FINAL

Send Orders for Reprints to reprints@benthamscience.ae

Mini-Reviews in Medicinal Chemistry, 2015, *15*, 000-000

Mitochondrial Dysfunction and Its Relationship with mTOR Signaling and Oxidative Damage in Autism Spectrum Disorders

Kunio Yui[1,*], Atsushi Sato[2,3] and George Imataka[4]

[1]*Research Institute of Pervasive Developmental Disorders, Ashiya University Graduate School of Education, Ashiya 659-8511, Japan;* [2]*Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan;* [3]*Tokyo Metropolitan Neurological Hospital, Tokyo, Japan;* [4]*Department of Pediatrics, Dokkyo Medical University School of Medicine, 880, Kitakobayashi, Mibu, 321-0293, Tochigi, Japan*

Abstract: Mitochondria are organelles that play a central role in processes related to cellular viability, such as energy production, cell growth, cell death via apoptosis, and metabolism of reactive oxygen species (ROS). We can observe behavioral abnormalities relevant to autism spectrum disorders (ASDs) and their recovery mediated by the mTOR inhibitor rapamycin in mouse models. In *Tsc2*$^{+/-}$ mice, the transcription of multiple genes involved in mTOR signaling is enhanced, suggesting a crucial role of dysregulated mTOR signaling in the ASD model. This review proposes that the mTOR inhibitor may be useful for the pharmacological treatment of ASD. This review offers novel insights into mitochondrial dysfunction and the related impaired glutathione synthesis and lower detoxification capacity. Firstly, children with ASD and concomitant mitochondrial dysfunction have been reported to manifest clinical symptoms similar to those of mitochondrial disorders, and it therefore shows that the clinical manifestations of ASD with a concomitant diagnosis of mitochondrial dysfunction are likely due to these mitochondrial disorders. Secondly, the adenosine triphosphate (ATP) production/oxygen consumption pathway may be a potential candidate for preventing mitochondrial dysfunction due to oxidative stress, and disruption of ATP synthesis alone may be related to impaired glutathione synthesis. Finally, a decrease in total antioxidant capacity may account for ASD children who show core social and behavioral impairments without neurological and somatic symptoms.

Keywords: Adenosine triphosphate, autism spectrum disorders, impaired glutathione synthesis, mTOR signaling, oxidative stress, rapamycin, tuberous sclerosis complex, mitochondrial dysfunction.

1. INTRODUCTION

Mitochondria are organelles that play a central role in processes related to cellular viability, such as energy production, cell growth, cell death via apoptosis, and metabolism of reactive oxygen species (ROS). A diverse number of human diseases, ranging from diabetes mellitus to neurodegenerative disorders, stem from and commonly coexist with mitochondrial dysfunction (MD) [1, 2]. Mutations in genes related to mitochondrial biogenesis affect multiple organs, particularly the brain and muscles, due to their high energy demands. These conditions are therefore known as “mitochondrial disorders” or “mitochondrial encephalomyopathies”, including MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) [3] and MERRF (myoclonic epilepsy associated with ragged-red fibers [4]. Neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease [5], and autism spectrum disorders (ASD) [6] are also closely related to MD with respect to disease occurrence and progression. In the Diagnostic Manual of Mental Disorders, Fifth Edition (DSM-5), ASD is defined as a set of heterogeneous neurodevelopmental conditions, characterized by early-onset difficulties in social communication and unusually restricted and repetitive behaviors and interests [7]. MD has been observed among a subgroup of ASD patients exhibiting elevated levels of lactate in their plasma and brain [6, 8, 9], as well as vulnerability to pro-oxidant micro environmental toxicants [9].

Some mitochondrial functions, such as oxygen consumption, adenosine triphosphate (ATP) production, fatty acid oxidation and gene transcription, are also regulated by mTOR [10, 11]. Impaired mitochondrial function is observed in association with ASD-related behavioral aberrations in mice that show mTOR activation [12]. In addition to excessive protein synthesis [14] and altered autophagy [15] related to neuronal alteration, MD may link dysregulated mTOR activity to ASD.

Converging lines of research have implicated the involvement of intracellular oxidative damage in mitochondrial diseases, a group of disorders caused by MD. Glutathione (L-γ-glutamyl-L-cysteinylglycine, GSH) plays a

*Address correspondence to this author at the Research Institute of Pervasive Developmental Disorders, Ashiya University Graduate School of Education, Ashiya 659-8511, Japan; Tel: +81 797-23-061; Fax: +81 797-23-1901; E-mail: yui16@bell.ocn.ne.jp

1389-5575/15 $58.00+.00

key role in the cellular defense against oxidant damage [16]. Glutathione deficiency has also been reported in MD [16]. The oxidative stress-related pathophysiology of ASD includes MD and impairment of the GSH synthesis pathway. In view of the fact that synthesized ATP inhibits MD [17], ATP may play a crucial role in the generation of ROS to impair GSH synthesis, resulting in a low detoxifying capacity and MD. A low detoxifying capacity, as indicated by a low urinary total antioxidant capacity, has been reported in ASD children without any of the neurological and somatic symptoms observed in children with MD or impaired glutathione synthesis.

The relationship among MD, impaired GSH synthesis and a low detoxifying capacity has yet to be elucidated. This review addresses recent advances in mitochondrial function in relation to mTOR. Moreover, this review attempts to untangle three aspects of the relationship of MD, impaired GSH biosynthesis and a low detoxifying capacity with oxidative stress. Firstly, we discuss whether children with ASD might have a certain subset of MD or a new type of MD. Secondly, we attempt to shed light on potential processes that may result from the production of ROS in two metabolic pathways, including MD and impaired GSH biosynthesis (Fig. **1**). Thirdly, there is growing evidence that intracellular oxidative damage associated with the pathophysiology of ASD appears to include MD, impaired glutathione synthesis, or a low detoxifying capacity, but the relationship among these processes in ASD has never been elucidated. This review also summarizes the clinical manifestations of ASD with MD as well as recent data on the regulation and role of biomarkers in MD, impaired glutathione synthesis, or a low detoxifying capacity.

2. MITOCHONDRIAL FUNCTION-RELATED MTOR SIGNALING IN AUTISM SPECTRUM DISORDERS

MD may be involved in ASD. As a neurodevelopmental disorder, ASD primarily affects reciprocal social interactions and is accompanied by restrictive, repetitive behaviors and interests according to the DSM-5 [7]. Like most psychiatric disorders, both genetic and environmental factors can influence the occurrence of ASD. Genetic research has provided an extensive list of genes, loci and copy number variations causing ASD, which is still growing [18-21]. Environmental causes, such as prenatal exposure to valproate [22-24] and inflammatory insults [25-27], act during the prenatal period in most cases and are thought to affect brain

