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# Autism and Mitochondrial Disease

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Autism spectrum disorder (ASD) as defined by the revised Diagnostic and Statistical Manual of Mental Disorders: DSM IVTR criteria (American Psychiatric Association [2000] Washington, DC: American Psychiatric Publishing) as impairment before the age of 3 in language development and socialization with the development of repetitive behaviors, appears to be increased in incidence and prevalence. Similarly, mitochondrial disorders are increasingly recognized. Although overlap between these disorders is to be expected, accumulating clinical, genetic, and biochemical evidence suggests that mitochondrial dysfunction in ASD is more commonly seen than expected. Some patients with ASD phenotypes clearly have genetic-based primary mitochondrial disease. This review will examine the data linking autism and mitochondria. 
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**Key Words:** autism spectrum disorder; mitochondrial disease; mitochondria; genetics; copy number variation; immune modulators

In recent years, evidence documenting mitochondrial dysfunction in some individuals with autistic spectrum disorder (ASD) has been growing. A role for the mitochondrion in the etiology of ASD in at least some individuals is suggested by a variety of lines of evidence. These include clinical, genetic and biochemical findings. Each of these lines of evidence will be discussed in detail. An apparent increasing incidence of ASD (stated by the CDC to be 1:110 [CDC, 2009] and an estimated birth incidence of genetic mitochondrial disease >1:2,000 in the population [Schaefer et al., 2008] will result in the occasional co-diagnosis of ASD and genetic mitochondrial disease. A recent study from the United Kingdom determined the frequency of 10 common pathogenic mitochondrial DNA (mtDNA) mutations in neonatal cord blood samples from an unselected population of 3,148 live neonates to be 1:200. The most common was the MELAS A3243G mutation [Elliott et al., 2008]. Using CDC autism estimates and a 1:2,000 incidence of definite mitochondrial disease, if there were no linkage of ASD and mitochondrial disease then it would be expected that 1 in 110 mitochondrial disease subjects would have ASD and 1 in 2,000 ASD individuals would have mitochondrial disease. The co-occurrance of autism and mitochondrial disease appears to be much higher than these figures would suggest. Our current knowledge of the relationship between autism and mitochondrial disease in children is illustrated in Figure 1. The role of mitochondrial dysfunction in the etiology of ASD, however, may be much more important than this Venn diagram would suggest.

Neurodegeneration in primary mitochondrial disease patients is frequently precipitated by infection, postulated to be mediated by metabolic decompensation and cytokine toxicity. More recently, autistic regression with resulting ASD in children who were thought to be previously normal has been reported following fever associated with infection or immunizations. Some of these children are subsequently recognized to have primary mitochondrial disease—"Mitochondrial Autism," a term suggested by Weissman et al. [2008].

## EVIDENCE LINKING MITOCHONDRIA AND AUTISM

Twenty-five years ago, elevated lactate levels were reported in some autistic subjects. This raised the question of oxidative phosphorylation (oxphos) defects in autism [Coleman and Blass, 1985]. Since then a number of case studies and retrospective chart reviews have identified evidence of 'mitochondrial autism', defined here as mitochondrial dysfunction and oxphos defects in ASD children (Table 1).

It is clear from the studies listed in Table 1 that patients with *definite* mitochondrial disease based on published diagnostic criteria [Bernier et al., 2002; Wolf and Smeitink, 2002] may have autistic phenotypes. The frequency of this co-morbidity is unknown but likely exceeds the 1:110 prevalence expected by chance alone. The studies in Table 1 identified 76 of these cases; of which 49 cases were reported by two groups [Weissman et al., 2008; Shoffner et al., 2010]. If autism (1:110) and mitochondrial disorder (1:2,000) occur independently, a patient population of almost 17 million would be needed to provide 76 patients with both conditions (110 × 2,000 × 76). Of note, *probable* or *possible* mitochondrial disease is far more common than *definite* primary mitochondrial disease.

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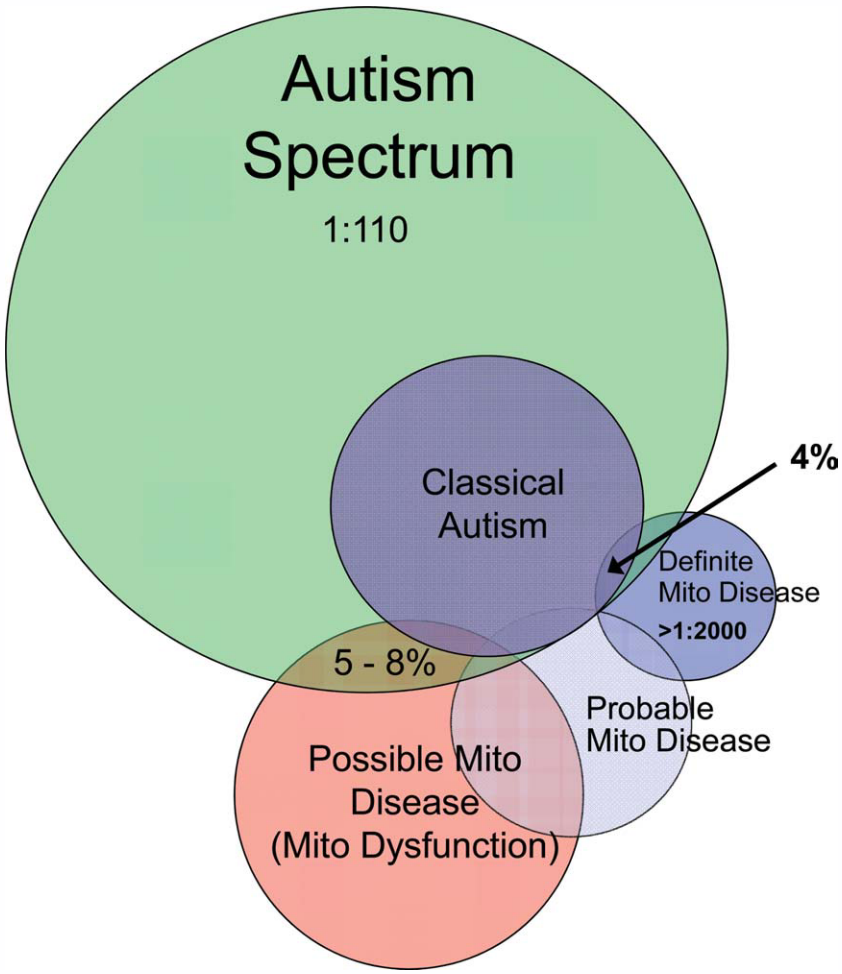


