Mitochondrial Dysfunction in Autism

Abha Chauhan, Feng Gu, and Ved Chauhan

Abbreviations

ADP	Adenosine diphosphate
AGC	Aspartate/glutamate carrier
ASDs	Autism spectrum disorders
ATP	Adenosine triphosphate
ETC	Electron transport chain
$FADH_2$	Dihydrogen flavin adenine dinucleotide
GPx	Glutathione peroxidase
GSH	Glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
HEADD	Hypotonia, intractable epilepsy, autism, and developmental delay
MD	Mitochondrial disease
MELAS	Mitochondrial encephalopathy with lactic acidosis and seizures
MMP	Mitochondrial membrane potential
MRS	Magnetic resonance spectroscopy
NADH	Reduced nicotinamide adenine dinucleotide
NO	Nitric oxide
PDHC	Pyruvate dehydrogenase complex
ROS	Reactive oxygen species
SNPs	Single-nucleotide polymorphisms
SOD	Superoxide dismutase
TCA	Tricarboxylic acid

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A. Dietrich-Muszalska et al. (eds.), *Studies on Psychiatric Disorders*,
Oxidative Stress in Applied Basic Research and Clinical Practice,
DOI 10.1007/978-1-4939-0440-2_17

1 Structure and Function of Mitochondria

Mitochondria, containing an inner and an outer plasma membrane, are very important cellular organelles that generate adenosine triphosphate (ATP), the energy carrier in most mammalian cells, by oxidizing glucose and fatty acids. Acetyl-CoA is a key intermediate generated from the oxidation of glucose and fatty acids, and it enters the tricarboxylic acid (TCA) cycle, also known as the Krebs cycle, in the mitochondrial matrix. The TCA cycle produces reduced flavin adenine dinucleotide ($FADH_2$) and reduced nicotinamide adenine dinucleotide (NADH), which donate electrons to the electron transport chain (ETC) located in the inner mitochondrial membrane. The ETC is an essential part of mitochondria, and generation of energy in the form of ATP is its most important function. More than 90 % of energy needed in the cell to maintain its physiological activities is supplied by the ETC through oxidative phosphorylation (Bertram et al. 2006; Szewczyk and Wojtczak 2002). The ETC consists of five multi-subunits of enzymes, i.e., complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome bc1 complex), complex IV (cytochrome c oxidase), and complex V (ATP synthase) (Boekema and Braun 2007; Dudkina et al. 2005). While complexes I–IV participate in the generation of proton gradient (membrane potential) in the intermembrane space of mitochondria, complex V transports protons from intermembrane space to the mitochondrial matrix (Fig. 1). The generated proton gradient is used by ATP synthase to catalyze the phosphorylation of adenosine diphosphate (ADP) to ATP. Ubiquinone (also known as coenzyme Q10) and cytochrome c are the electron carriers of the ETC and help in the transfer of electrons between ETC complexes.

The ETC is also a main source of free radicals, i.e., reactive oxygen species (ROS), which have important roles in cell signaling and homeostasis (Cadenas and Davies 2000; Lenaz 2001). ROS generation is a by-product of proton cycling between ubiquinone, cytochromes b and c1, and iron–sulfur protein (Sugioka et al. 1988). Complexes I and III are the main sites of mitochondrial superoxide (O_2^-) production (Barja 1999; Muller et al. 2004). While complex I releases superoxide exclusively into the mitochondrial matrix, complex III releases superoxide to both sides of the inner mitochondrial membrane, i.e., to the mitochondrial matrix and the intermembrane space (Muller et al. 2004). The superoxide radicals generated by complexes I and III are neutralized and converted to hydrogen peroxide (H_2O_2) by manganese superoxide dismutase (Mn SOD) in the mitochondrial matrix or by copper/zinc (Cu/Zn) SOD in the intermembrane space of mitochondria (Fig. 1). Under normal circumstances, there is a balance between ROS generation and the antioxidant capacity of the cell. However, in some situations (e.g., environmental exposure to air pollutants and toxins), ROS levels can increase dramatically and exceed the antioxidant ability of Mn SOD and Cu/Zn SOD, thus causing oxidative stress and triggering apoptosis. ROS-mediated lipid peroxidation, oxidation of proteins, and DNA damage are well-known outcomes of oxidative stress, leading to cellular damage and ultimately to cell death (Bandyopadhyay et al. 1999; Cadenas and Davies 2000; Lenaz 2001; Polster and Fiskum 2004). The ETC abnormalities may result in inhibition of ATP synthesis and acceleration of ROS generation, leading to