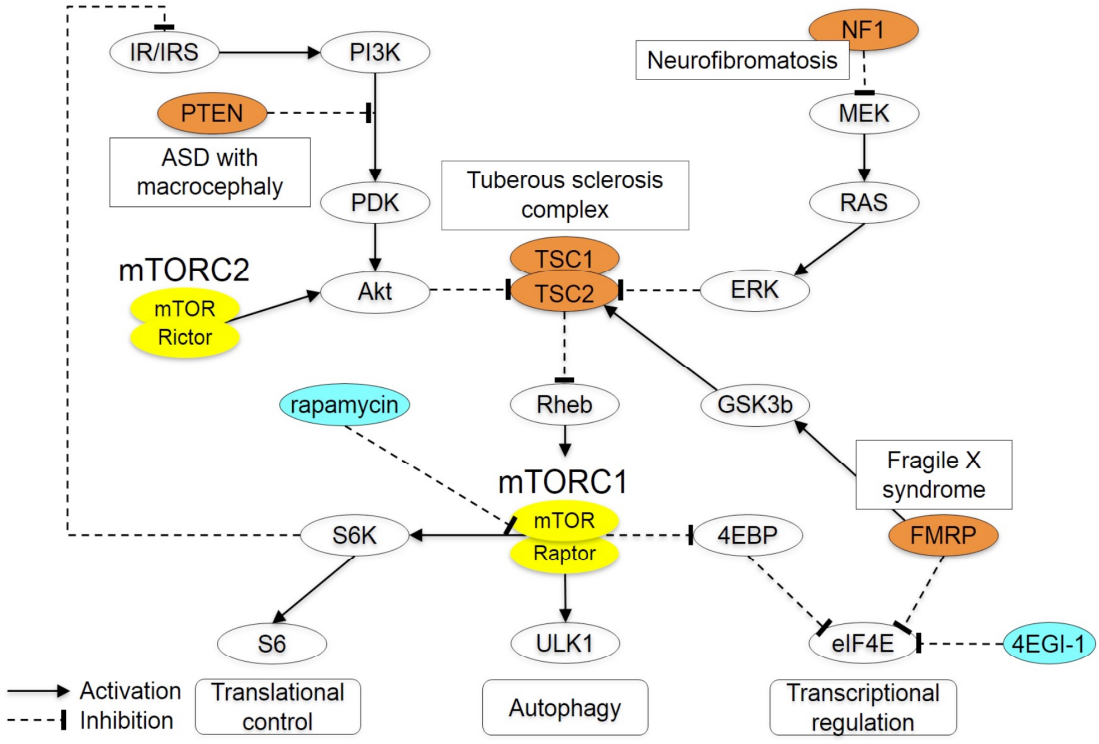

Fig. (1) Association of mTORC1 and mTORC2 in autism spectrum disorders. The genes colored in orange are discussed in the text for the causes of syndromic ASD. Hyperactivated mTORC1, caused by mutations in the highlighted genes, phosphorylates and alters the function of the three molecules. First, S6K is activated and global protein synthesis is elevated. Second, 4EBP is inactivated, resulting in release inhibition of eIF4E-mediated cap-dependent gene transcription. ULK1 is also activated and autophagy is stimulated though it is unclear whether this has influence on the genesis of ASD. Akt, protein kinase B; eIF4E, eukaryotic initiation factor 4E; 4EBP, 4E-binding protein; ERK, extracellular signal-regulated kinase; FMRP, fragile X mental retardation protein; GSK3b, glycogen synthase kinase 3b; IR, insulin receptor; IRS, insulin receptor substrate; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; NF1, neurofibromin; PDK, phosphoinositide dependent kinase; PI3K, phosphatidylinositol 3-kinase; phosphatase and tensin homolog deleted on chromosome 10; RAS, rat sarcoma; Rheb, Ras homolog enriched in brain; S6 ribosomal protein S6; S6K, S6 kinase; TSC, tuberous sclerosis complex; ULK1, unc51-like kinase 1.

development of brains. Interestingly, the genetic defects found in individuals with ASD converge on several common molecular alterations.

One representative alteration is the constitutive activation of mTOR-mediated signaling [14, 28, 29] (Fig. **1**). mTOR plays a central role in cell growth and differentiation. In neurons, mTOR critically controls synaptic function, and its alteration is therefore thought to disturb synaptic signal transmission and result in ASD.

2.1. mTOR Activation and ASD in Monogenic Disorders

2.1.1. mTOR Complexes

Mammalian target of rapamycin (mTOR) interacts with several proteins to form two distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The most distinctive difference between these complexes is that mTORC1 contains raptor, whereas mTORC2 contains rictor instead (Fig. **1**). mTOR inhibitors such as rapamycin block mTOR-raptor coupling and inhibit mTORC1 [30, 31] but not mTORC2, which does not contain raptor. Monogenic disorders associated with deregulated mTOR-mediated signaling have been investigated to reveal the pathogenic role of mTORC1 in ASD. In this context, tuberous sclerosis complex (TSC) and PTEN hamartoma tumor syndrome have been particularly intensively studied. Activated mTORC1 may participate in ASD in fragile X syndrome (FXS) and neurofibromatosis type 1 (NF1) [14, 28, 29].

2.1.2. The Role of TSC

Tuberous sclerosis complex (TSC) is caused by heterozygous mutations in the *TSC1* [32] and *TSC2* [33] genes. A hallmark of TSC is the presence of dysplastic and hamartomatous lesions in multiple organs, such as the brain, skin and kidneys [34]. Less specific symptoms frequently found among TSC individuals include neuropsychiatric problems such as agitation, hyperactivity and anxiety, which are collectively known as TSC-associated neuropsychiatric disorders (TAND) [35]. ASD is one of the most prominent conditions in TAND and makes TSC patients' daily life more challenging compared with patients without ASD, both in school and at home. Approximately half of TSC individuals have accompanying ASD [14, 36], whereas TSC-related ASD is found in up to 4% of general ASD cases [20]. TSC is therefore one of the most prevalent genetic causes of ASD along with FXS (see below). Risk factors for ASD include low intelligent quality scores, *TSC2* mutations, earlier age at seizure onset and infantile spasms (an epileptic encephalopathy characterized by spasms, hypsarrhythmia in EEG and developmental arrest or deterioration), whereas the influence of the number and location of cortical tubers remains controversial [14, 36, 38].

Analysis of a rodent model of TSC has contributed to our understanding of how hyperactivated mTOR signaling and the molecular pathomechanism of TSC participate in the genesis of ASD. Homozygous mutations in *Tsc1* and *Tsc2* are embryonically lethal [39, 40]. Haploinsufficient animals are viable and exhibit no signs of epilepsy, and obvious lesions are rarely observed in the brains of these mice [39, 40] and rats [41, 42]. These rodents show a reduced interest in social novelty when direct interaction is allowed [43-45], and an autism-like deficit in social learning is less prominent [21, 46]. They also exhibit signs of cognitive dysfunction during different behavioral tasks [43, 46, 47]. Treatment with rapamycin for just a few days completely reverses these autistic and cognitive deficits in adult mice, demonstrating the critical role of overactivated mTORC1 [45, 46]. The selective deletion of TSC genes results in different phenotypes depending on the targeted cells. Deletion of *Tsc1* in Purkinje cells aggravates the approach to a social stimulus [48]. Mice show far more severer phenotypes, such as epilepsy, brain enlargement and early mortality, when *Tsc1* or *Tsc2* is eliminated in astrocytes [49] and in neurons of the forebrain [46]. These features are also alleviated by the early implementation of life-long rapamycin treatment. In a recent clinical trial of the mTOR inhibitor everolimus, TSC patients with refractory epilepsy showed a reduction of seizure frequency as well as improvement of behavioral problems, including those related to ASD [50]. The above bench and bedside findings suggest that similar to the results in mice, ASD may be reversible with medication in humans.

2.1.3. Molecular Mechanisms of mTOR

The *PTEN* (phosphatase and tensin homolog) gene negatively regulates the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling pathway. Deletion of *PTEN* releases its inhibition of mTORC1 by activating Akt and inactivating TSC2. Heterozygous mutations in the *PTEN* gene can cause several syndromes, whose nomenclature depends on the phenotype. Collectively known as PTEN hamartoma tumor syndrome (PHTS), this group of syndromes includes Bannayan- Riley-Ruvalcaba syndrome, Lhermitte-Duclos syndrome and Cowden syndrome [51, 52].

Notably, a subset of ASD individuals with marked macrocephaly carry Phosphatase and tension homolog deleted on chromosome ten *(PTEN)* mutations, irrespective of the presence of hamartomas and tumors [53-55], implicating a direct link between *PTEN* mutations and macrocephaly-associated ASD. Similar to TSC, homozygous deletion of *Pten* results in embryonic lethality [56]. *Pten* heterozygosity in mice reproduces brain enlargement and ASD in certain settings [13, 57], though these conditions are subtler than expected compared with the human phenotype. Deletion of *Pten* in differentiated neurons of the cerebral cortex and hippocampus results in overt ASD-like behavioral deficits as well as epilepsy and early mortality, which are abrogated by chronic treatment with rapamycin beginning in the early postnatal period [58, 59] Considering the above evidence in relation to TSC and PTEN, mTOR activation is likely to be a common molecular event leading to ASD and represents a promising therapeutic target.

Several other monogenic disorders, such as FXS and NF1, are also often associated with ASD. The molecular basis of neurocognitive deficits in these diseases was initially attributed to synaptic transmission via glutamate for FXS and to the RAS signaling pathway for NF1. Activated mTORC1 was subsequently observed in these two disorders, and research is currently under way to examine its influence.