Fig. 1. The relationship between autism and mitochondrial disease. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**Co-occurrance of ASD and Mitochondrial Disease**

Epidemiological evidence from Portugal supports a prevalence of definite mitochondrial disease by Bernier et al.'s criteria [2002] of 4% in ASD children [Oliveira et al., 2007]. In this study, 120 children were diagnosed with ASD from a national survey of children attending elementary school in the year 1999 to 2000. This nationwide survey of approximately 20% of schools collected questionnaires from teachers for 58,399 children, of whom 120 were diagnosed with ASD by the autism diagnostic inventory revised (ADI-R) and/or DSM-IV criteria. Prevalence figures were biased by inclusion of all special schools in the country but only 20% of regular schools. Thus, the prevalence of ASD of 120 in 58,399 or 1:500 is likely an overestimate. It is interesting to compare this to the 2006 US CDC ASD prevalence figure of 1:110 [CDC, 2009] although in the year 2000, European studies estimated ASD prevalence at 1 in 247 to 1 in 817 [Fombonne, 2003]. Of the 120 children with ASD studied by Oliveira, detailed metabolic studies including plasma lactate were performed in 69. Elevated lactate was found in 14, of whom 11 underwent muscle biopsy. Five of these children were diagnosed with definite mitochondrial disease by Bernier et al.'s criteria [2002]. Thus, 4.2% of 120 children with ASD were determined to have definite mitochondrial disease. This is likely an underestimate as 51, or 43%, of the ASD children had no metabolic testing performed and three children with lactic acid elevation were not investigated further.

**Diagnosis of Mitochondrial Dysfunction**

*Markers of intermediary metabolism can be used to detect mitochondrial dysfunction*

Lactic acid accumulates when impaired mitochondrial metabolism leads to an accumulation of carbon skeletons from glycolysis. Lactate is in equilibrium with pyruvate in the cytosol through the action of lactic dehydrogenase. This reaction is controlled by the NADH/NAD ratio or redox state. The lactate:pyruvate ratio offers a means to monitor the cellular redox state. Accumulation of pyruvate, the transamination product alanine, and lactate are all markers of mitochondrial dysfunction. Impairment of fatty acid β-oxidation in the mitochondria may result in accumulation of fatty acids, some of which are shunted to the microsomes producing dicarboxylic acids. Excess fatty acid accumulation utilizes carnitine to detoxify these molecules, with excretion of acyl-carnitines in the urine. Plasma acyl-carnitine levels can indicate a variety of organic acid metabolic impairments. Free and total carnitine levels in blood and tissue may be secondarily reduced in mitochondrial dysfunction.

*Lactate.* Elevated plasma lactate is a useful screening marker for mitochondrial dysfunction. However, lactate levels are very prone to spurious elevation from a variety of causes (Table 2) [Haas et al., 2007]. Elevated lactate levels should be confirmed by collection of a free flowing sample from an indwelling IV line in a quiet child. In many cases of mitochondrial disease, CSF lactate will be elevated.

**Other metabolites**

Unpublished findings from metabolic studies in a series of 60 consecutive autistic subjects studied in a quantitative MRI protocol, ages 2 to 40 [Courchesne et al., 1994] identified evidence of mitochondrial dysfunction [Haas et al., 2008] in 5 of 60 (8.3%). Evidence of mitochondrial dysfunction included elevations of plasma lactate (four of five), urine organic acids such as 3-methyl-glutaconic acid (two of five), citric acid cycle intermediates, lactate, and dicarboxylic acids (variably seen in three of five), and high plasma alanine (two of five). Four of these five children (four males, one female) had well-documented regression with loss of language.

Oliveira found that plasma lactate was elevated in 14 of 69 (20%) of ASD children studied [Oliveira et al., 2005]. Eleven of these children had blood pyruvate measured with lactate/pyruvate ratios ranging from 22 to 54 (normal <20). Plasma and urine amino acids along with urine organic acids were normal in all 14 ASD children who had