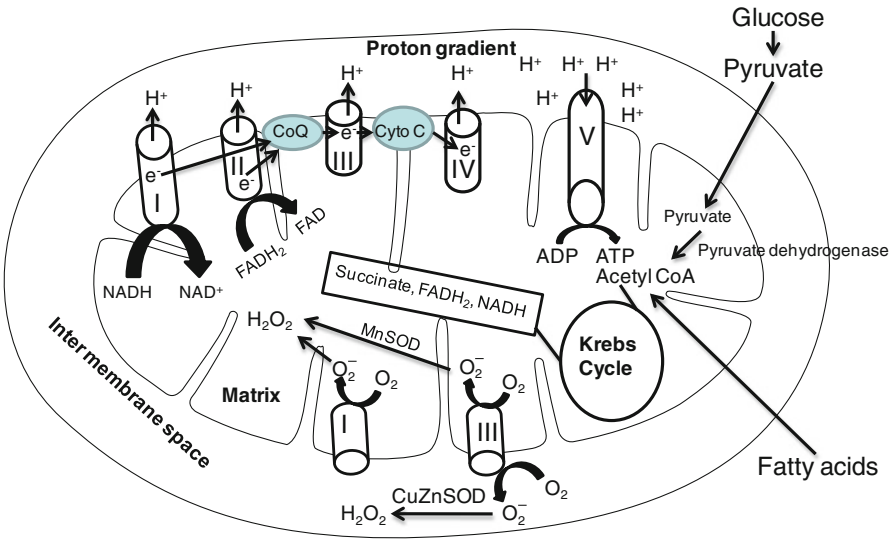

Fig. 1 ATP production and superoxide free radical generation by the electron transport chain (ETC) of mitochondria. Mitochondria have two membranes: an inner and an outer membrane. ETC consists of a series of metalloproteins bound to the inner membrane of the mitochondria, including five enzyme complexes, designated I–V, i.e., complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome bc1 complex), complex IV (cytochrome c oxidase), and complex V (ATP synthase). Electrons are transferred from NADH to O_2 through inner membrane ETC complexes I, III, and IV. Coenzyme Q (CoQ) and cytochrome c. CoQ shuttles electrons from complexes I and II to complex III, and cytochrome c transfers these electrons from complex III to IV. During this process, protons are pumped through the inner mitochondrial membrane to the intermembrane space to establish a proton gradient, which is used by complex V (ATP synthase) to phosphorylate ADP thereby generating ATP. Complexes I and III are also the main sites of mitochondrial free radical superoxide (O_2^-) production. Complex I-dependent O_2^- is exclusively released into mitochondrial matrix, where it is converted to hydrogen peroxide (H_2O_2) by the manganese superoxide dismutase (Mn SOD). On the other hand, superoxide generated at complex III can be released to both sides of the inner mitochondrial membrane. Superoxide released into the mitochondrial intermembrane space is converted to H_2O_2 by Cu/Zn SOD. NADH and $FADH_2$ are produced within the matrix of the mitochondria by the Krebs cycle, also known as the tricarboxylic acid (TCA) cycle. The redox energy from NADH and $FADH_2$ is then transferred to oxygen (O_2) via the ETC of mitochondria

impairment of energy metabolism, elevated oxidative stress, and disruption of mitochondrial functions and subsequently affecting neurons' function and plasticity, which may finally lead to abnormal neurodevelopment.

Although most DNA is packaged in chromosomes within the nucleus, mitochondria also have a small amount of their own DNA, known as mitochondrial DNA (mtDNA). Mitochondrial function is under the dual genetic control of both mtDNA and nuclear DNA (nDNA). mtDNA contains 37 genes and encodes for 13 subunits of complexes I, III, IV, and V (Anderson et al. 1981). The other subunits of the ETC complexes are coded by more than 850 nDNA genes (Cotter et al. 2004). The expression, replication, and maintenance of mtDNA also require the factors encoded by nuclear genes (Shadel 2008). Furthermore, the nuclear-encoded signaling pathway

genes play a role in mediating adaptive functions of mitochondria under altered conditions (Shadel 2008; Shadel and Pan 2009). Hence, mtDNA and/or nDNA genome mutations may lead to deficiencies of ETC complexes and subsequently to mitochondrial dysfunction.