FXS is an X-linked disorder caused by the unstable expansion of a trinucleotide repea in the fragile X mental retardation 1 (CGG repeat in the *FMR1*) gene, resulting in reduced production of the FMRP protein [60]. ASD is found in approximately half of all male FXS patients and up to 5% of ASDs derive from FXS; thus, FXS is considered to be the most common monogenic cause of ASD [20, 61]. Investigations on the molecular pathophysiology of FXS have mainly focused on metabotropic glutamate receptors, particularly concerning the activation of mGluR5 [62, 63], and altered mTORC1-mediated signaling has been proposed [14] (Fig. **1**). In fragile X mental retardation 1 (*Fmr1*) knockout mice, which is a mouse model of FXS, mTORC1 is hyperactivated in the hippocampus [64]. Inhibition of increased mTORC1 activity through the genetic removal of S6K1, a major effector of mTORC1, improves autistic behavior in these mice [65] (Fig. **1**), suggesting a causal role of the FMRP-mTORC1 interaction in FXS-related ASD. NF1, an autosomal dominant disorder caused by mutations in the *NF1* gene [66-68], is the most common neurocutaneous disorder worldwide. Individuals with NF1 often show signs of cognitive deficits in perceptual skills, executive functions, and attention [69]. Behavioral alterations relevant to learning deficits in $Nf1^{+/-}$ mice are associated with increased gamma-amino butyric acid (GABA)-mediated inhibition and specific deficits in long-term potentiation and can be reversed by decreasing Ras function [70]. NF1 constitutively activates Extracellular Signal-regulated Kinase (ERK) [71], and the downstream signaling pathway in turn suppresses the function of TSC1/TSC2, leading to mTORC1 activation [72]. Pharmacological inhibition of ERK using statins is effective in improving cognitive function in mice [73], but its efficacy is limited in humans [74]. Unlike other disorders mentioned above, NF1 in relation to ASD has rarely been investigated, partly because the relationship between NF1 and ASD is still insufficiently understood. NF1 has long been considered a risk factor for ASD [75]. It was recently revealed that 15-30% of NF1 patients meet the criteria for ASD [76, 77]. Very recently, it was demonstrated that *Nf1* haploinsufficiency causes ASD-like deficits in social learning and aberrant glutamate and GABA neurotransmission in the amygdala, which can be reversed by pharmacological inhibition as well as the additional genetic deletion of p21-activated kinase 1 (*Pak1*) [78]. Further research on whether mTORC1 activation is involved in the social deficits observed in NF1 is necessary.

2.2. How does mTORC1 Activation Result in ASD?

2.2.1. mTORC1 Overactivation

Despite the consensus opinion that mTORC1 activation results in ASD, the immediate molecular events causing ASD when mTORC1 is constitutively activated in the brain remain to be elucidated. Analysis of conditional knockout mouse models of TSC and PHTS revealed that significant morphological changes are followed by mTORC1 overactivation. *Pten* deletion in differentiated neurons results in neuronal and dendritic hypertrophy, a thickened cerebral cortex and brain enlargement, followed by profound phenotypic changes including a reduced survival rate [58, 59]. Inactivation of TSC genes in astrocytes increases astrocyte proliferation, neuronal disorganization and progressive macrocephaly [49]. Purkinje cell-specific *Tsc1* deletion causes earlier loss of Purkinje cells, an increased spine density and reduced excitability in Purkinje cells [48]. These genetic manipulations lead to autistic behavior that can be rescued by the continuous inhibition of mTORC1 using rapamycin [48, 52]. However, even mice without overt brain pathologies express social deficits that respond to rapamycin [39, 40, 45], demonstrating that morphological changes may not be essential for disrupting social behavior. Another question in this context is what follows mTORC1 activation? mTORC1 regulates many downstream proteins, including ribosomal p70S6 kinase (S6K1), and eukaryotic translation initiation factor-4E (4EBP), wchich binds the 5′ cap as a subunit of the eIF4E (Fig. **1**). Ribosomal p6 kinase 1 (S6K1), when activated by mTORC1, increases protein synthesis by phosphorylating ribosomal protein S6 [14]. Abnormally enhanced phosphorylation of S6K1 and Ribosomal protein S6 (rpS6) may disturb neuronal functions relevant to ASD via excessive protein production. Another possibility is altered long-term potentiation (LTP). Hippocampal slices obtained from $Tsc2^{+/-}$ mice show a reduced threshold of late-phase LTP [28]. Unexpectedly, S6K1 knockout mice, in which the mTORC1-S6K pathway is constitutively suppressed, also exhibit deficient social learning [65] as well as impaired early-phase LTP, without protein synthesis-dependent late-phase LTP [79]. It has been suggested that mTORC1-regulated LTP must be carefully balanced to avoid extremes to support normal social behavior.

2.2.2. Roles of 4EBPs and eIF4E

Cap-dependent mRNA translation is controlled by eukaryotic translation initiation factor-4E-binding protein (4EBPs) and their target eIF4E. eIF4E interacts with eIF4G and promotes cap-dependent translation. The 4EBPs bind to eukaryotic translation initiation factor 4G (eIF4E) and interrupt the eIF4E-eIF4G interaction, thus resulting in decreased translation. Here, mTORC1 phosphorylates and inhibits the binding of 4EBPs to eIF4E, releases the inhibition of eIF4E, and stimulates cap-dependent mRNA translation and protein synthesis [80-82]. An autism-like behavioral deficit is observed in mice with both 4EBP2 deletion [83] and eIF4E overexpression [84]. This genetic manipulation enhances the eIF4E-dependent translation of a subset of genes, including neuroligins, and increases spine density and synaptic activity, resulting in an altered excitatory/inhibitory (E/I) ratio. Pharmacological inhibition of eIF4E normalizes behavioral and neuronal abnormalities [83, 84]. Interestingly, knockdown of neuroligin 1 reverses neuronal changes and partially rescues social behavior [83]. Upon mTORC1 dysregulation, it has been suggested that increased synthesis of synaptic proteins, including neuroligins, disrupts the E/I balance and leads to the development of an ASD-like phenotype

2.2.3. The Relationship between mTORC1 and mGluR5

Understanding the molecular pathophysiology of FXS and TSC provides evidence of crosstalk between mTORC1

and metabotropic glutamate receptor 5 (mGluR5) in the brain. Deletion of the glutamine receptor metabotropic 5(*Grm5*) gene, encoding mGluR5, rescues abnormalities in synaptic density, hippocampal protein synthesis and learning in *Fmr1* knockout mice [63]. FXS brains show increased mTORC1 activity that may be region and age specific [64]. Genetic removal of S6K1 – to cancel out the effect of mTORC1 activation – reverses the ASD-like phenotype and enhances mGluR5-mediated long-term depression (LTD) [65]. In contrast, mGluR5-mediated LTD is rather attenuated in *Tsc2*$^{+/-}$ mice [47, 85]. However, the results have been inconsistent regarding whether mGluR5 activity is decreased [85] or increased [47] when mTORC1 is activated. The importance of the mTORC1 – mGluR5 relationship in ASD may hold true for general ASD. The BTBR mouse strain shows all of the symptoms of ASD [86], which are rescued by mGluR5 negative allosteric modulation [87]. Furthermore, rapamycin normalizes the associated social deficit [88]. It should be clarified in more detail how the ASD-like phenotype can be rescued by mGluR5 modulation (i.e., activated or inactivated, or kept within a certain range).

2.3. Mitochondria, Oxidative Stress and mTOR

2.3.1. mTOR in ASD

An accumulating body of evidence suggests an association between ASD and MD. ASD associated with mitochondrial disease is characterized by developmental regression, elevated plasma lactate and a lack of association with genetic abnormalities [8]. Elevated plasma lactate is also observed in general ASD [89, 90]. Analysis of brain tissue samples revealed that mitochondrial electron transport chain complexes are reduced in the frontal and temporal cortices of children with ASD [91]. Impairment of mitochondrial energy metabolism may underlie general ASD.

Recently, aberrant macroautophagy was reported in mTOR-mediated ASD [92]. The authors observed deficient dendritic pruning and mTOR-dependent macroautophagy in the post-mortem brains of ASD individuals. Analysis of mouse models of TSC revealed that mTOR-mediated macroautophagy regulates dendritic pruning. Moreover, deletion of *Atg7*, the gene required for autophagosome formation, results in deficient social learning and recapitulates the morphological changes observed in TSC. However, rapamycin fails to reverse these abnormalities in *Atg7*-deleted mice. The role of mTORC1-mediated macroautophagy in the development of ASD should be further investigated.

mTORC1 controls mitochondrial functions such as oxygen consumption, ATP production, fatty acid oxidation and gene transcription [11, 93]. Oxygen consumption and the oxygenation capacity are correlated with mTORC1 activity and are stimulated by knockdown of *Tsc2*, but attenuated by knockdown of raptor and S6K1. This is associated with reduced phosphorylation of enzymes involved in energy metabolism [93]. *Pten* deficiency exaggerates mTORC1-mediated cap-dependent gene transcription and protein synthesis, resulting in higher levels of ATP synthesis, coupled with elevated oxygen consumption [94]. In mice lacking *Pten* in the cerebellum and hippocampus, elevated respiratory complex activity is noted from early postnatal periods. These mice also exhibit autistic behavior at an older age. This is accompanied by a significant reduction of mitochondrial DNA levels in the cerebellum and hippocampus, but not in the cortex, where *Pten* is expressed normally [13]. It has been suggested that mitochondrial over activity induced by activated mTORC1 leads to mitochondrial loss and ASD.

