metabolic disorders are associated with “autistic features”
  - Usually metabolic disorders feature a constellation of signs and symptoms
  - Isolated autism rare
    - Disorders of purine/pyrimidine metabolism

- Other genetic conditions may be identified

Table 1

Partial list of genetic syndromes with a reported association with autism

<table>
<thead>
<tr>
<th>Syndrome Description</th>
<th>Autism Evaluation Indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>fragile X syndrome</td>
<td>X-linked dominant</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz syndrome</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td>Smith-Magenis syndrome</td>
<td>autosomal dominant</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td>CHARGE syndrome</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td>Sipplestein syndrome (22q11 deletion)</td>
<td>deletion/absence of gene</td>
</tr>
<tr>
<td>Sotos syndrome</td>
<td>autosomal dominant</td>
</tr>
<tr>
<td>Hypotrichosis of the ear</td>
<td>autosomal dominant</td>
</tr>
<tr>
<td>Lujan–Friedreich syndrome</td>
<td>autosomal recessive</td>
</tr>
</tbody>
</table>

Genet Med 10:4-12, 2008
Diagnostic Yield of Genetic Testing in Autism Spectrum Disorder

- 32 patients
  - Axis I diagnosis of autistic spectrum disorder made by a qualified specialist in autism
- 3 tiered diagnostic approach
  1) Dysmorphology evaluation + “standard” metabolic screen, audiogram
  2) Chromosomes, Fragile X, MRI, EEG
  3) MECP-2, FISH 22, 15, 17, purine/pyrimidine analysis, subtelomeric probes (IQ<50)

Genet Med 8:549-556, 2006

Diagnostic Yield of Genetic Testing in Autism

- 13/32 = 41%

Genet Med 8:549-556, 2006

Clinical genetics evaluation in identifying the etiology of autism spectrum disorders

Pre-evaluation
- Confirmation of diagnosis of autism by trained professional using objective criteria and tools
- Sensory screening (complete audiogram)
- Electroencephalogram (EEG) — if clinical questions
- Cognitive testing

Genet Med 10:4-12, 2008
Genetics and Autism Spectrum Disorder

Table 1  |  ASD-related syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s) associated with the syndrome</th>
<th>Proportion of patients with the syndrome that have an ASD</th>
<th>Proportion of patients with an ASD that have the syndrome</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>22q11 duplication</td>
<td>UBE3A (and others)</td>
<td>&gt;40%</td>
<td>1-2%</td>
<td>108-109</td>
</tr>
<tr>
<td>Haploinsufficiency</td>
<td>UBE3A</td>
<td>High</td>
<td>&gt;1%</td>
<td>20, 35, 44</td>
</tr>
<tr>
<td>22q11 deletion</td>
<td>SHANK3</td>
<td>High</td>
<td>&gt;1%</td>
<td>21, 22, 104</td>
</tr>
<tr>
<td>Cortical dysplasia-foveal hypoplasia syndrome</td>
<td>CNV8</td>
<td>Rare</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>FMR1</td>
<td>25% of males, 6% of females</td>
<td>1-2%</td>
<td>105</td>
</tr>
<tr>
<td>Infantile autism</td>
<td>SHANK3</td>
<td>25%</td>
<td>Rare</td>
<td>106</td>
</tr>
<tr>
<td>Poreci-Lupi syndrome</td>
<td>HCN1</td>
<td>&gt;90%</td>
<td>Unknown</td>
<td>107</td>
</tr>
<tr>
<td>Smith-Magenis syndrome</td>
<td>DLC1</td>
<td>50%</td>
<td>Rare</td>
<td>108</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>MECP2</td>
<td>All individuals have Rett syndrome</td>
<td>&lt;0.5%</td>
<td>109</td>
</tr>
<tr>
<td>Timothy syndrome</td>
<td>CA16</td>
<td>60-80%</td>
<td>Unknown</td>
<td>24</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>TSC1 and TSC2</td>
<td>20%</td>
<td>&gt;1%</td>
<td>110</td>
</tr>
</tbody>
</table>

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Second tier
- Fibroblast karyotype if leukocyte karyotype is normal and significant pigmentary abnormalities are noted
- Comparative genomic hybridization (chromosomal microarray)
- MECP2 gene testing (females only)
- PTEN gene testing (if the head circumference is 2.5 standard deviations greater than the mean)

Third tier
- Brain magnetic resonance imaging (MRI)
- Chromosome 15 methylation
- Serum and urine urea acid
  - If elevated, Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and Phosphoribosylpyrophosphate (PRPP) synthetase superactivity testing
  - If low, purine/pyrimidine panel (uric acid, xanthine, hypoxanthine)

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Table 1. Single-Gene Disorders with High Rates of Autism

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disorder</th>
<th>Rate of Autism</th>
<th>Rate of Autism</th>
<th>NR</th>
<th>Gene Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSC1</td>
<td>Tubular sclerosis complex</td>
<td>25%-60%</td>
<td>1%-4%</td>
<td>Inhibitor of mTOR</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>PTEN tumor syndrome (ASD with microcephaly)</td>
<td>ND</td>
<td>1%</td>
<td>Inhibitor of PI3K/AKT signaling</td>
<td></td>
</tr>
<tr>
<td>NF1</td>
<td>Neurofibromatosis type I</td>
<td>4%</td>
<td>0%-4%</td>
<td>Ras-GAP</td>
<td></td>
</tr>
<tr>
<td>MECP2</td>
<td>Rett syndrome</td>
<td>90%</td>
<td>2%</td>
<td>Global transcriptional repressor</td>
<td></td>
</tr>
<tr>
<td>UBE3A</td>
<td>Angelman’s syndrome</td>
<td>40%</td>
<td>1%</td>
<td>E3 ubiquitin ligase</td>
<td></td>
</tr>
<tr>
<td>CACNA2D</td>
<td>Timothy’s syndrome</td>
<td>60%</td>
<td>&lt;1%</td>
<td>L-type voltage-gated calcium channel (Ca2+)</td>
<td></td>
</tr>
<tr>
<td>NLGN4X</td>
<td>Familial ASD</td>
<td>ND</td>
<td>&lt;1%</td>
<td>Synaptic adhesion</td>
<td></td>
</tr>
<tr>
<td>SHANK4</td>
<td>Familial ASD</td>
<td>ND</td>
<td>&lt;1%</td>
<td>Synaptic adhesion</td>
<td></td>
</tr>
<tr>
<td>SHANK3</td>
<td>ASD (Overactivity syndrome)</td>
<td>ND</td>
<td>&lt;1%</td>
<td>PSD scaffolding</td>
<td></td>
</tr>
</tbody>
</table>