Mitochondria also play a pivotal role in the maintenance of intracellular calcium homeostasis and in amino acid, lipid, and steroid metabolism, thereby regulating developmental processes, including neurite outgrowth, axonal plasticity, and synaptic plasticity (Chinnery and Schon 2003; Fattal et al. 2006; Mattson and Liu 2002; Orth and Schapira 2011; Szewczyk and Wojtczak 2002). The brain has a high demand for energy, and it requires a high content of mitochondria. Neurons are highly dependent upon oxidative phosphorylation as the primary pathway for ATP generation, of which 40–60 % is utilized in the maintenance of ion gradients by ATPases. Neuronal synapses, in particular, are areas of high energy consumption, and therefore, they especially rely on mitochondrial function (Ames 2000; Mattson and Liu 2002). Mitochondria are concentrated in the dendritic and axonal termini, where they are involved in ATP production, calcium homeostasis, and synaptic plasticity (Li et al. 2004). Therefore, neurons' function and plasticity rely mostly on mitochondrial structure and number. Synaptic transmission is affected if there is alteration in the number, morphology, or function of synaptic mitochondria (Polster and Fiskum 2004). Metabolic and mitochondrial defects affect the function and plasticity of neurons, cause neuronal loss, and alter modulation of neurotransmission systems. Therefore, the brain is a prime target of mitochondrial dysfunction (Orth and Schapira 2011).

Mitochondrial dysfunction has been implicated in several human diseases, such as neurodegenerative diseases, neurodevelopmental disorders, and cardiac dysfunction, and it may play a role in the aging process. In addition, mitochondrial abnormalities are also associated with several other medical conditions (Fig. 2).

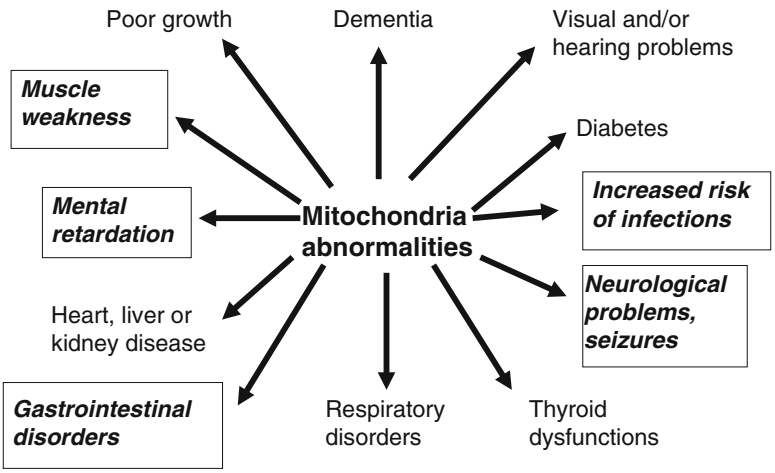

Fig. 2 Various medical conditions suggested to be associated with mitochondrial abnormalities. The medical conditions shown in *italics/bold* inside the boxes are also commonly observed in autism

Depending on how severe the mitochondrial disorder is, the symptoms can range in severity from mild to fatal. Some of the symptoms due to mitochondrial abnormalities shown in boxes in Fig. 2 are frequently observed in individuals with autism.

2 Autism

Autism belongs to a group of neurodevelopmental disorders known as the autism spectrum disorders (ASDs) that also include Asperger syndrome and pervasive developmental disorder-not otherwise specified. Autism is a heterogeneous disorder characterized by impairments in basic social and communicative behaviors such as eye contact, intonation, and facial expressions, as well as by repetitive and stereotyped patterns of behavior (Lord et al. 2000). The symptoms of ASDs are typically present before the age of 3 years. According to a report from the Centers for Disease Control and Prevention, 1 in 68 children in the USA is affected with autism (Wingate et al. 2014). Recently, researchers reported that the prevalence of ASDs in a South Korean community was as high as 3.74 % for boys and 1.47 % for girls among school-age children (Kim et al. 2011). It was 1.89 % in the general population of school-age children from regular schools and 0.75 % in a high-risk group from special education group and disability registry (Kim et al. 2011).

The exact cause of autism is still not known, although roles of genetic and environmental factors, oxidative stress, mitochondrial dysfunction, inflammation, and immune abnormalities have been suggested in ASDs (Chauhan et al. 2009a). While no single gene has been found to be associated with ASDs, multiple genetic components, including mtDNA mutations or deletions, nDNA mutations, and chromosomal defects, have been postulated in the ASDs (Abrahams and Geschwind 2010; El-Fishawy and State 2010; Holt and Monaco 2011; Miles 2011). However, the lack of complete concordance in monozygotic twins and the variation in severity in concordant pairs suggest that nongenetic factors also contribute to the etiology of ASDs. Some gene variants in ASDs may confer altered vulnerability to environmental stressors, and gene–environment interactions may alter the course of development of the central nervous system and lead to behaviorally defined symptoms of ASDs (Herbert 2010). Prenatal or postnatal environmental exposure to prooxidant factors such as metals, viruses, air pollutants, and toxins is known to increase the body burdens and production of ROS, which may trigger oxidative stress and the development of clinical symptoms of ASDs.

