Evidence of Mitochondrial Dysfunction in Autism and Implications for Treatment

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Abstract: Classical mitochondrial diseases occur in a subset of individuals with autism and are usually caused by genetic anomalies or mitochondrial respiratory pathway deficits. However, in many cases of autism, there is evidence of mitochondrial dysfunction (MtD) without the classic features associated with mitochondrial disease. MtD appears to be more common in autism and presents with less severe signs and symptoms. It is not associated with discernable mitochondrial pathology in muscle biopsy specimens despite objective evidence of lowered mitochondrial functioning. Exposure to environmental toxins is the likely etiology for MtD in autism. This dysfunction then contributes to a number of diagnostic symptoms and comorbidities observed in autism including: cognitive impairment, language deficits, abnormal energy metabolism, chronic gastrointestinal problems, abnormalities in fatty acid oxidation, and increased oxidative stress. MtD and oxidative stress may also explain the high male to female ratio found in autism due to increased male vulnerability to these dysfunctions. Biomarkers for mitochondrial dysfunction have been identified, but seem widely under-utilized despite available therapeutic interventions. Nutritional supplementation to decrease oxidative stress along with factors to improve reduced glutathione, as well as hyperbaric oxygen therapy (HBOT) represent supported and rationale approaches. The underlying pathophysiology and autistic symptoms of affected individuals would be expected to either improve or cease worsening once effective treatment for MtD is implemented.

Keywords: mitochondria, inflammation, oxidative stress, antioxidants, hyperbaric oxygen therapy, lactate, ammonia, Co-Enzyme Q10

INTRODUCTION

Autism and its related spectrum of disorders (ASD) are neurodevelopmental disorders characterized by substantial impairments in social interaction, difficulty with communication, and restrictive and repetitive behaviors[1]. However, this psychologically defined cluster of symptoms and behaviors represents a broad phenotypic expression which may result from numerous underlying biological mechanisms and genetic vulnerabilities interacting with environmental factors[2]. Three rigorously performed epidemiological studies demonstrate that the prevalence of autism has increased in recent years[3-5]. It is currently estimated that autism affects as many as 1 out of 150 children (67 per 10,000) in the United States[3, 6]. However since autism occurs four times as frequently in males as females[7], reporting the prevalence in children will underestimate the numbers of affected males significantly. A reasonable extraction of the overall data when applied to the male population finds 1 in 93 males (54 per 10,000) are likely affected with autism. This represents an extraordinarily high prevalence of autism and should generate concern about environmental factors that are more likely to adversely affect males.

Mitochondrial disorders, once thought to be rare, are now regarded as one of the most common metabolic diseases in children[8]. Classical mitochondrial diseases occur in a subset of autism cases and are usually caused by genetic anomalies or mitochondrial respiratory pathway abnormalities[9-15]. These diseases often result in severe symptoms including: limb weakness, cardiac conduction defects and cardiomyopathy, ataxia, and stroke-like episodes[8]. They have also been associated with developmental regression and growth retardation in ASD[16-18] including one boy who lost language skills, had hyperactivity, abnormal social interaction, restricted interests, stereotypical behaviors, seizures, and self-injurious behaviors[16]. However, within a significant subset of ASD, studies reveal evidence of mitochondrial dysfunction (MtD) without the classic features associated with mitochondrial disease[9, 16, 19, 20].

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For example, one study of 25 children with autistic-type features including developmental delay, seizures, and hypotonia found no mitochondrial structural changes upon muscle biopsy. These children did not fit any known categories of mitochondrial encephalo-myopathies[20].