2.3.2. mTORC1 Activation

The relationship is also implicated in oxidative stress, mTORC1 activation and ASD. Neurons lacking the *TSC1* and *TSC2* genes exhibit higher levels of ROS and expression of the antioxidant enzyme hemeoxygenase I (HO-1), indicating increased oxidative stress in the setting of TSC deficiency [95]. Analysis of lymphoblastoid cell lines (LCLs) revealed an association between an altered redox status and ASD. LCLs derived from children with ASD show decreased glutathione concentrations relative to oxidized disulfide glutathione as well as a decreased ratio of reduced nicotinamide adenine dinucleotide (NADH) to oxidized NAD^+ and higher levels of 3-nitrotyrosine, a marker of protein oxidation that is indicative of chronic oxidative stress [6, 9, 96]. Compared with control LCLs, exposure of ASD-derived LCLs to ROS markedly increases proton leak respiration and markedly depletes the reserve capacity of mitochondrial respiration. Furthermore, an increase in baseline ROS production is observed in a subgroup of ASD-derived LCLs [6]. Therefore, vulnerability to intrinsic and extrinsic ROS may play a role in the occurrence of ASD. mTORC1 activation can cause endoplasmic reticulum (ER) stress. Briefly, excessive protein synthesis, perturbations in calcium homeostasis, and nutrient deprivation are some causes of ER stress. Excessive ER stress activates the unfolded protein response (URP), which then stimulates signaling cascades to remove the ER overload. A failure to eliminate the ER overload results in ER stress-induced cell death via caspases and the proapoptotic transcription factor CHOP (C/EBP homologous protein) [95, 97]. Loss of the *TSC1* and *TSC2* genes coupled with mTORC1 activation causes ER stress and activates the unfolded protein response (URP) in fibroblasts [97] and in neurons [95]. Oxidative and ER stress renders neurons vulnerable to cell death in a CHOP-dependent manner. Cell death can be rescued by a CHOP inhibitor, but not by rapamycin [95]. Excessive exposure to oxidative and ER stress may underlie the genesis of ASD associated with mTORC1 activation, such as TSC. In these ASD patients, therapeutic approaches aimed at reducing cellular stress using antioxidant agents may be beneficial.

In the above sections, we reviewed the complex associations among ASD, mTORC1 hyperactivation and MD, particularly with regard to the link between ASD and mTORC1. Constitutive activation of mTORC1 may result in oxidative and ER stress as well as ASD associated with monogenic disorders such as TSC and FXS. In the following sections, the relationship between ASD and MD is discussed in more detail.

3. THE RELATIONSHIP BETWEEN MITOCHONDRIAL DYSFUNCTION AND ANTIOXIDANT ACTIVITY IN AUTISM SPECTRUM DISORDERS

MD includes various pathological features such as mitochondrial DNA mutations and mitochondrial-respiratory-chain disease. Clinical manifestations related to the nervous system in primary MD include developmental regression or delay, seizures, ataxia, movement disorders, myoclonus and basal ganglia diseases [98]. The prevalence of MD in the general ASD population has been reported as 5.0% [8], 13.0% [99] or 44% [100]. This indicates the possibility that a subgroup of children with ASD may have a new type of MD that is more prevalent and is distinct from classic MD [6, 101]. Another study proposed that a neurobiological subtype of ASD should undergo evaluation for MD [99].

Reports on ASD have generally indicated that oxidative stress induces ASD due to changes in the antioxidant status [102-104] and heightened vulnerability to oxidative stress [105, 106]. To prevent or reduce reactive oxygen species (ROS) - or MD-induced oxidative stress damage, the human body has developed an antioxidant defense system [107]. GSH plays a key role in the cellular defense against oxidant damage [16]. The oxidative stress-related pathophysiology of ASD includes MD and impairment of the GSH synthesis pathway. As mitochondrial oxygen consumption is coupled with ATP production [108], this review first suggests that the ratio of ATP production/oxygen consumption may play a crucial role in MD.

This chapter discusses four aspects of the relationship among MD, impaired GSH biosynthesis and a low detoxifying capacity against oxidative stress: (1) whether children with ASD and a concomitant MD might have a subset type of ASD and whether they have a new type of MD; (2) potential processes that may diverge from the production of ROS in the two metabolic pathways, such as MD and impaired GSH biosynthesis; (3) the relationship among the three types of oxidative stress-related pathophysiologies (MD, impaired GSH synthesis and a low detoxifying capacity) in ASD; and (4) a summary of the clinical manifestations of ASD with MD and recent data on the regulation and role and biomarkers of a low detoxifying capacity, glutathione deletion and MD?.

3.1. Relationship Among Mitochondrial Dysfunction, Impaired Glutathione Synthesis and A low Detoxifying Capacity

When antioxidant defenses are weakened, cells and tissues in the body become more prone to dysfunction and/or disease development [109]. Oxidative modification of biologically essential molecules by reactive oxygen species (ROS) has been implicated in the pathogenesis of various diseases [110]. ROS can be neutralized by antioxidant systems, resulting in a delicate balance that determines the fate and impact of ROS [111]. Oxidative stress-induced cell death or apoptosis may depend on the cell's ability to cope with oxidative stress, including its ROS-detoxifying capacity [112], GSH synthesis [113] and mitochondrial function [114].

3.2. Mitochondrial Dysfunction

Mitochondria are extraordinary organelles that play key roles in a number of fundamental cellular processes, including ATP synthesis, ion homeostasis, oxygen sensing [115], and redox homeostasis [116]. Mitochondria are important in apoptosis [115, 117], thus participating in neurodegenerative processes [117]. All of these pathways include redox-reactions as central elements [115]. Mitochondria play an important role in cellular energy by means of oxidative phosphorylation, which provides an efficient mechanism coupling electron transport to the synthesis of ATP [111]. Furthermore, the mitochondrial electron transport chain plays an important role in the generation and management of ROS and the production of ATP [118]. Thus, synthesis of ATP may be critical in mitochondrial function [119].

MD occurs as a result of the following changes: (1) loss of maintenance of the electrical and chemical transmembrane potential of the inner mitochondrial membrane, (2) alterations in the function of the electron transport chain, or (3) a reduction of the transport of critical metabolites into mitochondria [120].

3.2.1. Clinical Manifestations of MD in Individuals with ASD

Table **1** showed main clinical manifestations of MD. In Table **2** non-specific clinical signs of MD were presented. Table **3** showed clinical signs of ASD with a concomitant diagnosis of MD. MD may present with any symptoms in any organ at any age, but some symptoms and signs are more suggestive of MD than others. These core features warrant the initiation of a baseline diagnostic evaluation for mitochondrial disease (Table **1**). In contrast, there are a multitude of nonspecific symptoms that frequently occur in infants and children with mitochondrial disease but are associated with a broad differential diagnoses [98]. Nonspecific findings in MD include neurodegeneration [98], encephalopathy [98], epilepsy [98], ataxia [98], movement disorders [98], myoclonus, basal ganglia diseases [98], hypotrophic cardiomyopathy [98], metabolic cardiomyopathy [121], chronic or cyclic diarrhea and vomiting [121], pseudo-obstruction [121] and diabetes mellitus [121].

There have been a few prior cohort analyses of MD in ASD. One previous cohort study involving a review of the medical records of twenty-five ASD children according to the DSM-IV-TR identified core clinical manifestations of MD in individuals with ASD to provide information on the clinical spectrum of individuals with ASD and a concomitant diagnosis of MD (Table **3**) [100]. This cohort study indicated that among the twenty-five ASD children aged 2-20 years, twenty-one had histories of major non-neurological medical problems. Additionally, twenty-one (84%) exhibited the most common gastrointestinal symptom of constipation; seventeen (68%) presented excessive fatigability and exercise intolerance; seven (28%) displayed functional cardiovascular abnormalities (28%); and several exhibited abnormal findings in physical examinations, including microcephaly (n=4, 16%), macrocephaly (n=4, 16%) and

Table 1. Main clinical characteristics of mitochondrial dysfunction.