nature reviews | genetics volume 9 | May 2008 | 341 Brett S. Abrahams and Daniel H. Geschwind
Immunizations and Autism Spectrum Disorder

• There is no convincing biological evidence that the measles virus or MMR vaccine is related to autism
  – Wakefield study reviewed
• There is no convincing evidence that the ethylmercury found in thimerosal has an etiological role in autism


Wakefield Study

• 12 children with history of normal development
• Regression co-existing with GI complaints (diarrhea, pain, bloating, food intolerance)
  – 8/12 onset coincided with MMR vaccine
• All had abnormalities on endoscopy

Lancet 1998;351:637-41

From The Sunday Times
February 8, 2009
MMR doctor Andrew Wakefield fixed data on autism

http://www.timesonline.co.uk/tol/life_and_style/health/article5683671.ece
Sunday Times  
*February 8, 2009*

- Ailments described in *The Lancet* were different from hospital and GP records
- Medical concerns raised before vaccination in many cases
- Only one case had a temporal relationship to vaccination
- “Through his lawyers, Wakefield...denied the issues raised by our investigation, but declined to comment further.”

http://www.timesonline.co.uk/tol/life_and_style/health/article5683671.ece

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**Acute Metabolic Crisis Induced By Vaccination in Seven Chinese Patients**

Yanling Yang, MD, Sayami Sujan, MD, Fang Sun, MD, Yuehua Zhang, MD, Yusu Jiang, MD, Jingjing Song, MS, Jiong Qin, MD, and Xiru Wu, MD

*Pediatr Neurol 35:114-118, 2006*

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**“Vaccine-induced acute metabolic crisis”**

- Acute crisis between 3-12 hours after vaccination
  - Leigh disease (n=3)
  - 21-hydroxylase deficiency (n=2)
  - Methylmalonic acidemia (n=1)
  - Glutaric acidemia type 1 (n=1)
- “…although the vaccines were not the primary cause of the metabolic crisis, the severe acute episodes occurred coincidentally.”

*Pediatr Neurol 35:114-118, 2006*
Vaccination in Metabolic Disease

- “Transient metabolic changes associated with fever or anorexia may tip the balance in the child whose clinical status is fragile or not well controlled.”
- Immune response affected?
- Risk v. Benefit

Oxidative Stress in Autism

- Valproate-induced neurobehavioral deficits are attenuated by vitamin E in mice
- Oxidative stress may lead to aberrant behavior
Oxidative Stress in Autism

- ↑RBC and serum lipid peroxides, urine isoprostanes, cortical brain lipofuscin
- Abnormal retinograms
- ↓RBC and plasma GSHPx, RBC and platelet SOD, RBC catalase, plasma GSH/GSSG
- ↓Plasma vitamin C, E, A, zinc, RBC magnesium, selenium, activated B6
- ↑Plasma perchlorethylene, hexane, pentane, copper, nitrite + nitrate, RBC mercury, lead, arsenic, xanthine oxidase

Alter Ther 2004, 10:22-36

Biomarkers in Autism

- 20 autistic children age 6.4 ± 1.5 years
- Evidence for impaired methylation
  - ↓methionine, S-adenosylmethionine, homocysteine, cystathionine, cysteine
  - ↓total glutathione
  - ↑oxidized glutathione (GSSG)
  - ↑S-adenosylhomocysteine, adenosine
- Folinic acid, betaine, methylcobalamin corrected the imbalance in a subset of children
- Oxidative stress and impaired methylation may contribute to the manifestations of autism

Am J Clin Nutr 2004;80:1611-7

Biomarkers in Mitochondrial Disease


Biomarkers in Mitochondrial Disease

Atkuri et al. PNAS, 2009 (in press)
Ascorbic Acid as a Supplemental Therapy for Autism

- 30 week double-blind, placebo-controlled trial
- Ascorbate 8g/70kg/day
- Residential children with autistic features (n=18)
- Randomly assigned to ‘ascorbate-placebo-ascorbate’ or ‘ascorbate-ascorbate-placebo’ groups
- 10 week treatment phases
- Behaviors rated weekly using Ritvo-Freeman Real Life Rating Scale for autism
- Reduction in symptom severity associated with ascorbate treatment

L-Carnosine Supplementation in Children with Autistic Spectrum Disorders

- 31 children
- 8-week, double-blinded study
- 800 mg L-carnosine daily
- Significant improvements in Gilliam Autism Rating Scale and Receptive One-Word Picture Vocabulary test
- Improved trends in Childhood Autism Rating Scale, Clinical Global Impressions of Change

So What’s the Connection?

Autism, Metabolic Diseases, and Immunizations

- Plausible biochemical/biological hypotheses
- Not all metabolic diseases are created equal
- Need more data
  - Clinical Immunization Safety Assessment (CISA) Network
  - Further clinical studies
  - NIH Mitochondrial Roadmap Initiative