MtD in autism was first suggested by overlapping symptoms of both conditions and led Lombard to hypothesize that autism may be caused by MtD[21]. In comparison to classical mitochondrial disease, MtD occurs more commonly in autism[22], is not as severe in symptomatology, and is not associated with any discernable mitochondrial abnormality upon muscle biopsy. It is, however, associated with laboratory evidence of lowered mitochondrial functioning. This MtD is likely precipitated by environmental toxins, and could contribute to a number of diagnostic symptoms and comorbidities of autism including: cognitive impairment, language deficits, abnormal energy metabolism, chronic gastrointestinal problems, abnormalities in fatty acid oxidation, and increased oxidative stress. Although biomarkers for mito-chondrial deficiency have been identified, it appears that MtD is significantly under-recognized in autism, and a widespread under-prescribing of potentially beneficial therapies often occurs. Interestingly, MtD has been implicated in other chronic neurological diseases, including Alzheimer's disease[23, 24], Parkinson's disease[25], amyotrophic lateral sclerosis[25], Huntington's disease[26], and Friedreich's ataxia[26]. This may be due to the fact that the aerobic metabolic demand of the brain is very high, making it extraordinarily vulnerable to any decline in mito-chondrial efficiency[19], largely due to the extraordinary dependence of synaptic transmission on high energy output from mitochondria[27].

MtD in Autism

Evidence of MtD in autism: One of the first studies suggesting MtD in autism used ^{31}P nuclear magnetic resonance spectroscopy (MRS) to investigate energy metabolism in the brain of individuals with autism by measuring steady-state levels of phosphocreatine (PCr), adenosine triphosphate (ATP), adenosine diphosphate (ADP), and inorganic orthophosphate and comparing these levels to neurotypical individuals. The study found that PCr was lower in the autism group, consistent with "increased utilization of PCr to maintain brain ATP levels," and this finding was correlated with language and neuropsychological problems. The group with autism also had lower levels of esterified end-products (αATP, αADP, dinucleotides, and

diphosphosugars). The authors speculated that the clinical abnormalities found in autism may be secondary events that result from these "alterations in brain bioenergetics"[28]. A subsequent study also confirmed evidence of "altered energy metabolism" in the brains of children with autism[29]. These studies are consistent with impaired mitochondrial function and document the fact that some individuals with autism have overall lowered cellular energetics and deficient reserve mitochondrial energy capacity that could lead to cognitive impairment, language deficits, and abnormal energy metabolism.

Impairments in mitochondrial function can also deplete glutathione levels[30] and lead to chronic gastrointestinal (GI) problems [19] because the GI tract is highly dependent on glutathione to function properly[31]. Several studies demonstrate that chronic GI problems are commonly found in autism[32, 33] including chronic constipation[34]. Constipation is frequently observed in patients with mitochondrial disorders[35] and the chronic constipation found in some ASD cases may be a manifestation of MtD. Seizures[36] and hypotonia[37] in autistic individuals may also be manifestations of MtD.

Abnormalities in fatty acid oxidation may also reflect underlying MtD in autistic individuals. Fatty acid oxidation is noted in autism[38], as well as impairments in beta oxidation and oxidative phosphorylation associated with carnitine deficiency and reduced production of phospholipids containing 22:6n-3 fatty acids (docosahexaenoic acid)[39]. For example, in one case, a child with autism and hypo-tonia, but with normal muscle strength, demonstrated elevated blood lactate, low bicarbonate, and an abnormal acyl-carnitine profile. This child was eventually diagnosed with a dysfunction of mito-chondrial beta oxidation[40]. In another study of 15 autistic children, compared to 18 control mentally retarded children, the autism population displayed a "marked reduction" in 22:6n-3 levels[41]. Taken together, these findings suggest that MtD may be common in autism and that the specific changes described in this section may be diagnostic of this effect.

MtD and oxidative stress in autism: During mitochondrial respiration, an inability to neutralize reactive oxygen species and free radicals leads to oxidative stress[23, 42]. Oxidative damage in humans is thought to contribute to aging and neurological diseases[43]. The inner membrane of the mitochondria contains a large amount of free radical scavengers including glutathione, vitamin C, and vitamin E, as well as anti-oxidant enzymes such as superoxide dismutase