3 Mitochondrial Dysfunction in Autism

3.1 Prevalence of Mitochondrial Disease (MD) in Autism

MD is often caused by a gene mutation or deletion and is the most frequent cause of metabolic disease. The diagnosis of MD is complicated and is based on several clinical and laboratory tests. The prevalence of MD is approximately 0.01 % in the

general population (Skladal et al. 2003). However, according to meta-analyses from two large prospective studies (Correia et al. 2006; Oliveira et al. 2005) and one retrospective study (Scaglia et al. 2009), the prevalence of MD in the general population of ASDs is 5.0 % (Rossignol and Frye 2012), which is much higher than its prevalence in the general population. There are many biochemical markers such as lactate, pyruvate, lactate/pyruvate ratio, ubiquinone, alanine, alanine-to-lysine ratio, and acyl-carnitine that may directly suggest mitochondrial dysfunction. Other biomarkers, e.g., creatine kinase, carnitine, aspartate aminotransferase, alanine aminotransferase, and ammonia, may also indirectly suggest mitochondrial dysfunction. However, there is no reliable biomarker to identify all cases of MD (Haas et al. 2007).

3.2 Abnormal Energy Metabolism in Autism

Because of the frequent association of lactic acidosis and carnitine deficiency in autistic subjects, Lombard (1998) presented a hypothesis that mitochondrial dysfunction and defects in neuronal oxidative phosphorylation may be involved in the etiology of autism. Several reviews have recently shed light on mitochondrial dysfunction in autism (Chauhan and Chauhan 2012; Chauhan et al. 2012b, Gargus and Imtiaz 2008; Haas 2010; Palmieri and Persico 2010; Rossignol and Bradstreet 2008; Rossignol and Frye 2012). However, it is not yet known whether mitochondrial dysfunction in ASDs is the primary etiology or pathology secondary to other causes.

Many lines of evidence from biochemical, genetic, anatomical, and neuroradiographical studies indicate a relationship between the dysfunction of brain energy metabolism and autism (Chauhan et al. 2011b; Chugani et al. 1999; Filiano et al. 2002; Guevara-Campos et al. 2010; Lombard 1998; Minshew et al. 1993). In 1993, a ^{31}P-magnetic resonance spectroscopy (MRS) study showed decreased synthesis and increased membrane degradation as well as decreased synthesis of ATP in the dorsal prefrontal cortex of the brain in 11 high-functioning autistic men compared to age-matched control subjects (Minshew et al. 1993). The alterations in brain energy and phospholipid metabolism in autism correlated with the neuropsychologic and language deficits, i.e., the severity of autism symptoms. In 1999, another MRS study showed decreased N-acetyl-aspartate and increased lactate levels in the frontal lobe, temporal lobe, and cerebellum of nine children with autism (Chugani et al. 1999). These studies suggested a disturbance of brain energy metabolism in autism.

In several investigations with blood and/or muscle biopsy samples from individuals with ASD, analysis of biochemical markers of mitochondrial dysfunction showed high lactate, increased lactate to pyruvate ratio, increased alanine levels, and low carnitine levels in autism (Correia et al. 2006; Filipek et al. 2003; Mostafa et al. 2005; Oliveira et al. 2005; Weissman et al. 2008). For example, significantly lower serum carnitine and higher plasma lactate were reported in a study of 30 children with autism compared with the control subjects (Mostafa et al. 2005). The levels of carnitine and lactate correlated with the severity of autism, i.e., individuals with severe autism had significantly lower carnitine and higher lactate concentrations than those with mild or moderate autism.

A population-based survey of children with ASD conducted by Oliveira et al. (2005) showed that 14 of 69 (20.3 %) autistic children had hyperlactacidemia and 5 of 11 autistic children (who also had muscle biopsies) were classified with definite mitochondrial respiratory chain disorder, suggesting that this might be one of the most common disorders associated with autism. However, these investigators did not find any mtDNA mutation and/or deletion associated with known mitochondrial disorders in these children. Another study of 210 autistic subjects reported hyperlactacidemia in 36 subjects (17 %) and elevated lactate/pyruvate ratio in 27 % (53 of 196 subjects) (Correia et al. 2006). MD was also confirmed in 7 of the 30 fully assessed subjects (Correia et al. 2006). The results of a meta-analysis by Rossignol and Frye (2012) showed the prevalence of elevated lactate to be 31.1 % from six studies (Correia et al. 2006; Germanò et al. 2006; Laszlo et al. 1994; Moreno et al. 1992; Mostafa et al. 2005; Oliveira et al. 2005) and of elevated pyruvate to be 13.6 % from two studies (Germanò et al. 2006; Laszlo et al. 1994) in the general population of individuals with ASDs. However, classical MD only occurs in a few autistic individuals and is generally accompanied by genetic abnormalities and defects in the respiratory chain.

