Summary – Autism, Mito & Oxidative Stress

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Pervasive Development Disorders (PPDs) (slide 2) is the umbrella diagnosis encompassing three disorders: Autism Spectrum Disorders (ASDs), Rett Syndrome (known genetic mutation), and Childhood Disintegrative Disorder (CDD) (later onset, autism-like presentation with some type of medical issues such as epilepsy, mitochondrial disorders, or metabolic disorders). Autism Spectrum Disorders (ASD) present before age 3 and include Autistic disorder, Asperger Syndrome, and Pervasive Disorders - Non-Specified (PPD- NOS).

Three areas of impairment with Autistic Disorder (slide 3) include:

- Social Impairment
- Communication Impairment - Not only language impairment, but the inability to communicate both verbally and non-verbally.
- Repetitive Behaviors or Restrictive Interests

Where a child falls within these areas of impairment differentiates between PPD-NOS and Asperger's Disorder (slide 4). For example, a child with Asperger's Disorder would have little communication impairment when young, but would struggle with social impairment and limited interests, whereas a child with PPD-NOS would have a large communication impairment. Since communication and social impairment are strongly interrelated, the DSM is now combining those impairments.

Age of Onset of Autistic Disorder and PPD (slide 5)

- DSM-IV/ICD-10 definition - before 36 months

1. 33% Regression from normal development - usually between 12-14 months
2. 33% Symptoms from early infancy
3. 33% Symptoms obvious after one year - developmental plateau

- Onset after 36 month - receive other diagnosis
- Regression after 36 months - CDD

Asperger's Syndrome

- No age criteria for diagnosis
- Typically diagnosed in later childhood because symptoms are less obvious when language development is normal.

Studies have outlined early abnormal behaviors, which are primarily social behaviors, in children who later develop autism, differentiating a child with autism from both developmental delayed and typical children (slide 6):

- Responding to name
- Looking at other people and looking for parents
- Showing objects for shared enjoyment

- Joint attention - pointing and following a point (slide 7). Pointing (8-10 months) - commonly deficient in children with Autism, yet remains a vital, early, non verbal communication tool. Types:

1. Protoimperative - point at desired object, communicating wants and needs.
2. Protodeclarative - more evolved and social, used to show others something interesting (Sharing). Further evolves to giving when child places object in another's hand to share object of interest (Slide 8).

- Decreased social interactions, such as Peek-a-Boo
- Looking at others with good eye contact for communication. Even a shy child will use gaze to begin and end interactions, even if just briefly.

Causes of Autism

- **Genetic** - Estimated prevalence of genetic abnormalities is only about 21%, leaving nearly 80% of children with ASD without an identified genetic diagnosis (slide 10)
- I**nherited Metabolic Disorders** (only reported in case studies) (slide 11) -- mitochondrial disease (~25% have known genetic cause), and many other disorders of metabolism as listed on the slide.
- **Non-inherited Metabolic Conditions** fall into three major categories (slide 12) which interact with each other: 1) Mitochondrial disorders - with no genetic abnormalities (75% of those with mito have no known mutations), electron transport chain deficiencies in immune cells and brain tissue, Acyl-carnitine elevations; 2) Redox Abnormalities- Decreased reduced Glutathione and Cysteine, reduced Glutathione Peroxidase function, increase oxidized Glutathione, DNA, protein, and lipids; 3) Folate Abnormalities - cerebral folate insufficiency, autoantibodies to folate receptor and Mitochondrial disease dysfunction.

ASDs are often tied to mitochondrial diseases. Non-traditional causes of autism, including toxin exposure, immune dysfunction, mitochondrial disease, and oxidative stress, are gaining more recognition over the past 10-20 years (slide 13).

Genetic and environmental factors were studied in twins (slide 14) to determine how each contributed to the development of ASD. Genetic heritability accounted for only 38% of the risk of developing ASD, where as the environmental component was much higher at 58%, but parents and professionals have to consider the impact of both important factors. *(Hallmayer,J, Cleveland, BS, et.al., Genetic Heritability and Shared Environmental Factors among Twin Paris with Autism, Archives of General Psychiatry, July 2011).* **http://www.ncbi.nlm.nih.gov/pubmed/21727249**

New Understanding of Autism (slide 15)

- Autism is defined as a collection of symptoms, with no specific condition or blood test able to diagnose ASD.
- Symptoms are associated with underlying medical disorders in many cases, but exactly how these disorders are related to ASD is not yet clear.
- In many cases, autism is a multisystemic disorder with primary neurological manifestations, with abnormalities in multiple parts of the body such as the brain, gut, and immune system.

- The rise in autism cases is likely due to complex interactions between genetics, environment, and the dynamics of physiological development, not just one single cause.