(SOD)[26]. Animal studies demonstrate that mice that are deficient in SOD have molecular defects in mitochondrial respiration, and that improvement of symptoms occur with administration of SOD[44]. Some children with autism have lower levels of reduced glutathione[45, 46] and SOD[47]; both could lead to mitochondrial damage and dysfunction[48]. Impairments in mitochondrial function lead to further oxidative stress[49] and continued lower glutathione levels[30], and a vicious negative cycle can ensue. Since increased oxidative stress is commonly found in autism[50], this harmful cyclical process is likely to persist. In a pivotal autism study, plasma oxidized glutathione, a reliable marker of intracellular oxidative stress[51], was 72% higher in children with autism when compared to neurotypical children[45]. Furthermore, decreased activities of certain antioxidant enzymes including SOD[47], glutathione peroxidase[47], catalase[52], and paraoxonase[53] are found in some individuals with autism. Some ASD children also express increased lipid peroxidation[52, 54], including increased malondialdehyde, which is a marker of oxidative stress and lipid peroxidation[55]. One group of researchers has actually suggested that "oxidative stress (endogenous or environmental) may contribute to the development and clinical manifestations of autism"[46]. As an example, in one study, clinical regression (loss of previously acquired skills) in some autistic children was associated with lower levels of serum antioxidant enzymes[55].

Oxidative stress may also play a role in the increased male to female ratio observed in autism. Because of lower levels of reduced glutathione, mitochondria from males, when compared to females, are more vulnerable to oxidative stress[56]. In animal studies, due to lower enzyme activity levels of SOD and glutathione peroxidase in males, oxidative damage to mitochondrial DNA is 4-fold higher in males compared to females[56]. Oxidative stress, as measured by a loss in tissue ascorbate after cerebral ischemia, is greater in the brain of male animals compared to females[57]. The activity of catalase, an anti-oxidant enzyme, is also higher in females than in males[58], and estrogen induces glutathione peroxidase activity[59]. In one animal model, in cerebral arteries, estrogen attaches to estrogen receptor-α and increases mitochondrial capacity for ATP production by increasing mitochondrial enzyme activity including citrate synthase and complex IV. Estrogen also decreases production of hydrogen peroxide thereby reducing oxidative stress[60]. Therefore, males with increased oxidative stress may manifest more MtD and more severe clinical symptoms when compared to females.

Biomarkers of classical mitochondrial disease and MtD in autism: Classical mitochondrial diseases are commonly associated with abnormal laboratory findings including: elevated lactate, ammonia, aspartate aminotransferase, pyruvate, and creatine kinase, and lowered carnitine levels. These markers can be useful in evaluating mitochondrial diseases[20]. However, many of these laboratory abnormalities have also been described in autistic individuals with MtD and without any other evidence of classical mitochondrial disease. For example, elevated blood lactate levels consistent with MtD have been described in numerous autistic individuals[29, 40, 61-65], sometimes affecting as many as 43% of children with autism studied when compared to neurotypical children[17]. In one study, ASD children with an elevated blood lactate level underwent deltoid muscle biopsy and approximately 35% (5 out of 14) were found to have classical mitochondrial disease[22]. However, this study is significant because although some of the children with autism and elevated lactate had a true mitochondrial disease, most had normal muscle biopsies which were felt to be consistent with MtD.

In several studies of individuals presenting with autism, but without classical mitochondrial disease, increased ammonia has also been described; this led some authors to conclude that MtD was present[40, 63, 64]. Typically, these ammonia levels are not nearly as elevated as those that have been observed in some conditions that can cause autism, such as urea cycle disorders[66]. Ammonia requires three ATP molecules to be converted into urea and in animal studies, elevated ammonia can lead to brain ATP depletion[67]. Therefore, the increased ammonia noted in autism may be both consequentially and causally related to ATP depletion. One study of 100 children with autism and a history of clinical regression, but without classical mitochondrial disease, found evidence of MtD that was expressed by significantly reduced levels of free and total carnitine and increased ammonia, alanine, and lactate blood levels[64].