**Table 1. Reports of Mitochondrial Disease and Dysfunction in Autism**

| Author | Year | No. ASD Cases | Number with "Definite" Mito Disease [Bernier et al., 2002] | Gene/Chromosome Defect | Evidence of Mito Dysfunction | Type of Report |
|---|---|---|---|---|---|---|
| Coleman [Coleman and Blass, 1985] | 1985 | 4 | NA | NA | ↑Plasma lactate | Case series |
| Laszlo [Laszlo et al., 1994) | 1994 | 30 | 1/30 | 18q deletion (1/30) | ↑Plasma lactate (13/30). ↑Pyruvate (9/30) | Controlled case series |
| Chugani [Chugani et al., 1999] | 1999 | 15 and 9 | NA and NA | NA and NA | ↑Plasma lactate (15/15). Brain MRS ↑lactate (1/9) | Prospective case series |
| Graf [Graf et al., 2000) | 2000 | 1 | 1 | G8363A mtDNA | High CI | Case report |
| Filiano [Fillano et al., 2002] | 2002 | 12 | 12 | mtDNA deletion (4/12) | Low CI (1/12). Low CIII (6/12). Low CIV (1/12). Low CV (3/12) | Case series |
| Filipek [Filipek et al., 2003] | 2003 | 2 | 2 | 15q11-q13 inverted duplication (2/2) | ↓Carnitine (2/2). ↑Plasma alanine (1/2). ↑Plasma lactate (1/2). ↑CPK (1/2). Muscle mito proliferation (2/2). Low fibroblast CIII (2/2) | Case series |
| Filipek [Filipek et al., 2004] | 2004 | 100 | NA | NA | Relative to control mean: 36% plasma carnitine + 85% serum pyruvate 1 SD below; 78% ammonia + 80% serum alanine 1 SD above | Retrospective case series |
| Pons [Pons et al., 2004] | 2004 | 5 | 3 | Family history of A3243G mtDNA in 4; A3243G in 2/5; mtDNA depletion in 1 | ↑Plasma lactate (2/3). ↑CSF lactate (1/1). Low muscle CI, CII, CII/III, and CIV (1/2) | Retrospective case series |
| Oliveira [Oliveira et al., 2007, 2005) | 2005 | 69 | 5 | None | ↑Plasma lactate (14/69). Low muscle CI (2/11), CIV (2/11), CV (3/11) | Epidemiological study |
| Poling [Poling et al., 2006] | 2006 | 1 | 1 | None | ↑Blood lactate, abnormal urine organic acids and muscle CI, CII/III, and CIII deficiency | Case report |
| Tsao [Tsao and Mendell, 2007] | 2007 | 2 | 2 | NA | Muscle CI and CoQ deficiency (1/2). Muscle CII, C II/III, and CIV deficiency (1/2) | Retrospective case series |
| Weissman [Weissman et al., 2008] | 2008 | 25 | 21 | 7 mtDNA mutations: 1 tRNA mutation, 2 probable pathogenic mtDNA point mutation, 4 possible pathogenic mtDNA point mutations | ↑Blood lactate (19/25). ↑Blood pyruvate (9/17). ↑CPK (8/25) Abnormal urine organic acids (10/24). Brain MRS ↑lactate (2/5). Muscle CI (16/23), CII (2/23), CIII (5/23), CIV (1/23), deficiency | Retrospective case series |
| Shoffner [Shoffner et al., 2010] | 2010 | 28 | 28 | Muscle mtDNA depletion 1/20 CI | Blood, urine or CSF ↑lactate, pyruvate or alanine. Muscle CI (14/28), CV (4/28), CI + CIII (5/28), CI + CIII + CIV deficiency with Abnormal oxphos proteins (20/28) | Retrospective case series |

CI = complex I, CII = complex II, CIII = complex III, CIV = complex IV (cytochrome c oxidase), CV = complex V.

elevated lactate [Oliveira et al., 2005]. Other studies have noted abnormalities of a number of intermediate metabolites in a subset of ASD children. Markers of mitochondrial dysfunction were reported in two patients with 15q inverted duplications including modest lactic acidosis in one, a twofold elevation in plasma alanine in one, modest hyperammonemia in one, low plasma carnitine in both, as well as urine organic acid elevations of pyruvate and glutarate in one and lactate, pyruvate and fumarate in the other. Both children had evidence of mitochondrial proliferation on muscle biopsy and complex III deficiency on fibroblast assay [Filipek et al., 2003].

A random retrospective chart review of 100 autistic children (by DSM-IV criteria) revealed group mean levels differing from controls in several metabolites, which together suggest mild mitochondrial impairment. Mean serum levels < than 1 SD from the control mean were found in ASD subjects for total carnitine levels (36%), free carnitine levels (27%), and mean pyruvate levels (85%). Lactate levels were not significantly raised for the ASD cohort but serum alanine and ammonia levels were >1 SD above the control in 80% and 75% of ASD subjects, respectively [Filipek et al., 2004]. Whilst lactate and ammonia levels are quite susceptible to the difficulty of blood collection in ASD children, carnitine, alanine, and pyruvate levels will not be affected by these problems. Taken as a whole, these data provide evidence supporting mitochondrial dysfunction in many ASD children. In a retrospective study of "Mitochondrial Autistic" children, 24 of 25 (96%) had biochemical evidence of mitochondrial dysfunction

**Table 2. Causes of Plasma Lactate Elevation**

| |
|---|
| Erroneous elevation |
| Poor collection technique (use of a tourniquet) Struggling child |
| Poor sample handling (wrong collection tube or processing delay) |
| Physiological |
| Anaerobic exercise |
| Systemic diseases that increase blood lactate levels |
| Hypoxia |
| Hypotension |
| Shock |
| Sepsis |
| Cardiac failure/cardiomyopathy |
| Renal failure |
| Short bowel syndrome (D-lactate) |
| Metabolic diseases |
| Amino acid disorders |
| Organic acidemias |
| Urea cycle defects |
| Pyruvate metabolism defects |
| Citric acid cycle defects |
| Mitochondrial OXPHOS disorders |
| Fatty acid oxidation disorders |
| Disorders of liver glycogen metabolism |
| Disorders of liver gluconeogenesis |
| Biotinidase deficiency |
| Other |
| Thiamine deficiency |
| Toxin exposure (carbon monoxide, methanol) |

in blood; 76% had elevated lactate, 53% had elevated pyruvate, 36% had elevated alanine, and 42% had abnormal urine organic acids. Serum CPK was elevated in 32% [Weissman et al., 2008]. It is important to note that not all children with ASD and mitochondrial disease have abnormalities in the common blood and urine markers of mitochondrial dysfunction. This is also the case for children with definite mitochondrial disease without ASD features. Partial deficiency of one or more electron transport enzymes in muscle (and coenzyme $Q_{10}$ in one) were reported in two girls with ASD who underwent a neurodegenerative course. However, in both girls blood lactate, ammonia, and amino acids were normal as were the urine organic acids [Tsao and Mendell, 2007].