Mitochondria and Autism

Mitochondrial disease is a relatively new field, with the first well-defined mitochondrial disease described in 1988 (slides 16-17). Since mitochondrial disease is medically "young," not much is understood by practitioners, nor taught in medical schools. Mitochondrial disease is typically described as having extreme clinical symptoms, which can vary, with a progressive course. The tissues usually affected are the high-energy dependent tissues, such as the neurological/brain, gastrointestinal, and immune systems. Dysfunction with these systems are seen often with autism as well. Keep in mind that mitochondria are not just the "powerhouse" energy-makers, but are also important in programmed cell death (apoptotic) and oxygen radical regulation.

Each cell has many mitochondria with the actual range dependent on the energy needs of that specific type of cell. Skin cells, for example, with lower energy needs, have hundreds of mitochondria, where as muscle cells, with higher energy needs, may have thousands of mitochondria (slide 18). Mitochondria are a complicated structure with a double membrane and folds of cristae where the energy is produced. Glucose (carbohydrates) and small, medium, and long chain fatty acids (fats) enter the mitochondria, and, through a complicated process involving multiple metabolic pathways and the TCA cycle, ATP (the energy currency of the cell) is produced (slide 19). Measuring metabolites and molecules in the electron transport or respiratory chain, complexes I - IV, may indicate where mitochondrial dysfunction exists. If the mitochondria is not working, glucose will turn into pyruvate, which will turn into lactate, which will turn into alanine. These metabolites become some of the markers to indicate that the mitochondria is not working properly. Blood and urine organic acids, which are part of the TCA cycle, can also build up when not working efficiently. The respiratory chain complexes are made from genes both within the nucleus (nDNA) and within the mitochondria (mtDNA) (slide 20), which adds complexity when studying genes for mitochondrial disorders. Studies have examined the prevalence estimates for mitochondrial disease in autism and for abnormal biomarker for mito in ASD (slide 21-24). For example, 31% of the general ASD population have elevated lactate, with one study showing that up to 90% have a low carnitine. Using a strict definition of mitochondrial disease, however, only 5% of children with autism also have mitochondrial disease, although abnormalities in lactate, pyruvate, carnation, ubiquinone, and other biomarkers is clear (55.6%).

Oxidative Stress and Autism - Abnormalities in redox metabolism and signs of oxidative stress are seen in children with ASD (slide 27-29).
Oxidative stress - a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. The creation of this reactive species (both oxygen and nitrogen) creates unstable molecules which can react with other molecules, thus damaging those molecules. Causes:

- Body itself creates free radicals though the mitochondria

- Other body systems create free radicals
- Environmental toxin exposure, air pollution
- Environmental stressors - ultraviolet light and radiation
- Smoking
- Inflammation

A body needs a balance between having some free radials but not too many. Aging, disease, and cell death are believed to be caused by too many free radicals, but when too few free radicals exist, problems with growth and regulation of the body's systems occur. Autism has evidence of both too many and too few free radicals. The major antioxidant in the body, glutathione or GSH, requires energy from the mitochondria to produce (slide 30-33) and is important in eliminating toxins. The production of glutathione depends on methylation and the folate cycle, again, producing striking abnormalities in specific metabolites that are often found in children with autism as outlined in research by Melnyk, et.al., titled: Metabolic Imbalance Associated with Dysregulation and Oxidative Damage in Children with Autism (http://www.ncbi.nlm.nih.gov/pubmed/21519954). Decreased antioxidants and increased pro-oxidants both lead to oxidative stress in autism (Slide 34-36). Children with Mito tend to have problems making glutathione when compared to children with autism who tend to have problems with pro-oxidants, both causing oxidative stress. In summary, oxidative stress weakens mitochondrial function and causes programmed cell death, resulting in dysfunction and reduced production of energy for the cell (ATP) as well as creating a cycle of more free radical production. A strong connection between mitochondrial disease and autism and oxidative stress has been found, possibly due to an interaction between genetics and the environment.

Acquired Mitochondrial Dysfunction in Autism

The Seahorse, a machine that measures mitochondrial function within the cells, measured cell function in people with and without autism (slides 37-41). By measuring oxygen consumption (OCR), the seahorse gives a bioenergetic profile, depicting mitochondrial function and glycolysis (a non- mitochondrial energy source within the cell) by mapping four fundamental parameters:

1. Basal respiration
2. ATP turnover
3. Proton leak
4. Maximal respiratory capacity

Agents are added to manipulate ATP production, and OCR is measured at specific points. Oxidative stress can cause proton leak, which is waste of cell energy. The difference between *basal respiration* (how much energy the mitochondria use in their regular state) and *maximal respiratory capacity* (how much a cell can work under stress) measures reserve capacity, or the reserve or extra energy the mitochondria have when needed. Reserve capacity is important to measure because when the reserve hits zero, the mitochondria are signaled that they are no longer viable, which in turn, signals

apoptosis or programmed cell death. Increasing oxidative stress decreases reserve capacity, causing harm to the cell.