3.3 Activities and Expression Defects of Mitochondrial ETC Complexes in Autism

Several case studies showed alterations in the activities or expression levels of ETC complexes in autism. For example, two children with autism had deficiencies in respiratory chain enzymes such as complexes I–III and coenzyme Q_{10} (CoQ) (Tsao and Mendell 2007). In a recent review and meta-analysis, Rossignol and Frye (2012) reported deficiencies of complexes I, III, V, IV, and II in 53 %, 30 %, 23 %, 20 %, and 9 % of children with ASD and concomitant MD, respectively. Multiple complex deficiencies were found in 36 % of the children with ASD/MD.

The onset of autism is gradual in many children. However, in regressive autism, children first show signs of normal social and language development, but they lose these developmental skills at 15–24 months and develop autistic behavior (Ozonoff et al. 2005). This pattern may be different in some children with regressive autism. The reported incidence of regressive autism varies in different studies from 15 to 62 % of cases (Goldberg et al. 2003; Hansen et al. 2008; Stefanatos 2008). A huge reduction of the enzymatic activities of complexes I and III was reported in a 19-month-old autistic girl with developmental regression (Poling et al. 2006). These investigators also performed a retrospective study, which included 159 subjects with autism and 94 age-matched control subjects. They reported increased levels of aspartate aminotransferase and creatine kinase in the serum, suggesting abnormal oxidative phosphorylation in autism (Poling et al. 2006). In another study, Shoffner et al. (2010) reported autistic regression in 61 % (17 of 28) ASD subjects with definite MD and that fever was associated with the onset of regression in 12 of these children.

Weissman et al. (2008) performed a retrospective analysis of cases with autism. In addition to clinical symptoms for autism, these 25 individuals also presented

enzyme- or mutation-defined mitochondrial ETC dysfunction. Complex I activity was decreased in 16 of 25 (64 %) autistic subjects, and this was the most prevalent enzyme defect. It was followed by complex III deficiency, which was affected in 5 of 25 (20 %) autistic subjects. Deficiency of complexes II and IV was reported in 5 % and 4 % of autism cases, respectively. They reported that 40 % of this group demonstrated an unusual pattern of regression (multiple episodes, loss of motor skills, or regression after the age of 3), and six children had the mtDNA mutation (Weissman et al. 2008). Another report on the mitochondrial respiratory chain in the muscle homogenate of a 3-year-old girl with autism also showed a partial deficiency of complex III and a slightly diminished complex IV (Guevara-Campos et al. 2010).

Mitochondrial abnormalities have also been reported in the lymphoblastoid cells and lymphocytes from peripheral blood in autism. Inhibition of complex I was reported in the lymphoblasts from 7 of 9 autistic subjects, and a 40–50 % higher mitochondrial maximal respiratory rate was found in all nine autistic cases compared to lymphoblasts from unaffected subjects (Holtzman 2008). Increased respiratory rate in autism was suggested to be a compensatory response to the partial inhibition of ATP synthesis (Holtzman 2008). We reported that mitochondrial membrane potential (MMP) is reduced, and free radical generation is elevated in the lymphoblasts from autistic subjects (obtained from the Autism Genetic Resource Exchange) compared to lymphoblasts from age-matched control subjects (Chauhan et al. 2009b). In another study, exposure to physiological concentrations of nitric oxide (NO) reduced MMP to a greater extent in the lymphoblasts from autistic subjects than from control subjects (James et al. 2009). Giulivi et al. (2010) examined mitochondrial functions in the lymphocytes from the blood of 10 children with autism and 10 typically developing children (ages 2–5 years). The activities of ETC complexes I–V and pyruvate dehydrogenase complex (PDHC), the mitochondrial rate of H_2O_2 production, and mtDNA copy number were analyzed. PDHC is the critical regulatory enzyme of cell metabolism because it catalyzes oxidative decarboxylation of pyruvate to form acetyl-CoA. They reported reduced activities of complexes I, IV, and V in six, three, and four of ten autistic children, respectively. The activity of PDHC was also significantly reduced, while plasma pyruvate levels and mitochondrial rate of H_2O_2 production increased in children with autism. However, the diagnostic criterion for a definite MD was fulfilled in only one child with autism.