## The Mitochondrial Autism Phenotype

*Is there a characteristic phenotype of Mitochondrial Autism?*

Published studies suggest that there are features which should alert the clinician to the possibility of mitochondrial disease. These include a history of regression and multiorgan system involvement. In Weissman's study of Mitochondrial Autistics, 14 of 28 (50%) suffered regression, 36% had multiple regressions, and 24% experienced regression after the age of 3 years [Weissman et al., 2008]. Shoffner et al. reported that autistic regression occurred in 17 of 28 (61%) of ASD subjects with definite mitochondrial disease and in 12 of these children fever was associated with the onset of regression [Shoffner et al., 2010]. Of these 28 Mitochondrial Autistics, 46% had motor developmental delay and hypotonia, 43% fatigued with activity, 39% had epilepsy, 11% had abnormal growth or weight gain, and 36% had affected siblings. Filano et al. described a more severe phenotype in 12 children with hypotonia, intractable epilepsy, autism and developmental delay which they termed HEADD syndrome [Filano et al., 2002]. All children were autistic by DSM IV criteria. It should be noted that hypotonia is the most common motor finding in ASD [Haas et al., 1996].

Multiorgan system disorder was noted by Weissman et al. Of their 25 Mitochondrial Autistics, 96% had at least one major clinical finding uncommon in ASD, 84% had at least one, and 32% had two non-CNS organs involved. Sixty percent had at least one neurological finding uncommon in ASD, 32% had marked gross motor delays, and 20% had seizures. The most common non-CNS organ system affected was the gastrointestinal (GI) tract with GI dysfunction noted in 64% of subjects [Weissman et al., 2008].

*Neurodegeneration in mitochondrial disease and autistic regression*

Primary mitochondrial disease may be defined as a genetic defect in mitochondrial function that results in an impairment of oxidative phosphorylation (oxphos) [Haas et al., 2007]. It is frequently the case that metabolic decompensation associated with neurodegeneration occurs in primary mitochondrial disease following (often mild) infection. This common phenomenon was reported in a series of mitochondrial disease patients followed at the University of California San Diego. Intercurrent infection was recognized as a precipitant of neurodegenerative events in 13 of 40 (33%) patients. Seventy-two percent of episodes of metabolic decompensation were associated with infection [Edmonds et al., 2002]. The mechanism of infection-mediated metabolic decompensation in mitochondrial disease is unknown. As noted above, a retrospective analysis of definite mitochondrial disease subjects identified 28 with an autism diagnosis. In this population, fever was associated with neurodegeneration in 12 of 28 (43%) [Shoffner et al., 2010].

Regression is a common event in the autistic population. The definition of autistic regression requires loss of language skills. Frequently, autistic children also exhibit loss of fine and gross motor skills, eye contact, and socialization. Fombonne and Chakrabarti noted a lack of association of autistic regression with MMR immunization, with a higher incidence of regression preimmunization (18.4%) than post (15.6%) [Fombonne and Chakrabarti, 2001]. The California CHARGE study group reported loss of language and social skills in 15% of 333 children with autism or ASD [Hansen et al., 2008]. Lord et al. noted that 20% of autistic children underwent specific regression patterns and language loss was associated with low cognitive functioning [Lord et al., 2004]. Kurita reported speech loss in 21.7% of autistic children in Japan [Kurita, 1996]. Mitochondrial disease patients, as noted above, may undergo loss of cognitive and motor skills following encephalopathy induced by infection and fever. This neurodegeneration shares some features with autistic regression.

*Multiorgan system disease*

A common feature of mitochondrial disease is multi-organ system involvement predominantly of organ systems requiring a high energy supply. The most common organ affected is the brain but heart, skeletal muscle, gut, and endocrine systems are also frequently involved [Haas et al., 2007]. Recently, a retrospective cohort analysis found that 24 of 25 children with a primary diagnosis of ASD by DSM IV criteria and enzyme- or mutation-defined mitochondrial electron transport chain dysfunction had one or more major clinical abnormalities in addition to autism [Weissman et al., 2008]. In this group fatigability/exercise intolerance (68%) and GI dysfunction (64%) were the most common findings in organ systems other than brain.

*Gastrointestinal disease*

Gl dysfunction is a commonly reported comorbidity of ASD. In a cross-sectional study comparing lifetime prevalence of GI symptoms in 50 ASD children with age/sex and ethnicity matched normal controls and children with developmental disabilities, 70% of ASD children had a history of GI

symptoms compared with 28% of normal controls and 42% of children with other developmental disabilities [Valicenti-McDermott et al., 2006]. However, a sample of 172 ASD children participating in clinical trials conducted by the Research Units on Pediatric Psychopharmacology (RUPP) Autism Network found that only 22.7% were positive for GI symptoms, which were primarily constipation and diarrhea prior to starting treatment [Nikolov et al., 2009]. This finding more closely matches the findings from the Danish Hospital Register with an average 30 years of observation of hospital contact (inpatient or outpatient) in 118 subjects with infantile autism compared with 336 matched controls from the general population. This study found no evidence of an increased frequency of defined GI diseases in the autistics with a prevalence of 30.5% compared with 30.7% in controls [Mouridsen et al., 2009]. Thus, whilst GI symptoms in ASD are a common complaint it is not clear that their prevalence exceeds that of the general population. However, GI symptoms are more likely to be distressing to ASD subjects and present significant management issues [Nikolov et al., 2009]. Some studies which show no difference in GI symptoms between autistic children and controls do not consider severity and longevity of the problem. Recent workshops on GI dysfunction in ASD children address this issue [Buie et al., 2010a,b].