Organs	Manifestations	References
Neuromuscular symptoms	Encephaloathy Basal ganglia disease Epilepsia partialis continua Ataxia Neurodegeneration Myoclonus Ptosis Ophthalmologic abnormalities Retinal degeneration Ophthalmoplegia/paresis	Hass *et al.*, 2007
	Sensorineural deafness Exercise intolerance or myalgia	Mattman *et al.*, 2011
	Developmental delay Loss of skill Seizure Recurrent or familial neuropathy Muscle weakness	Fry and Rossignol, 2011
Gastrointestinal symptoms	Severe dysmotility Pseudo-obstructive episode	Hass *et al.*, 2007
	Constipation Diarrhea Delayed gastric emptying with nausea and vomiting	Matman *et al.*, 2012
Cardiovascular	Hypertrophic cardiomyopathy	Hass *et al.*, 2007
	Unexplained heart block Metabolic cardiomyopathy	Mattman *et al.*, 2011
Endocrine	Diabetes mellitus	Mattman *et al.*, 2011

Table 2. Non-specific clinical signs of mitochondrial dysfunction[a].

Organs	Manifestations	References
Neurologic symptoms	Axonal neuropathyIntractable epilepsy Hypotonia Infantile spasms Coma Hearing loss	Hass *et al.*, 2007
Gastrointestinal	Chronic or cyclic vomiting	Hass *et al.*, 2007
Cardiovascular	Tachycardia	Hass *et al.*, 2007
Endocrine	Hypothyroidism Hypoparathyroidism Idiopathic growth hormone deficiency	Hass *et al.*, 2007
Renal	Renal tubular dysfunction Nephrotic syndrome	Hass *et al.*, 2007

a) Non-specific clinical sighs indicate other clinical characteristics that frequently occur in children with mitochondrial dysfunction but have a broad different diagnosis, and more often lead to other clear diagnosis (Haas *et al*., 2007).

Table 3. Clinical signs of autism spectrum disorders accompanied by mitochondrial dysfunction.

	Weissman *et al.*, 2008 (N = 25)	Rossignol and Frye, 2012 (N = 68)	Palieri and Persico, 2010 (N = 25)	Shoffner *et al.*, 2010 (N = 28)
At least 1 non-CNS organ system	84	-	-	-
Fatigability or exercise intolerance	68	54	76	42.9
Gastrointestinal dysfunction	64	74	64	-
Constipation	20	-	-	-
At least 1 neurological finding of uncommon ASD	60	58[a)]	-	-
Multiple regression	38	-	-	-
Gross developmental motor delay	32	51	32	46.4
Regression	24	52	-	-
Seizure	20	41	39.3	-
Growth retardation	20	21	20	10.7
Oculomotor abnormality	16	-	-	-
Macrocephaly	16	16	-	-
Microcephaly	16	16	-	-
Sensorineural hearing deficit	12	-	-	-
Dysarthria	12	-	-	-
Movement disorders	8	-	-	-
Hypotonia	62	-	-	-
Ataxia	58	-	-	-
Cardiomyopathy	24	-	-	-
Endocrine dysfunction	8	-	-	-
Renal dysfunction	8	-	-	-
Hematologic abnormality	8	-	-	-
Ptosis	8?	-	-	-

Values are expressed as percent of patients. a) ataxia.

growth retardation (n=5, 20%). In this study, twelve patients presented neurological findings that are uncommon in ASD (n=12, 48%), including oculomotor abnormalities (n = 4, 16%), sensorineural hearing deficits (n = 3, 12%), dysarthria (n = 3, 12%), ptosis (n = 2, 8%), and movement disorders (n = 2), and five patients (20%) had seizures, one of whom had medically refractory epilepsy. Eight patients exhibited markedly delayed early motor milestones (32%). Fourteen patients showed regression of previously acquired skills (56%), with nine presenting multiple regressions (36%), six presenting regression at ages older than three years (24%), and seven presenting regressions occurring with infections or other metabolic stresses (28%). In six patients, gross motor skills were lost in addition to language skills (24%). In one case, the timing of regression coincided with a recent vaccination (4%) [100] (Table **3**). Overall, the main clinical manifestations in individuals with ASD/MD were found to be the gastrointestinal symptom of constipation, regression of previously acquired skills, neurological abnormalities, delayed motor milestones, excessive fatigability, exercise intolerance, functional cardiovascular abnormalities, dysmorphisms such as macrocephaly and microcephaly, and medically refractory epilepsy.

These features are similar to the features of MD, as shown in Tables **2** and **3**. In previous studies, MD features have been diagnosed in ASD patients with a range of incidences: 5.0% of 68 ASD children and adolescents, with the oldest being 20 years of age [8]; 13.0% of individuals with ASD aged 5 to 60 years of age [99]; 19% of 210 ASD patients [122]; and 45% of ASD children aged 11 to 14 years of age [123] were diagnosed as having MD features. The above findings, together with a previous report indicating that that MD presents clinically in at least one in 10,000 adults [124], suggest that individuals with ASD and a

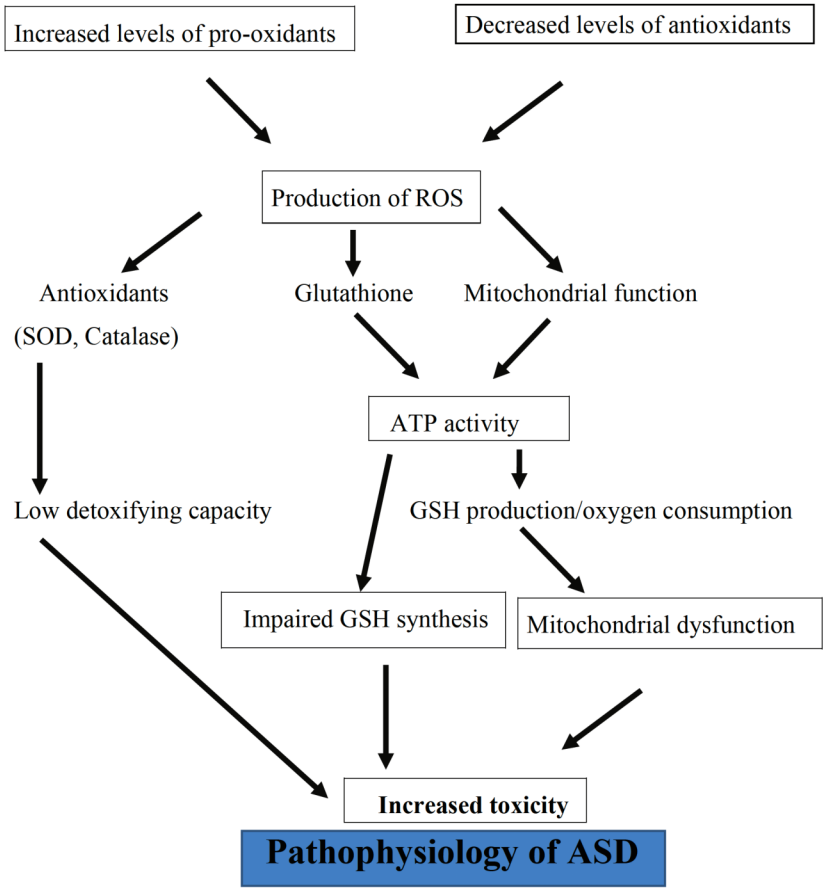

Fig. (2). Oxidative stress in autism spectrum disorder. The arrows in the figure represent the advanced oxidation processes. The ratio of ATP production/oxygen consumption may be a potential candidate target for preventing MD due to oxidative stress. On the other hand, disruption of ATP synthesis alone may be related to impaired GSH synthesis. In this regard, oxygen consumption may be important in preventing MD. ROS, reactive oxygen species; ATP, adenosine triphosphate; SOD, superoxide dismutase; ASD, autism spectrum disorders.

concomitant diagnosis of MD are more prevalent than patients with typical MD. Collectively, a significant subgroup of ASD children may have a new type of MD that is more prevalent and is distinct from classic MD [7, 9]. Meanwhile, another study suggested that children with ASD and a concomitant diagnosis of MD may constitute a subset of the ASD population who have distinct abnormalities in redox metabolism such as MD [101]. In this regard, a previous study reported the interesting finding that seventy-five individuals with ASD (simplex type) aged 5-60 years exhibited lactate-positive voxels in the cingulate gyrus more frequently, indicating that these autistic individuals with a concomitant diagnosis of MD may be a neurobiological subtype of ASD [99]. However, only 60% of autistic individuals ASD with a concomitant diagnosis of MD show developmental regression including impaired social communication, but not stereotyped or repetitive behaviors [100]. Moreover, autistic individuals with a concomitant diagnosis of MD display clinical manifestations including prevalent seizures [98] and exhibit abnormal biochemical markers of MD [8, 98]. The DSM-IV criteria do not include major neurological and non-neurological medical problems (e.g., constipation in the gastrointestinal system, excessive fatigability, exercise intolerance, and cardiovascular functional abnormalities). It would therefore be logical to propose that autistic individuals with MD may have a subtype of MD that is more prevalent and is distinct from classic MD. Considering that tuberous sclerosis and the Landau-Kleffner syndrome manifest with similar symptoms to ASD, individuals with ASD/MD are more likely to represent a new type of MD. This review proposes that individuals with ASD and a concomitant diagnosis of MD may exhibit a subtype of MD rather than a subtype of ASD.