In our recent study, we have reported brain region-specific deficits in the expression levels of mitochondrial ETC complexes in children with autism (Chauhan et al. 2011b). As compared to age-matched control subjects, the levels of complexes III and V in the cerebellum, of complex I in the frontal cortex, and of complexes II, III, and V in the temporal cortex were significantly lower in children with autism (ages 4–10 years) (Chauhan et al. 2011b). Analysis of the scattered plots showed that there was no overlap in the levels of these ETC complexes in the cerebellum and temporal cortex between autistic and control groups. In the frontal cortex, lower levels of ETC complexes were observed in a subset of autism cases, i.e., 60 % (3 of 5) for complexes I, II, and V and 40 % (2 of 5) for complexes III and IV. Interestingly, no change in the levels of any of the five ETC complexes was detected in the parietal and occipital cortices in the children with autism compared to control subjects,

suggesting that the ETC defect in autism is specific to the cerebellum and the frontal and temporal lobes. When adult cases (ages 14–39 years) were examined, no significant difference in the levels of ETC complexes in any brain region was observed between autistic subjects and age-matched control subjects. These results suggest that the expression of ETC complexes is decreased in the cerebellum and the frontal and temporal regions of the brains of children with autism (Chauhan et al. 2011b).

3.4 Mitochondrial Dysfunction in Autistic Subjects with Genetic Abnormalities

Many studies have revealed mitochondria-related gene abnormalities in autism, which may be caused by chromosome depletion, mtDNA mutation or depletion, and/or decreased levels of mRNA. Duplications of the proximal long arm of chromosome 15, including inverted duplication and interstitial duplication, are associated with autism. This abnormality has high frequency in autism, and 1–5 % of individuals with autism carry it (Gillberg 1998; Schroer et al. 1998). Mitochondrial hyperproliferation and deficiency in complex III of ETC were found by muscle biopsies in two autistic children with a chromosome 15q11-q13 inverted duplication (IDIC 15) (Filipek et al. 2003). In another study, two autism cases associated with sudden infant death syndrome showed mild mitochondrial hyperproliferation and a possible complex II defect (Gargus and Imtiaz 2008). The risk of sudden death in individuals with IDIC 15 is approximately 1 % per year.

The results of buccal swab ETC analysis in a 12-year-old boy with autism, epilepsy, and leg weakness showed a severe decrease in complex IV activity and a mild reduction in complex I activity (Ezugha et al. 2010). Chromosomal microarray analysis revealed 1-Mb deletion in the 5q14.3 region in this child. It was suggested that (i) this chromosomal deletion may be related to complex I and IV deficits, thereby manifesting in a mitochondrial disease and autism, and (ii) genes located within the deleted region of 5q14.3 may encode or regulate the expression and/or assembly of complex I or IV subunits (Ezugha et al. 2010).

Several studies have reported mtDNA mutations in autism. In a group of 12 children who presented clinically with hypotonia, intractable epilepsy, autism, and developmental delay (HEADD syndrome), five showed increased levels of large-scale mtDNA deletions that were not related with mitochondrial encephalomyopathies, and three of four children who were further examined had structural mitochondrial abnormalities (Filiano et al. 2002). Activities of mitochondrial respiratory enzymes were reduced in seven of eight children who had muscle biopsy (Filiano et al. 2002). In another report of five children with autism, the mtDNA A3243G mutation was observed in two of these children and in the mothers of two other children (Pons et al. 2004). This mutation is often associated with MELAS syndrome (mitochondrial encephalopathy with lactic acidosis and seizures). Another child in this group had 72 % mtDNA depletion in skeletal muscle and reduction in activities of complexes I, III, and IV to 34 %, 23 %, and 25 % of control

values, respectively (Pons et al. 2004). Another study reported the G8363A mutation in the mtDNA $tRNA^{lys}$ in blood and skeletal muscle from a boy with autism who also showed complex IV defect (Graf et al. 2000).

The protein encoded by the NADH-ubiquinone oxidoreductase 1 alpha subcomplex 5 (NDUFA5) gene is involved in the mitochondrial ETC complex I. In a Japanese case-control study that included 235 subjects with autism and 214 control subjects, Marui et al. (2011) examined three single-nucleotide polymorphisms (SNPs) of this gene and reported a significant association of two SNPs with autism. However, the mRNA level of another subunit of complex I (75-kDa subunit) was similar in the whole blood from autistic children compared to the controls (Taurines et al. 2010).