GI symptoms that may be severe are commonly reported in mitochondrial disease. GI dysmotility is the most common manifestation with the syndrome of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) due to thymidine phosphorylase deficiency that results in mtDNA depletion [Hirano et al., 1994; Giordano et al., 2008]. Mitochondrial polymerase γ deficiency is another cause of GI dysmotility [Amiot et al., 2009]. Intestinal pseudo-obstruction can be life-threatening in mtDNA disease including MELAS [Verny et al., 2008]. GI dysmotility can be an early manifestation of oxphos failure [Chitkara et al., 2003]. GI dysmotility often shows a maternal inheritance pattern, and mtDNA single nucleotide polymorphisms in the control region and elsewhere are frequently associated [Camilleri et al., 2009]. Severe GI symptoms are "red flags" for mitochondrial disease [Haas et al., 2007]. It follows that mitochondrial disease should be considered in ASD children with severe GI symptoms.

*The high-risk ASD population for possible mitochondrial disease*

Given that neurodegeneration is a feature of mitochondrial disease, it is a reasonable supposition that ASD children who undergo regression and children with symptoms of multisystem disorders (particularly GI dysfunction) are the populations which will contain most mitochondrial disease subjects.

*Is there a characteristic oxphos finding in mitochondrial autistics?*

Findings reported in published literature to date are listed in Table 1. Shoffner et al. noted that complex I deficiency was the most common isolated electron transport chain (ETC) defect in 14 of 28 (50%) of children and found in association with either complex III or complex IV deficiency in an additional 10 of 28 (36%) [Shoffner et al., 2010]. The two children reported by Filipek et al. had partial complex III deficiency in fibroblasts in both and muscle in one [Filipek et al., 2003]. Filiano et al. reported decreased ETC enzyme activities in seven of the eight children who underwent muscle biopsy, in whom six had complex III deficiency and one had complex IV deficiency. Five children had large mtDNA deletions and three had a histochemical or ultrastructural mitochondrial abnormality [Filano et al., 2002]. Oliveira et al. found muscle oxphos abnormalities in 7 of 11 children tested, of whom two had complex I deficiency, two had complex IV deficiency, and three had complex V deficiency [Oliveira et al., 2005]. Tsao and Mendell reported two ASD cases who had ETC deficiencies of either complex I or complexes II/III and IV in muscle [Tsao and Mendell, 2007]. Weissman et al. reported muscle biopsy results on 23 ASD patients of whom 16 (64%) had CI defects, 2 (8%) had complex II defects, 5 (20%) had complex III defects, and only 1 (4%) had a complex IV defect. In summary, published series to date report the majority of ASD children with ETC defects having had complex I or complex III defects.

*Do Mitochondrial Autistics have typical or atypical ASD?*

Weissman found that 11 of 25 (44%) of Mitochondrial Autistics met DSM IV-TR criteria for autistic disorder (typical) and the remainder (54%) met criteria for PDD-NOS (atypical) [Weissman et al., 2008]. These study findings do not differ markedly from the measured ratio of typical (36%) to atypical (PDD-NOS) (64%) autism in a recent meta-analysis of ASD prevalence reports [Williams et al., 2006]. However, all of Oliveira's 11 *definite* mitochondrial disease cases from Portugal were classified as severe typical autistics with learning disabilities ranging from moderate to severe [Oliveira et al., 2005]. The other published case series of Mitochondrial Autistics do not clearly indicate ratios of typical to atypical autism.

**Genetics of Autism**

Autism is known to have a strong genetic component. In 1985, a Swedish multicenter study reported that 13 of 83 boys with ASD (16%) were found to have fragile X syndrome [Blomquist et al., 1985]. This was an unusually high frequency. In a recent large collaborative study fragile X was found in 0.46% of ASD cases [Shen et al., 2010]. A variety of chromosomal defects have been found to be associated with ASD. One of the most common is duplication of the 15q11-15q13 region, which when deleted causes Angelman or Prader-Willi phenotypes [Procter et al., 2006]. Duplications in this region account for 1% to 2% of ASD cases [Abrahams and Geschwind, 2008]. These genetic defects affect a number of candidate genes that seem to be causal in individual families. Twin studies showing 70% to 90% concordance rate for monozygotic twins and up to 10% for dizygotic twins, a 25 fold increased prevalence of ASD in siblings of ASD children, and a high incidence of familial autistic behavioral traits clinch a genetic basis in many ASD cases [Abrahams and Geschwind, 2008]. There are an estimated 1,500 mitochondrial proteins encoded in the nucleus [Meisinger et al., 2008]. The total human genome is estimated to contain 30,000 genes and 5% are mitochondrial genes. It is not surprising that mitochondrial genes can be affected by copy number variations or pathogenic structural chromosomal changes occurring in ASD.