3.2.2. Biomarkers of ASD with a Concomitant Diagnosis of MD

Table **4** shows the number of subjects exhibiting abnormal results for biochemical markers of ASD with a concomitant diagnosis of MD, along with the main references published during 2008-2012 [8, 100, 125-127]. The abnormal biochemical parameters that are frequently detected in ASD with MD include elevated blood or plasma levels of lactate and an increased lactate/pyruvate ratio [8, 100, 126]. Lactate has long been regarded as an end product of anaerobic energy production and appears to play an

Table 4. Biochemical data from individuals with ASD accompanied by MD.

Biochemical markers	Number of abnormal
Weissman *et al.*, 2008	Number abnormal/number tested
Increased blood lactate	19/25 (76%)
Increased serum aspartate transaminase or alanine transaminase	13/25 (53%)
Increased plasma pyruvate acid	9/17 (53%)
Abnormal urinary organic acid analysis	10/24 (42%)
Increased plasma alanine	9/25 (36%)
Increased serum creatine kinase	8/25 (32%)
Increased fibroblast lactate/pyruvate ratio	3/15 (20%)
Increased lactate on cranial MRS	10/21 (40%)
Shoffner *et al.*, 2010	
Increased blood lactate, pyruvate and alanine	13/28 (46.4)
Palmieri *et al.*, 2010‡	
Increased plasma lactate	15/15 (100.0%)
Decreased plasma GSH	148/233 (63.5%)
Increased plasma GSSG	118/203 (58.4%)
Increased MDA	67/67 (100.0%)
Essa *et al*., 2012	
19 ASD children vs 19 normal controls	
Plasma levels of PCO, LPO, and lactate/pyruvate ratio significant decreases in ASD children	
Rossignol and Frye, 2012†	
Elevated lactate	31.1%
Elevated pyruvate	13.6%
Elevated lactate/pyruvate ratio	27.6%
Elevated creatine kinase	46.8%
Elevated ammonia	35.0%
Elevated AST	45.6%
Elevated ALT	7.0%
Low total carnitine	90.0%

†, The total number of subjects was not described (Weissman *et al.*, 2012); ‡, total number who exhibited abnormal values (Palmieri *et al.*, 2010).

PCO, protein carbonyls; LPO, lipid peroxidation; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

important role in the antioxidant defense [129]. The plasma lactate concentration is a marker of the severity of the tissue oxygen supply-to-demand imbalance [130]. Pyruvate is the end product of cytosolic glycolysis and has a variety of possible fates, with the major one being mitochondrial oxidation [131] (Table **4**). Thus, a high blood lactate/ pyruvate ratio is a potential biomarker of MD [132]. Additionally, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are biochemical markers of organ dysfunction, including MD [133].

3.2.3. Medical Treatment of MD

Accumulating evidence indicates that essential cofactors in mitochondria and certain types of flavone and plant compounds are effective for treating MD. Beta-amyloid (Aβ) protein is a key factor in the pathogenesis of Alzheimer's disease. Coenzyme Q10, an essential cofactor involved in the mitochondrial electron transport chain and DHA, has been suggested to be a potential therapeutic agent for Alzheimer's disease, as it may restore the decreased oxygen consumption rate and ATP turnover [134]. Quercetin potentially prevents Huntington's disease and other neurodegenerative disorders in which mitochondrial function is perturbed [135]. Resveratrol treatment functionally impacts oxygen consumption and mitochondrial ATP production while decreasing lactate contents, thus showing potential clinical applications in selected Parkinson's disease patients [136].

3.3. Impairment of Glutathione Biosynthesis

3.3.1. Glutathione Synthesis

GSH in the mitochondria controls compartment-specific needs and functions [111], representing the main mechanism of antioxidant defense against ROS [111]. Thus, GSH

deficiency can lead to impaired mitochondrial function [111, 128]. The synthesis of GSH from glutamate, cysteine, and glycine is catalyzed sequentially by two cytosolic enzymes, γ-glutamylcysteine synthetase and GSH synthetase [137].

Upon reaction with ROS, GSH becomes oxidized to GSSR by GSSH reductase (GR), a process that decreases GSH [111, 138]. The ratio of reduced GSH to the oxidized disulfide form of glutathione (i.e., glutathione disulfide, GSSH) is considered to be a reproducible indicator of the systemic redox status that can be used to clinically assess and treat individuals with ASD, who may be at risk of oxidative stress-related pathology [139]. Moreover, the GSH/GSSH ratio reflects the oxidative state and interactions with ROS to the maintain redox balance in the cell [111]. A shift in the GSH redox rate towards to the oxidized state may lead to DNA damage and increased apoptosis [138].

3.3.2. Clinical Findings

As a decreased GSH redox ratio has been reported in individuals with ASD, it has been hypothesized that a shift in the GSH redox ratio may play a role in the etiology of ASD [138, 140]. Some evidence has suggested that glutathione redox ratios, such as the GSH/GSSH ratio, can be used as biomarkers of impaired GSH synthesis and treatment status in ASD [141, 142]. An early study examined 20 children with ASD with a mean age of 6.4±1.5 years who met the DSM-IV criteria for autistic disorders. Most of these children exhibited symptoms of gastrointestinal distress, in addition to impaired speech and socialization skills. In this study, plasma levels of GSH and the GSH/GSSH ratio were found to be reduced [143]. Two other studies examined glutathione redox status and glutathione metabolism, in 40 children [143] and 48 children [142] who met the DSM-IV criteria of autistic disorders. In these two studies, Asperger's disorder, pervasive developmental disorders (not otherwise specified), severe gastrointestinal distress, genetic disorders and chronic seizures (epilepsy) were excluded. In these studies, the total GSH/GSSH ratio was shown to be significantly reduced compared with 42 (James *et al.*, 2009) [144] and 40 age-matched normal controls [142]. The most recent of these studies, in which the diagnostic criteria included the DSM-5 criteria for autistic disorders but excluded epileptic seizure and any additional psychiatric and neurological disorders, revealed lower plasma levels of glutathione-transferase compared with 30 age-matched normal controls [145]. Another earlier study examined frozen samples from the cerebella and temporal cortexes of fifteen individuals with ASD and twelve unaffected controls [141]. In this study, the diagnosis of ASD was confirmed based on the Autism Diagnostic Interview-Revised, which matches the DSM-5 criteria for ASD symptoms without any neurological or somatic symptoms [146]. This study reported that the cerebella of ASD individuals show significantly decreased GSH levels, GSH/GSSH ratios and mitochondrial superoxide production as well as significantly increased levels of biomarkers of oxidative stress, such as 3-nitrotyrosine (3-NT), and inflammation, such as 3-chlorotyrosine (3-CT) [141] Considering these findings together, this review is the first to suggest that impaired GSH synthesis is evident in relation to the core social and behavioral sequences described in the DSM-5 criteria for autistic disorders.

3.3.3. Biomarkers of Impaired GSH Synthesis

Plasma 3CT [142], S- adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) [144, 147] are useful biomarkers of impaired GSH synthesis and treatment status in ASD.