Normally, each mitochondrion has two to 10 copies of mtDNA (Robin and Wong 1988). This copy number can vary depending on the energy needs of the cells under different physiological conditions (Shay et al. 1990). Using lymphocytes, mtDNA over-replication was reported in five of 10 children with autism (Giulivi et al. 2010). This difference was not lymphocyte-specific. Similar results were also obtained with granulocyte cells. It was suggested that increased copy number of mtDNA in autism may be a compensatory mechanism to the defects of mtDNA replication or repair so that mtDNA can maintain the normal transcript's levels. The defects of mtDNA in autism may be caused by primary gene deficiency or higher oxidative stress of cells.

3.5 Oxidative Stress in Autism

The free radicals are generated endogenously during oxidative metabolism and energy production by mitochondria, and the ETC in mitochondria is a prime source of ROS generation (Cadenas and Davies 2000; Lenaz 2001). ROS can attack vital components of the cell, such as polyunsaturated fatty acids, proteins, and nucleic acid. These reactions can alter intrinsic membrane properties such as fluidity, ion transport, enzyme activities, protein cross-linking, and protein synthesis, ultimately resulting in cell death (Bandyopadhyay et al. 1999). Neuronal cells are very vulnerable to oxidative stress as a result of the high rate of oxygen delivery and consumption in the brain. Extensive evidence from our and other groups suggests a role of oxidative stress in the development and clinical manifestation of autism. Prenatal or postnatal exposure to environmental factors such as air pollution, organophosphates, and heavy metals may act as a trigger to increase the production of ROS, induce oxidative stress, and cause mitochondrial dysfunction, leading to the development of autism in the children (Chauhan and Chauhan 2006; Chauhan et al. 2009a; Herbert 2010).

The levels of oxidative stress markers for lipid peroxidation, protein oxidation, and DNA oxidation are increased in the blood (Chauhan and Chauhan 2006; Chauhan et al. 2004; Mostafa et al. 2010; Zoroglu et al. 2004), urine (Ming et al. 2005) and brains (Chauhan et al. 2011a, b; Chauhan and Chauhan 2012b; Evans et al. 2009; López-Hurtado and Prieto 2008; Muthaiyah et al. 2009; Sajdel-Sulkowska et al. 2011) of individuals with autism as compared to control subjects. In addition, antioxidant defense is decreased in autism. Levels of major antioxidant

proteins, namely, transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein), are decreased in the serum of children with autism, particularly in regressive autism (Chauhan et al. 2004). Glutathione (GSH) is another major endogenous antioxidant produced by the cell, which neutralizes ROS and participates in the detoxification and elimination of environmental toxins. Lower levels of GSH and decreased ratio of GSH/ oxidized glutathione (GSSG) in the blood of individuals with autism have been reported in many clinical studies (Adams et al. 2011; Al-Gadani et al. 2009; Bertoglio et al. 2010; James et al. 2004; Pastural et al. 2009), which could be raised by methyl B12 treatment (Bertoglio et al. 2010) or vitamin/mineral supplementation (Adams et al. 2011). We reported reduced levels of GSH, increased levels of GSSG, and a decrease in the ratio of GSH/GSSG in the cerebellum and temporal cortex of autistic subjects (Chauhan et al. 2012a). James et al. (2009) also reported a decreased ratio of GSH/GSSG in both cytosol and mitochondria in the lymphoblastoid cells from autistic subjects. Glutathione peroxidase (GPx) is an enzyme involved in the direct elimination of ROS. It catalyzes H_2O_2 reduction to H_2O and also reduces lipid hydroperoxides to their corresponding alcohols. Decreased GPx activity of plasma was reported in a group of 44 Egyptian children with autism compared to 44 normal children (Mostafa et al. 2010) and in another group of 20 Egyptian autistic children compared to 25 age-matched control subjects (Meguid et al. 2011). We also reported increased oxidative damage and free radical generation, coupled with reduced activities of antioxidant enzymes in the lymphoblastoid cells from autistic subjects compared to age-matched control subjects (Essa et al. 2009).

3.6 Calcium-Signaling Abnormalities in Autism

Not only do mitochondria play a central role of maintaining Ca^{2+} homeostasis, but intracellular Ca^{2+} levels also modulate mitochondrial activity. Many cellular functions are regulated by intracellular free Ca^{2+} concentration. Calcium is essential for neurotransmitter release, and Ca^{2+} influx is essential for neuronal excitability. Mitochondrial activity and Ca^{2+} signaling have an intense cross talk. Therefore, abnormal calcium signaling can affect normal mitochondrial function and is considered a mitochondrial dysfunction.