*Nuclear copy number variations in ASD*

The finding of susceptibility loci such as Xq,1p, 5q, 7q,16p,17q, and 19q as well as duplication of the maternally derived 15q11-q13 region have been known for many years. However, no single genomic change accounts for more than 1% to 2% of ASD cases. All genetic defects considered together may explain 10% to 20% of ASD cases [Abrahams and Geschwind, 2008; Kakinuma and Sato, 2008]. Over the last 3

years, a number of laboratories carrying out microarray studies have confirmed nuclear copy number variations (CNV) in ASD subjects that are rarely found in the normal population [Sebat et al., 2007; Szatmari et al., 2007; Glessner et al., 2009]. These CNVs are often microdeletions but can be duplications and may involve genes which are important for central nervous system development. These studies have suggested putative roles for three genes acting at the synapse (*SHANK3, NLGN4, NRXN1*) [Marshall et al., 2008], and *ubiquitin* [Glessner et al., 2009], *APBA2* [Babatz et al., 2009] and *contactin 4* [Roohi et al., 2009] as candidate genes in ASD. CNVs in ASD can be inherited or arise de novo. De novo CNVs were found in 10% of simplex ASD families, 3% of multiplex ASD families, and 1% of controls [Sebat et al., 2007]. The cause(s) of these nuclear CNV changes in ASD are not known, nor is it known whether mitochondrial DNA has aberrant epigenetic modifications. The intracellular localization of many of the putative genes affected by CNVs and structural chromosome defects in ASD are unknown, although some of these genes do affect mitochondrial function. The ubiquitin conjugation system is important for mitochondrial function and mitochondrial membrane dynamics. A number of ubiquitin genes including *UBE3A*, *PARK2*, *RFWD2*, and *FBXO40* are candidate genes affected by CNVs that are unique to ASD subjects [Glessner et al., 2009]. Loss or mutation of *UBE3A* encoding for E3 ubiquitin protein lyase is the cause of Angelman syndrome. Four of the many RING finger E3 ubiquitin ligases have been shown to localize to mitochondria, of which two are involved in mitochondrial fission (*MARCH5*) or fusion (*MRF1*) [Neutzner et al., 2008]. *PARK2* encodes Parkin, which is a cytosolic protein that moves into uncoupled mitochondria to assist in their destruction. Overexpression of Parkin with the phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (*PINK1*) causes collapse of the mitochondrial network, encouraging autophagy of even nondamaged mitochondria having normal membrane potentials [Vives-Bauza et al., 2010].

There is also some evidence of increased mitochondrial function in ASD subjects. Brain tissue homogenates from ASD subjects showed significantly higher aspartate/glutamate reduced nicotinamide adenine dinucleotide shuttle (AGC1) activity than in controls which appeared to be induced by higher neocortical calcium levels [Palmieri et al., 2010]. AGC1 activation is expected to provide increased NADH and result in increased oxphos activity. A moderate increase in cytochrome oxidase activity was also noted in these samples. The authors noted a marked increase in carbonylated mitochondrial proteins in four of the six brain samples, speculating that oxidative damage results from increased oxidative stress associated with higher oxphos activity. No mutations in *SLC25A12* encoding ACG1 were found. Information on copy number was not provided.

*Biochemical abnormalities in ASD*

Although not commonly measured, glutathione is an important mitochondrial antioxidant. Alteration of the glutathione redox balance with lower levels of reduced glutathione is seen in mitochondrial disease [Atkuri et al., 2009]. Low levels of reduced glutathione and increased oxidized glutathione was reported in lymphoblastoid cell lines and lymphoblast mitochondria from ASD children [James et al., 2009].

The metabolomic markers of mitochondrial dysfunction include elevations of lactate, pyruvate and ammonia in blood along with several amino acid elevations that result from accumulation of these metabolites. Elevations of plasma alanine (the transamination product of pyruvate) above 450 μM, particularly when compared with the level of essential amino acids alanine:lysine (normal ratio <3:1) and alanine: phenylalanine + tyrosine (normal ratio <4), can be seen in mitochondrial dysfunction [Hoffmann et al., 2001]. Several urine organic acids can also indicate mitochondrial dysfunction when elevated. These include fumarate and malate [Barshop, 2004], ethylmalonic acid, and 3-methyl glutaconic acid. In this study urinary lactate correlated poorly with mitochondrial disease. Secondary impairment of mitochondrial β-oxidation leads to shunting of fatty acids to microsomal ω-oxidation resulting in excretion of dicarboxylic acids in the urine. Caution should be exercised in the interpretation of dicarboxylic aciduria, as fasting that provokes ketosis will also cause this. Other indicators of impaired mitochondrial β-oxidation include elevations of plasma acyl-carnitine esters and depletion of plasma carnitine, which is esterified to excess fatty acids and lost in the urine. Any of these metabolic abnormalities can be seen in some ASD subjects to suggest but not confirm possible mitochondrial dysfunction.

## Mitochondrial Dysfunction, Autism, and Immunization

A recent Vaccine Compensation Board decision in one ASD case [Sugarman, 2007] highlights growing concerns about the possibility that vaccination acts as a trigger for neurodegeneration in predisposed individuals, with a resulting autistic phenotype. In this case, mitochondrial disease was identified as the predisposing condition [Poling et al., 2006]. The recent retrospective study by Shoffner et al. concluded that fever was a predisposing factor for regression in Mitochondrial Autistics in 12 of the 17 subjects (70%) who suffered a developmental regression [Shoffner et al., 2010]. Four of these 12 had fever following immunization. Regression following immunization without fever was not noted. An NIH/CDC/FDA workshop was held to explore the putative association between mitochondrial disease and autism (NINDS, 2008). It is clear that vaccine induced autism is a rare situation. Several studies from around the world have failed to show an association between immunization and autism [Halsey and Hyman, 2001; DeStefano and Thompson, 2004; Fombonne et al., 2006; Honey, 2008]. A link between MMR vaccine, measles virus in the GI tract, and autism was proposed by Wakefield in 1998 leading to widespread fear among the lay community and a fall in immunization rates in the UK and elsewhere. This work is now discredited and the original article was retracted by the Lancet [Lancet, 2010]. As noted earlier, Fombonne and Chakrabarti noted that the incidence of autistic regression was 18.4% preMMR immunization and 15.6% postMMR immunization in autistic children [Fombonne and Chakrabarti, 2001]. A recent study of 25 autistic children undergoing endoscopy found no association of autism with the measles live virus immunization [Hornig et al., 2008]. Despite these studies, public anxiety about vaccination toxicity is widespread in part fostered by the internet [Zimmerman et al., 2005].