Other markers of MtD that are elevated in autism without evidence of classical mitochondrial disease include pyruvate[17] and creatine kinase[18]. One study of 159 children with autism, compared to 94 neurotypical children, demonstrated elevations in aspartate aminotransferase and creatine kinase suggestive of possible defects in oxidative phosphorylation. The authors concluded that "further metabolic evaluation is indicated in autistic patients and that defects of oxidative phosphorylation might be prevalent." They also commented that MtD occurring during early childhood would lead to additional

oxidative stress that could impair the body's ability to handle certain infections or immune activation from immunizations received during that time[18].

Possible causes of MtD in autism: The cause of MtD in some autistic individuals is unknown but could be a secondary manifestation resulting from exposure to certain environmental toxins. These agents could directly damage the mitochondria and reduce their efficiency, or impair functional activity by increasing body burden of free radicals and resultant oxidative stress. Drugs like valproic acid and thalidomide[68, 69], as well as exposure to viruses such as rubella and cytomegalovirus[70, 71] are examples of environmental factors that have a known causal role in autism. However, many more environmental toxins including pesticides[53, 72], polychlorinated biphenyls (PCB's)[73], industrial chemicals[73-76], and toxic metals such as mercury[77, 78], lead[79, 80], cadmium, nickel[74], and tin[81] have also been implicated in ASD. Notably, many of these toxins including mercury[82, 83], arsenic, cadmium, lead[84], pesticides[85], and industrial chemicals[86] inhibit mitochondrial function. In fact, mitochondria may be the earliest target of metal neurotoxicity[82]. Other substances may also hinder mitochondrial function. Propionic acid is produced by *Clostridia* in the gastrointestinal tract and impairs mitochondrial function by directly inhibiting oxidative phosphorylation[87]. Higher amounts of clostridia are typically present in the gastrointestinal tract of autistic individuals, and injection of propionic acid into rats has recently been shown to produce an autistic-like illness[87]. By increasing oxidative stress[49] and lowering glutathione levels[30], MtD may actually lead to problems in detoxifying environmental contaminants[46] and consequently increase susceptibility to metal toxicity[88].

Testing autistic individuals for MtD: Based upon these findings, some researchers have proposed that mitochondrial disorders may be the most common metabolic cause of autism[10, 65] and that MtD should be suspected and tested for in children with autism who have associated neurological findings or a family history of mitochondrial problems[14, 22]. Certain biomarkers may be useful for evaluating possible MtD in autistic individuals. Some researchers suggest pyruvate levels should be measured during routine evaluation of ASD children[61]. However, measuring a pyruvate level is not always practical because typically it is only available in teaching or specialty hospitals. The plasma alanine to lysine ratio (normal range is 1.5-2.5) is a surrogate marker for pyruvate; a ratio above 2.5 is

consistent with an elevation in pyruvate[18]. A lactate level is "a simple, non-invasive, low-cost means for the initial diagnostic approach" of mitochondrial problems[8]. Mitochondrial problems can also be screened for by examining urinary organic acids[19]. Assessing carnitine and ammonia levels[64] and oxidative stress markers may also be useful. Screening for any of these blood and urine tests may be helpful in identifying MtD in ASD.

Potential Treatments of MtD in Autism

Nutritional supplements and antioxidants: Treatment of oxidative stress with antioxidants and other nutritional supplements may ameliorate MtD in some individuals with autism[26]. In animal studies, acetyl-L-carnitine restored partial mitochondrial function and muscle activity[89]. Coenzyme Q10 administration in rats increased mitochondrial concentrations, extended survival times, and exhibited neuroprotective effects[90]. In human studies, coenzyme Q10 improved symptoms in patients with mitochondrial disease. For instance, in one patient with mitochondrial disease and coenzyme Q10 blood concentrations below 25% of normal, coenzyme Q10 administration led to subjective improvements[91]. In another study of 2 patients with mitochondrial encephalomyopathy, administration of coenzyme Q10 and idebenone led to clinical improvements as measured by EEG and standardized psychological testing. Lactate and pyruvate levels also decreased after this treatment[92]. In one double-blind, crossover study of 8 patients with mitochondrial encephalomyopathy, coenzyme Q10 improved fatigue and muscle exercise endurance, and decreased serum lactate and pyruvate levels[93]. Thiamine, pyridoxine, and riboflavin may also improve mitochondrial function[21]. In one study, pyridoxine improved language, affection, and behavior in 3 out of 8 autistic children with MtD[62]. Ginkgo biloba extract also appears to lessen oxidative stress in mitochondria[94]. Furthermore, treatment with antioxidants to decrease oxidative stress may improve mitochondrial function and energy production[95]. In fact, treatment with antioxidants in ASD children raised the levels of reduced glutathione in the serum and appeared to improve symptoms[45]. In one double-blind study, the use of vitamin C improved behavior in autistic individuals when compared to a placebo[96]. Once MtD is identified, treatments with these nutritional supplements may lead to improvements in mitochondrial function and reductions of negative symptoms.