Mitochondrial aspartate/glutamate carrier (AGC), which is physiologically activated by calcium, plays an important role in energy metabolism and neuron development by transporting glutamate into mitochondria (Napolioni et al. 2011). AGC transport rates and expression levels of AGC1 protein were significantly higher in the brain of autistic subjects compared with the control subjects (Palmieri et al. 2010). Neocortical calcium levels were also increased in these autistic subjects. Excessive calcium levels were responsible for high AGC1 activity and the activation of mitochondrial metabolism in autism (Napolioni et al. 2011; Palmieri et al. 2010). Furthermore, AGC1-encoding SLC25A12 gene is also reported to be associated with autism (Ramoz et al. 2004; Segurado et al. 2005).

After a stimulus, calcium flows rapidly into neurons through various types of membrane channels, including voltage-dependent and receptor-coupled channels. Intracellular Ca^{2+} concentrations are quickly restored to resting levels, primarily through Ca^{2+}/Mg^{2+} ATPase and Na^{+}/Ca^{2+} exchange. Several studies have reported calcium-signaling abnormalities in autism (Chauhan and Chauhan 2009; Gargus 2009). The L-type voltage-gated Ca^{2+} channel is important for excitation of neurons and activation of various Ca^{2+}-regulated signaling cascades (Catterall et al. 2005). Gain-of-function mutations in the L-type voltage-gated Ca^{2+} channel CaV1.2 (CACNA1C) was revealed in Timothy syndrome, a multisystem disorder including mental retardation and autism (Splawski et al. 2004, 2005). This mutation causes these channels to remain open longer and allow the influx of more Ca^{2+} than wild-type channels, resulting in increased intracellular Ca^{2+}. These studies supported the importance of the L-type voltage-gated Ca^{2+} channel in autism. The mutation in the CACNA1F gene, which encodes the L-type voltage-gated Ca^{2+} channel, CaV1.4, was reported to cause autistic symptoms in a New Zealand family with an X-linked retinal disorder (Hemara-Wahanui et al. 2005; Hope et al. 2005). In addition, the mutation of T-type voltage-gated Ca^{2+} channel was also found in six of 461 individuals with autism (Splawski et al. 2006). The function of T-type channels in the brain is related to the regulation of the oscillatory behavior of neurons in the cortex and thalamus (Perez-Reyes 2003). ASD-associated mutations have also been identified in some genes, which encode ion channels whose activity is directly regulated by Ca^{2+}, such as Ca^{2+}-dependent Na^{+} or K^{+} channel. Several point mutations in SCN1A and SCN2A genes, which encode the voltage-activated K^{+} channels NaV1.1 and NaV1.2, respectively, have been reported in autism (Kamiya et al. 2004; Weiss et al. 2003). Laumonnier et al. (2006) reported functional deficit of Ca^{2+}-activated K^{+} channel (BKCa), a synaptic regulator of neuronal excitability in autism. Disruption of the BKCa gene (KCNMA1) led to the haploinsufficiency and reduced BKCa activity in autism.

Several neurological diseases are caused primarily by malfunctioning of Ca^{2+} channels or Ca^{2+}/Mg^{2+} ATPase (Cooper and Jan 1999; Jacobsen et al. 1999). Ca^{2+}/Mg^{2+} ATPase is a membrane-bound enzyme involved in maintaining the electrochemical gradient of the cells in an energy-dependent manner and the concentration of intracellular calcium by extrusion of calcium from cytosol. We reported increased activity of Ca^{2+}/Mg^{2+} ATPase in the cerebellum of autistic subjects compared with age-matched control subjects (Ji et al. 2009). Increased Ca^{2+}/Mg^{2+} ATPase activity may be a compensatory mechanism in response to increased intracellular calcium levels in autism. Taken together, the studies above suggest that calcium-signaling abnormalities may also contribute to mitochondrial dysfunction and pathophysiology of ASDs.

4 Conclusion

Mitochondria play a vital role in many pathways such as energy generation, developmental metabolism, calcium homeostasis, free radical generation, and cell survival. Mitochondrial dysfunction can be caused by alterations in activities or

expression levels of ETC complexes, genetic abnormality, oxidative stress, or calcium-signaling abnormalities. Collectively, several independent studies have provided evidence of impaired mitochondrial function and, as a result, impaired cellular energy state as one of the mechanisms involved in the pathophysiology of autism. Any abnormal change in the mitochondrial activities may affect the cellular energy production and neurotransmission system and destroy the balance between ROS generation and antioxidant capacity of the cell, thus leading to abnormal neurodevelopment in children with autism.

Acknowledgments This work was supported in part by funds from the New York State Office for People With Developmental Disabilities, the Department of Defense Autism Spectrum Disorders Research Program AS073224P2, the Autism Research Institute, and the Autism Collaboration.

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