## Immune Modulators, Autism, and the Role of Mitochondria

The pathogenesis of most autism spectrum disorders (ASDs) remains unclear. One plausible explanation is the occurrence of abnormal immune responses to several putative antigens in genetically vulnerable children [Ashwood et al., 2006]. This theory is sup-

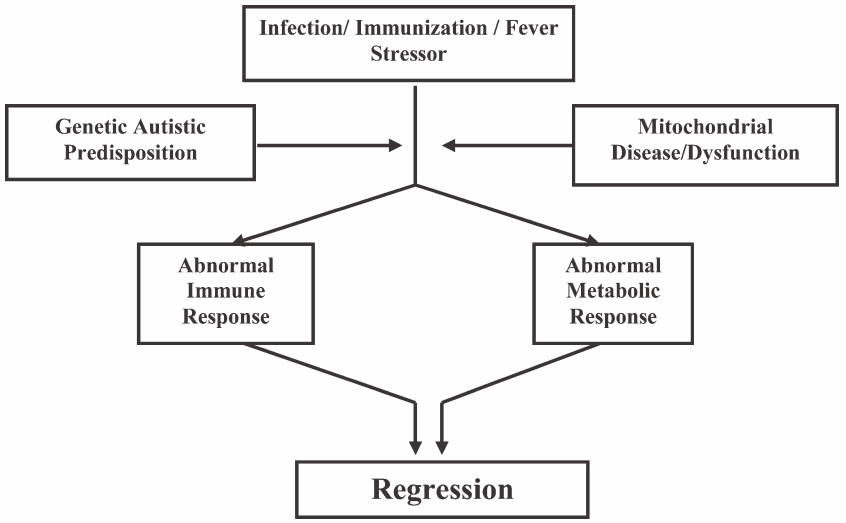


Fig. 2. Hypothesized interaction of immune stressors and mitochondria in autistic regression.

ported by evidence of abnormalities in several components of the immune system in ASD children, including elements of innate and adaptive immune responses. These abnormalities could lead to abnormal brain growth and development by disrupting the delicate balance between the immune system, neural growth factors, neural stem cells, and neurotransmitters. Figure 2 illustrates a hypothesis for the role of inflammation and mitochondrial dysfunction in the pathogenesis of ASD regression.

A number of studies have identified abnormalities in T-lymphocyte number and function, including abnormal accumulation of T lymphocytes in tissues such as the gastrointestinal tract [Ashwood et al., 2004], reduced numbers of circulating CD4+ T cells, abnormal lymphocyte responsiveness, and abnormal production of Th1 and Th2 cytokines. In particular, proinflammatory Th1 cytokines, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-1β, IL-6, and IL-12 have been the focus of several investigations. Two Th1/Th2 cytokines that have been implicated in ASD are TNF-α and IFN-γ. In ASD, constitutive [Ashwood et al., 2004] and inducible [Jyonouchi et al., 2001] production of TNF-α by peripheral blood mononuclear cells was elevated in comparison with normal children. TNF-α concentrations were also elevated in the serum [Croonenberghs et al., 2002] of ASD patients and their siblings [Ashwood et al., 2006]. IFN-γ is a pleiotropic cytokine produced by T cells and is one of the most potent macrophage activating molecules in vivo. In adults, IFN-γ has been implicated in a wide variety of neurological disorders. In ASD, IFN-γ levels were increased in plasma from 29 children [Sweeten et al., 2004] and markedly increased in CSF from a separate group of six others [Vargas et al., 2005]. Importantly, IFNs can induce the expression of over 300 genes, some of which are likely to be mitochondrial. These genes may be the actual mediators of the antiviral effects and anti-tumor effects of IFNs but they may also promote proapoptotic actions that could lead to neurodegeneration. One chemokine, CCL2 [or monocyte chemotactic protein (MCP)-1] seems to play an important role in several neurological disorders. In ASD, CCL2 levels are markedly elevated in CSF [Vargas et al., 2005].

Is there, in fact, inflammation in the autistic brain? Recently, Vargas et al. provided the first such evidence: Histological analyses demonstrated activated microglia and astroglia in prefrontal and cerebellar gray and white matter tissue from autistic brains [Vargas et al., 2005]. Glia play essential roles during brain development. Stimuli such as infection, injury, or immune activation can cause them to become excessively or persistently activated. Such abnormal glial activation is a feature of many neurological disorders, including Alzheimer's disease, AIDS, dementia, amytrophic lateral sclerosis, multiple sclerosis, ischemic injury, and prion diseases [Skaper, 2007]. In addition to TGF-β1, the Vargas analysis identified high levels of several growth factors, including VEGF, FGF-9, and IGFBP-1, among others. A number of studies have implicated growth factors in the pathogenesis of ASD, suggesting that ASD individuals manifest an abnormal or abnormally robust immunological response.

*Cytokine responses in immunization*

For the last 60 years, inducible factors released by leukocytes have been known to induce fever. The fever producing property of IL-1 was the first observed cytokine effect [Dinarello, 2010]. The mechanism of fever production is not fully understood but involves hypothalamic and vasomotor changes, along with increased heat production through the effects of mitochondrial uncoupling proteins [Ricquier and Bouillaud, 2000]. Heat stress can damage mitochondria, opening the permeability transition pore and leading to apoptosis [Qian et al., 2004]. It is likely that cells with compromised oxphos activity resulting in impaired proton motive force will be more susceptible to effects of high temperature. Immunization vaccines can be classified as killed vaccines and live attenuated vaccines. In both cases, the aim of vaccination is to increase immune responses to natural infections. To be effective, vaccines must activate similar immunological responses to the natural infection that they are designed to protect against. Cytokines are key components of the immune response. In some cases, vaccines can produce an enhanced cytokine response when compared with natural virus infection. This is the case with measles vaccines, which induce IRF-3 production and subsequent IFN-β release in an enhanced manner compared with wild-type infection with this RNA virus. Enhanced innate or adaptive immune responses could theoretically play a role in vaccine reactions, febrile response, and damage to susceptible mitochondria.