DMNQ is the agent used to increase oxidative stress in the cells by depleting glutathione (slide 42). The higher the DMNQ, the more the cells are stressed. "Oxidative Stress Induces Mitochondrial Dysfunction in a Subset of Autism Lymphoblastoid Cell Lines in a Well-Matched Case Control Cohort" is a paper about the first step in examining mitochondrial stress and autism (slide 43-45). Lymphoblastoid cell lines are immortalized cells derived from humans that can be used over and over again, making them ideal for research. Cells were also aged matched. The results of the research showed that autistic cell lines seemed to have higher mitochondrial function than the control cell lines. ATP linked OCR and proton leak OCR were both higher. Autistic cells lines initially had a higher maximal OCR and reserve capacity, but dropped below the control lines under oxidative stress. Change in reserve capacity seemed to fall into two groups (slide 46-47). Some autistic cells lines had normal reserve capacity, yet some were very deviant from the controls with dramatic reserve capacity losses. These cells are very sensitive to oxidative stress, which would happen with a mitochondrial disorder. Glycolysis was also very abnormal in the autistic cells lines with the sensitivity to oxidative stress (slide 48), suggesting that, at baseline, these cells were already under stress and using every metabolic pathway they have to function. The electron transport train moves protons (H+) to one side of the membrane throughout the first four respiratory chain complexes, creating an imbalance (slide 49). With any imbalance, the system wants to correct itself to return to homeostasis. The mitochondria uses that imbalance to synthesize ATP at Complex V. Too much oxidative stress can be controlled by proton leak, acting as a release valve by letting protons come back through the membrane. The autistic cells with the sensitivity to oxidative stress also have an increase in proton leak. UCP2, is a protein that is very helpful in regulating oxidative stress, helps control proton leak. Control cells lines responded much differently to manipulation of UCP2 as compared to the autistic cell lines (slides 50-52). In abnormal autistic cell lines, UCP2 is up-regulated and could be one important piece of the puzzle.

Other parameters were also examined and graphed (slides 53-55), such as the differences in number of mitochondrial DNA copies between cell lines. The relative number of genes, however, remained the same between the groups of autistic cell lines as also did the redox profiles, outlining the way glutathione was working. However, the autistic cell line with abnormal sensitivity to oxidative stress seemed to have have poor regulation of redox metabolism *within* the cells with higher levels of oxygen reactive species. Fresh immune cells were then derived from children with autism to see if the cells would share oxidative abnormalities with the initial cell lines (slide 56). When looking at different measures of mitochondrial functions in terms of oxidative stress, the cells did, in fact, react very differently from normal cells with a subset of about one-third of the children responding very poorly to oxidative stress. The numbers were similar between the initial cells lines and the children with autism, but researchers questioned how that information correlates to the health of the child? When looking at differences in development, the children who had mitochondrial function that was sensitive to

oxidative stress scored 10 points lower on average on the Vineland Adaptive Developmental Scale, suggesting a lag in development as compared to those children without the mitochondrial dysfunction (slide 56). Glutathione levels were also worse in children with mitochondrial dysfunction (slide 57).

Mild and severe oxidative stress insults and the effect on the cell are depicted on slide 58, including the effect on ATP turnover, UCP2 uptake, proton leak, maximal and reserve capacity, and cell death. Children with autism are under chronic oxidative stress and when additional stress is imposed, some may react normally, but others, about one-third, will not react normally with cells becoming dysfunctional and damaged, manifesting as mitochondrial dysfunction. These children have a predisposition to reacting poorly to even mild oxidative insults, such as a viral illness or environmental exposure. When cells lines with abnormal responses to oxidative stress are pretreated with N-Acetyl-Cysteine, a precursor to glutathione, the reserve capacity is normalized under stress, however the cells tended to work harder, with a higher proton leak and ATP production (slide 62-64). This treatment is not a cure, but way to protect the cells. As a final summary, mitochondrial dysfunction and autism is complex. Environmental and genetic code influences, mitochondrial dysfunction, redox regulation, oxidative stress, and immune dysfunction all impact cell function, sometimes creating a vicious cycle which causes cell harm and deleterious developmental consequences (slide 66). N-Acetyl-Cysteine may prove to be protective to those children with a poor response to oxidative stress. Further studies are underway, such as defining subgroups of mitochondrial disease and dysfunction in ASD (not yet published) and determining if giving Folinic Acid to improve mitochondrial function is safe and effective for ASD: (http://www.ncbi.nlm.nih.gov/pubmed/25019065 & http://www.ncbi.nlm.nih.gov/pubmed/22230883)(Slides 65-72).

Additional Reading

(Hallmayer,J, Cleveland, BS, et.al., Genetic Heritability and Shared Environmental Factors among Twin Paris with Autism, Archives of General Psychiatry, July 2011).

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About Mito and Autism, MitoAction: http://www.mitoaction.org/about-autism-and-mito
Frye, R.E. and Rossignol, D.A., Pediatr Res. 2011 May; 69(5 Pt 2): 41R–47R. Mitochondrial dysfunction can connect the diverse medical symptoms associated with autism spectrum disorders.