Hyperbaric oxygen therapy (HBOT): Several studies have described cerebral hypoperfusion in autism[97-99] and this decreased blood flow has been reported to correlate with core autistic symptoms[100] and could lead to relative cerebral hypoxia. In fact, several studies have demonstrated evidence of hypoxia in the brains of some individuals with autism as measured by a reduction in brain Bcl-2 and an increase in brain p53[101, 102]. Elevated p53 is induced by hypoxia[103] and a decrease in Bcl-2 is associated with increased apoptosis provoked by hypoxia[104]. Hypoxia can impair mitochondrial function[105] and diminish ATP production[106]. Since only approximately 0.3% of inhaled oxygen is ultimately delivered to mitochondria[107], increasing the oxygen delivery to mitochondria by hyperbaric oxygen therapy (HBOT) may actually improve mitochondrial function[108, 109]. For example, in a mouse model with an intrinsic impairment of mitochondrial complex IV, HBOT at 2 atmospheres (atm) "significantly ameliorate[d] mitochondrial dysfunction" and delayed the onset of motor neuron disease when compared to control mice[108]. In animal studies, HBOT has been shown to increase the amount of work done by mitochondria[110], improve mitochondrial function after brain injury[109], and prevent mitochondrial deterioration[111] when compared to room air pressure and oxygen levels. HBOT also increased sperm motility by augmenting mitochondrial oxidative phosphorylation in fructolysis-inhibited sperm cells. Fructose is the sugar used by sperm for energy production[112]. Furthermore, the use of HBOT prevented apoptosis and improved neurological recovery after cerebral ischemia by opening mitochondrial ATP-sensitive potassium channels[113]. In one animal model, hypoxia and ischemia led to diminished ATP and phosphocreatine production; the addition of HBOT restored these levels to near-normal and increased energy utilization when compared to room air oxygen and pressure levels[106]. Finally, HBOT has recently been shown to activate mitochondrial DNA transcription and replication, and increase the biogenesis of mitochondria in the brains of animals[114]. One small case series suggested that HBOT may improve symptoms in some autistic children[115].

CONCLUSION

Classical mitochondrial diseases affect a small number of individuals with autism and typically occur due to genetic anomalies and mitochondrial respiratory chain deficits. However, MtD, a condition that does not have the classic features of mitochondrial disease, is found among some individuals with autism and is associated with abnormal biomarkers of mitochondrial function. A variety of environmental toxins could be disrupting both the structure and function of the mitochondria in these individuals, and these toxins should be considered in the evaluation of children with autism. MtD may contribute to a number of symptoms and comorbidities observed in autism including: cognitive impairment, language deficits, abnormal energy metabolism, chronic gastrointestinal problems, abnormalities in fatty acid oxidation, and increased oxidative stress. Additionally, since there is increased vulnerability of males to these dysfunctions, MtD may provide an explanation as to why the high male to female ratio in autism exists. MtD can be diagnosed via certain biomarkers and is potentially treatable. Therefore, increased efforts at MtD screening of all individuals affected with autism should be encouraged. Potentially, there are a large number of children with autism affected by MtD, and since the possibility of available treatments for this aspect of autism exist, investigation of methodologies for correcting the underlying mechanisms of MtD should be urgently undertaken.

ACKNOWLEDGEMENTS

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