*Immune modulation and mitochondria*

Corticosteroid release by the autonomic nervous system and hypothalamic-pituitary-adrenocorticalneuroaxis is an important modulator of the immune response. Glucocorticoids inhibit production of proinflammatory cytokines, chemokines, and cytokine receptors including TNF-α, IL-2, IL-6, IL-1β, and IL-8. They also upregulate production of anti-inflammatory cytokines including IL-10, IL-4, and TGF-β [Psarra et al., 2006]. Both nuclear and mitochondrial encoded oxphos subunits are regulated by glucocorticoids. Cyto-

kines and their receptors are known to be important components of the stress response, triggering apoptosis through both intrinsic and extrinsic pathways. There is accumulating evidence that mitochondria are important mediators of cytokine-induced apoptosis and efficiency of mitochondrial function helps to determine apoptotic response. Indeed, mitochondrial-nuclear interactions play a role in cytokine gene expression. Cybrid studies with 143BT.K-osteosarcoma cells have shown that the mtDNA background of polymorphisms modulates cytokine gene expression of interleukin-6 (IL-6) under stress as well as expression of IL-1β and tumor necrosis factor receptor 2 in the basal state [Bellizzi et al., 2006]. Recently, a mouse model of autism produced by injection of double-stranded RNA poly(I:C) into pregnant mice to cause maternal immune activation (MIA) identified IL-6 as a key mediator of the effects of MIA on fetal brain development [Smith et al., 2007]. Recent evidence also suggests that IL-6 regulates mitochondrial fusion interactions by increasing Bcl-2 preventing the dissociation of the proapoptotic protein Bak from the mitofusin protein Mfn, thereby increasing survival in mice exposed to hyperoxia [Waxman and Kolliputi, 2009].

*Intrinsic and extrinsic apoptosis pathways and autism*

Bcl-2 anti-apoptotic protein was decreased 34% to 51% in autistic cerebellum compared with age, sex matched controls [Fatemi, 2001]. This finding was recently confirmed utilizing expression arrays and expression of the proapototic proteins caspase-3 and cathepsin D were significantly increased in autistic brain [Sheikh, 2010]. These findings suggest that increased apoptosis may contribute to the pathogenesis of autism. Mitochondria are involved in apoptosis at several levels [Psarra et al., 2006]. The study of oxidative phosphorylation (oxphos) deficient fibroblasts has shown that oxphos is necessary for both the extrinsic and intrinsic apoptotic pathways. Knock-out mouse cytochrome-c (cyt c) derived fibroblasts are resistant to both staurosporine-induced (intrinsic) and TNF-α induced (extrinsic) apoptosis. The mechanism involves a caspase cascade that is independent of the mitochondrion. TNF-α also is known to produce caspase-8-dependent Bid cleavage to truncated Bid, which works through a mitochondrial mechanism to stimulate mitochondrial formation of Bax oligomers which results in cyt c release. IL-1β and IFN-γ, secreted by T cells, have been shown to induce apoptosis in a rat insulinoma cell line both by induction of nitric oxide (NO) and by an independent mitochondrial mechanism that involves a fall in mitochondrial membrane potential, release of cytochrome c, and downstream cleavage of procaspases −9, −7, and −3. In a study of a Jurkat cell variant H123 that undergoes apoptosis when incubated with INF-α/β, several lines of evidence confirm a mitochondrial-dependent (intrinsic) pathway as the mechanism of INF-α induced apoptosis. In these cells, INF-α activates caspase-9 but not caspase-8 and JC-1 studies confirmed a fall in mitochondrial membrane potential with INF-α treatment and cyt c release [Gamero et al., 2006].

*Immune modulators and mitochondrial disease*

Remarkably, there is very little literature on the role of cytokines in mitochondrial disease. Nonalcoholic steatohepatitis (NASH) is a disorder with documented low ETC activities, which plays an important role in its pathogenesis. When associated with diabetes, NASH displays over-production of TNF-α and modulation by IL-10 [Hashem et al., 2008]. An obese, leptin deficient mouse model, the ob/ob mouse, was found to be protected from ETC deficiency by administration of anti-TNFα [Garcia-Ruiz et al., 2006]. The neurological deterioration so commonly seen in mitochondrial disease following infections and fever may well involve cytokines and other immune modulators. This is an area worthy of future research attention. A better understanding of these mechanisms may help both mitochondrial disease patients and autistic children.

## SUMMARY

There is convincing evidence for a mitochondrial role in a subset of children with ASD. Evidence of mitochondrial dysfunction is seen in at least 8% of ASD subjects and a number clearly have mitochondrial disease. Children with Mitochondrial Autism often suffer multiorgan system disease and regression. A variety of biomarkers of intermediary metabolism can help identify mitochondrial dysfunction in ASD subjects. It is unknown whether mitochondria might contribute to the pathogenesis of autism in children without biomarkers of mitochondrial dysfunction. Gene defects and CNVs in ASD have the potential to affect nuclear genes controlling mitochondrial function. The underlying mechanisms of mitochondrial involvement in ASD are unknown but likely involve neuroinflammation, glial activation and cytokine release. Efficient mitochondrial energy production is also important in both neuronal function and neurodevelopment. Damaged mitochondrial energy production triggers apoptosis which may be important in the pathogenesis of ASD. The mitochondrial role in autism is a fertile area for future research which may lead to opportunities for therapeutic intervention. ■

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