3.4. The Role of ATP in MD and Impaired GSH Synthesis

Mitochondria play an important role in ATP generation [148]. An imbalance between energy intake and expenditure leads to MD, characterized by a reduced ratio of energy production, such as ATP production [149]. In the cytosol, GSH synthesis includes two consecutive ATP-dependent enzymatic reactions. The first step in GSH synthesis involves the formation of glutamylcysteine from glutamate and cysteine in an ATP-dependent reaction catalyzed by glutamate-cystein-ligase [138]. In the second step, γ-glutamylcysteine (GCL) is coupled to glycine to form GSH via glutathione synthetase [138, 140]. The major determinants of GSH synthesis are the availability of cysteine and glutamate cysteine ligase [140, 150]. When oxidative stress coincides with excess dietary nutrients, glycolytically synthesized ATP inhibits mitochondrial oxygen consumption [119]. Importantly, when nutrient oxidation is insufficient, the ratio of ATP synthesis/oxygen consumption is low, leading to increased production of ROS, such as superoxide anions [149]. Mitochondrial oxygen consumption is uncoupled from ATP production during the formation of reactive oxygen species by components of the mitochondrial electron transport chain [108]. It therefore appears that ATP production/oxygen consumption may be closely associated with MD. Hence, this review provides a potential framework to explain how the ratio of ATP production/oxygen consumption may be a potential candidate target for preventing MD due to oxidative stress. On the other hand, disruption of ATP synthesis alone may be related to impaired GSH synthesis. In this regard, oxygen consumption may be important in preventing MD.

3.5. Impairment of the Antioxidant Capacity

An adequate equilibrium between the production of ROS and the antioxidant capacity plays an important role in brain function. In this context, early studies on ASD mostly reported that changes in the blood levels of SOD, Tf and Cp indicate alterations of the antioxidant status [102-104], vulnerability to oxidative stress [105, 106], and copper dyshomeostasis [151, 152] in children with ASD. Although ASD consists of a group of developmental disorders with symptoms that present in a continuum ranging from mild to severe expression [153], the core symptoms of ASD include two domains according to the DSM-5 [7]: negative symptoms of impairment of social and communication function impairment and positive symptoms of restricted and repetitive behaviors in the DSM-5. The DSM-5 does not includes any major neurological and non-neurological medical problems in the diagnostic criteria for ASD [7].

Participants in these earlier studies exhibited the core symptoms, such as social and communication impairment.

The plasma [106, 152, 154, 155] and urinary [156, 157] total antioxidant capacity (TAC) are useful biomarkers of reduced antioxidant capacity. A few studies have examined plasma or urinary levels of TAC in ASD. A previous study found that plasma TAC levels were reduced in 35 individuals with Asperger's disorders compared with 34 age-matched normal healthy controls [106]. In addition, urinary levels of TAC were reported to be elevated in 45 autistic children aged 4-12 years [156] and 29 children with ASD aged 6-12 years [157]. Salivary TAC was shown to be significantly reduced in 101 children aged 2-12 years [158]. The exclusion criteria for these studies included neurological disorders and a history of head trauma [106]. Participants in the remaining two studies met the DSM-IV-TR criteria for autism [157] or were scored using the Childhood Autism Rating Scale [156] and therefore did not exhibit any major neurological or non-neurological medical problems. Collectively, these findings appear to indicate that reduced plasma or urinary TAC levels occur in ASD children without any neurological or somatic symptoms.

4. CONCLUSIONS

Mitochondria are organelles that play a central role in processes related to cellular viability, such as energy production, cell growth, cell death via apoptosis, and metabolism of reactive oxygen species (ROS). Constitutive activation of mTORC1 may result in oxidative and ER stress as well as ASD associated with monogenic disorders such as TSC and FXS. We can observe behavioral abnormalities relevant to autism spectrum disorders (ASDs) and their recovery mediated by the mTOR inhibitor rapamycin in mouse models. In *Tsc2*$^{+/-}$ mice, the transcription of multiple genes involved in mTOR signaling is enhanced, suggesting a crucial role of dysregulated mTOR signaling in the ASD model. Moreover, The mTOR inhibitor such as rapamycin may be useful target for the pharmacological treatment of treatment resistant ASD.

This review offers three insights into the relationship between MD and impaired GSH synthesis. Firstly, children with ASD and concomitant diagnosis of MD have been reported to manifest clinical manifestations similar to those of MD, and it therefore appears that the clinical manifestations of ASD with a concomitant diagnosis of MD are more likely to be due to MD. Secondly, the ATP production/oxygen consumption ratio may be a potential candidate target for preventing MD due to oxidative stress, and the disruption of ATP synthesis alone may be related to impaired GSH synthesis. Third, changes in TAC have been reported in most ASD children without any neurological and somatic symptoms. In future studies, these three types of pathophysiologies should be considered throughout the pathophysiology and medical treatment of ASD. This information provides insight into the pathophysiology of ASD and can guide the design of medical treatments for various subtypes of ASD.

LIST OF ABBREVUATIONS

ALT	=	Alanine aminotransferase
ASD	=	Autism spectrum disorders
AST	=	Aspartate aminotransferase
ATP	=	Adenosine triphosphate
CGG repeat in the *FMR1*	=	Trinucleotide repeat in the fragile X mental retardation
CHOP	=	C/EBP homologous protein
3-CT	=	3-chlorotyrosine
eIF4E	=	Eukaryotic translation initiation factor 4E
eIF4G	=	Eukaryotic translation initiation factor 4G
ER	=	Endoplasmic reticulum
ERK	=	Extracellular Signal-regulated Kinase
4EBPs	=	Eukaryotic translation initiation factor 4E (eIF4E)-binding proteins
Fmr1	=	Fragile X mental retardation 1
FMRP	=	Fragile X Mental Retardation Protein
FXS	=	Fragile X syndrome
GABA	=	Gamma-aminobutyric acid
GCL	=	γ-glutamylcysteine
GMR 5	=	Glutamine receptor metabotropic 5
GR	=	GSSH reductase (GR)
GSH	=	Glutathione
GSSH	=	Glutathione disulfide
HO-1	=	Heme oxygenase I
LCLs	=	Lymphoblastoid cell lines
LTD	=	Long-term depression
MD	=	Mitochondrial dysfunction
mGluR5	=	Metabotropic glutamate receptor 5
mTOR	=	Mammalian target of rapamycin
NAD	=	Nicotinamide adenine dinucleotide
NADH	=	Nicotinamide adenine dinucleotide
NF1	=	Neurofibromatosis type 1
3-NT	=	3-nitrotyrosine
PHTS	=	PTEN hamartoma tumor syndrome
PTEN	=	Phosphatase and tension homolog deleted on chromosome ten
ROS	=	Reactive oxygen species
rpS6	=	Ribosomal protein S6
S6K1	=	Ribosomal p6 kinase 1

SAH = S-adenosylhomocysteine

SAM = S-adenosylmethionine

TAC = Total antioxidant capacity

TSC = Tuberous sclerosis complex

URP = Unfolded protein response

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Dr. Kunio Yui received a Grant-in-Aid For Scientific Research on Innovative Areas (Grant No 21200017) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (2009-2012), and a Grant-in-Aid For Scientific Research C (2014-2017)

REFERENCES

[1] De Felice, F.G.; Ferreira, S.T. Inflammation, defective insulin signaling, and mitochondrial dysfunction as common molecular denominators connecting type 2 diabetes to Alzheimer disease. *Diabetes*, **2014**, *63,* 2262-2272.

[2] Stroh, M.; Swerdlow, R.H.; Zhu, H. Common defects of mitochondria and iron in neurodegeneration and diabetes (MIND): a paradigm worth exploring. *Biochem. Pharmacol*., **2014**, *88*, 573-583.

[3] Goto, Y.; Horai, S.; Matsuoka, T.; Koga, Y.; Nihei, K.; Kobayashi, M.; Nonaka, I. Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): a correlative study of the clinical features and mitochondrial DNA mutation. *Neurology*, **1992**, *42*, 545-550.

[4] Shoffner, J.M.; Lott, M.T.; Lezza, A.M.; Seibel, P.; Ballinger, S.W.; Wallace, D.C. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. *Cell*, **1990**, *61*, 931-937.

[5] Chaturvedi, R.K.; Flint Beal, M. Mitochondrial diseases of the brain. *Free Radic. Biol. Med*., **2013**, *63*, 1-29.

[6] Rose, S.; Frye, R.E.; Slattery, J.; Wynne, R.; Tippett, M.; Melnyk, S.; James, S.J. Oxidative stress induces mitochondrial dysfunction in a subset of autistic lymphoblastoid cell lines. *Transl. Psychiatry*, **2014a**, *4*:e377.

[7] American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders*, 5th ed.; Arlington, VA: American Psychiatric Publishing, 2013.

[8] Rossignol, D.A.; Frye, R.E. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol. Psychiatry*, **2012**, *17*(3), 290-314.

[9] Rose, S.; Frye, RE.; Slattery.; Wynne, R.; Tippett, M.; Pavliv, O.; Melnyk, S.; James, S.J. Oxidative stress induces mitochondrial dysfunction in a subset of autism lymphoblastoid cell lines in a well-matched case control cohort. *PLoS One*, **2014b**, *9*(1), e85436.

[10] Schieke, S.M.; Phillips, D.; McCoy, J.P.; Jr, Aponte, A.M.; Shen, R.F.; Balaban, R.S.; Finkel, T. The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. *J. Biol. Chem*., **2006**, *281*, 27643-27652.

[11] Cunningham, J.T.; Rodgers, J.T.; Arlow, D.H.; Vazquez, F.; Mootha, V.K.; Puigserver, P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature*, **2007**, *450*, 736-740.

[12] Goo, C.K.; Lim, H.Y.; Ho, Q.S.; Too, H.P.; Clement, M.V.; Wong, K.P. PTEN/Akt signaling controls mitochondrial respiratory capacity through 4E-BP1. *PLoS One*, **2012**, *7*(9), e45806.

[13] Napoli, E.; Ross-Inta, C.; Wong, S.; Hung, C.; Fujisawa, Y.; Sakaguchi, D.; Angelastro, J.; Omanska-Klusek, A.; Schoenfeld, R.; Giulivi, C. Mitochondrial dysfunction in Pten haplo-insufficient mice with social deficits and repetitive behavior: interplay between Pten and p53. *PLoS One*, **2012**, *7*(8), e42504.

[14] de Vries, P.J.; Howe, C.J. The tuberous sclerosis complex proteins - a GRIPP on cognition and neurodevelopment. *Trends Mol. Med*., **2007**, *13*, 319-326.

[15] Tang, B.; Dong, X.; Wei, Z.; Qiao, H.; Jiang, H.; Liu, B.; Sun, X. Enhanced autophagy by everolimus contributes to the antirestenotic mechanisms in vascular smooth muscle cells. *J. Vasc. Res*., **20**14, *51*(4), 259-68.

[16] Enns, G.M.; Moore, T.; Le, A.; Atkuri, K.; Shah, M.K.; Cusmano-Ozog, K.; Niemi, A.K.; Cowan, T.M. Degree of glutathione deficiency and redox imbalance depend on subtype of mitochondrial disease and clinical status. *PLoS One*, **2014**; *9*(6), e100001.

[17] Naviaux, R.K. Oxidative shielding or oxidative stress? *J. Pharmacol. Exp. Ther.*, **2012**, *342*(3), 608-618.

[18] Abrahams, B.S.; Geschwind, D.H. Advances in autism genetics: on the threshold of a new neurobiology. *Nat. Rev. Genet*., **2008**, *9*, 341-355.

[19] Pinto, D.; Pagnamenta, A.T.; Klei, L.; Anney, R.; Merico, D.; Regan, R.; Conroy, J.; Magalhaes, T.R.; Correia, C.; Abrahams, B.S.; Almeida, J.; Bacchelli, E.; Bader, G.D.; Bailey, A.J.; Baird, G.; Battaglia, A.; Berney, T.; Bolshakova, N.; Bölte, S.; Bolton, P.F.; Bourgeron, T.; Brennan, S.; Brian, J.; Bryson, S.E.; Carson, A.R.; Casallo, G.; Casey, J.; Chung, B.H.; Cochrane, L.; Corsello, C.; Crawford, E.L.; Crossett, A.; Cytrynbaum, C.; Dawson, G.; de Jonge, M.; Delorme, R.; Drmic, I.; Duketis, E.; Duque, F.; Estes, A.; Farrar, P.; Fernandez, B.A.; Folstein, S.E.; Fombonne, E.; Freitag, C.M.; Gilbert, J.; Gillberg, C.; Glessner, J.T.; Goldberg, J.; Green, A.; Green, J.;. Guter, S.J.; Hakonarson, H.; Heron, E.A.; Hill, M.; Holt R.; Howe, J.L.; Hughes, G.; Hus, V.; Igliozzi, R.; Kim, C.; Klauck, S.M.; Kolevzon, A.; Korvatska, O.; Kustanovich, V.; Lajonchere, C.M.; Lamb, J.A.; Laskawiec, M.; Leboyer, M.; Le Couteur, A.; Leventhal, B.L.; Lionel, A.C.; Liu, X.Q.; Lord, C.; Lotspeich, L.; Lund, S.C.; Maestrini, E.; Mahoney, W.; Mantoulan, C.; Marshall, C.R.; McConachie, H.; McDougle, C.J.; McGrath, J.; McMahon, W.M.; Merikangas, A.; Migita, O.; Minshew, N.J.; Mirza, G.K.; Munson, J.; Nelson, S.F.; Noakes, C.; Noor, A.; Nygren, G.; Oliveira, G.; Papanikolaou, K.; Parr, J.R.; Parrini, B.; Paton, T.; Pickles, A.; Pilorge, M.; Piven, J.; Ponting, C.P.; Pose, D.J.; Poustka, A.; Poustka, F.; Prasad, A.; Ragoussis, J.; Renshaw, K.; Rickaby, J.; Roberts, W.; Roeder, K.; Roge, B.; Rutter, M.L.; Bierut, L.J.; Rice, J.P.; Salt, J.; Sansom, K.; Sato, D.; Segurado, R.; Sequeira, A.F.; Senman, L.; Shah, N.; Sheffield, V.C.; Soorya, L.; Sousa, I.; Stein, O.; Sykes, N.; Stoppioni, V.; Strawbridge, C.; Tancredi, R.; Tansey, K.; Thiruvahindrapduram, B.; Thompson, A.P.; Thomson, S.; Tryfon, A.; Tsiantis, J.; Van Engeland, H.; Vincent, J.B.; Volkmar, F.; Wallace, S.; Wang, K.; Wang, Z.; Wassink, T.H.; Webber, C.; Weksberg, R.; Wing, K.; Wittemeyer, K.; Wood, S.; Wu, J.; Yaspan, B.L.; Zurawieck, D.; Zwaigenbaum, L.; Buxbaum, J.D.; Cantor, R.M.; Cook, E.H.; Coon, H.; Cuccaro, M.L.; Devlin, B.; Ennis, S.; Gallagher, L.; Geschwind, D.H.; Gill, M.; Haines, J.L.; Hallmayer, J.; Miller, J.; Monaco, A.P.; Nurnberger, J.I Jr.; Paterson, A.D.; Pericak-Vance, M.A.; Schellenberg, G.D.; Szatmari, P.; Vicente, A.M.; Vielan, V.J.; Wijsman, E.M.; Scherer, S.W.; Sutcliffe, J.S.; Betancur, C. Functional impact of global rare copy number variation in autism spectrum disorders. *Nature*, **2010**, *466*, 368-372.

[20] Caglayan, A.O. Genetic causes of syndromic and non-syndromic autism. *Dev. Med. Child Neurol*., **2010**, *52*, 130-138.

[21] Misra, V. The social brain network and autism. *Ann. Neurosci*., **2014**, *21*, 69-73.

[22] Wagner, G.C.; Reuhl, K.R.; Cheh, M.; McRae, P.; Halladay, A.K. A new neurobehavioral model of autism in mice: pre- and postnatal exposure to sodium valproate. *J. Autism Dev. Disord*., **2006**, *36*, 779-93.

[23] Meador, K.J.; Baker, G.A.; Browning, N.; Cohen, M.J.; Bromley, *R.L.;* Clayton-Smith, J.; Kalayjian, L.A.; Kanner, A.; Liporace, J.D.; Pennell, P.B.; Privitera, M.; Loring, D.W.; NEAD Study Group. Fetal antiepileptic drug exposure and cognitive outcomes at age 6 years (NEAD study): a prospective observational study. *Lancet Neurol*., **2013**, *12*, 244-252.

[24] Christensen, J.; Grønborg, T.K.; Sørensen, M.J.; Schendel, D.; Parner, E.T.; Pedersen, L.H.; Vestergaard, M. Prenatal valproate

exposure and risk of autism spectrum disorders and childhood autism. *J.A.M.A.*, **2013**, *309*, 1696-1703.

[25] Shi, L.; Fatemi, S.H.; Sidwell, R.W.; Patterson, P.H. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J. Neurosci*., **2003**, *2*, 297-302.

[26] Smith, S.E.; Li, J.; Garbett, K.; Mirnics, K.; Patterson, P.H. Maternal immune activation alters fetal brain development through interleukin-6. *J. Neurosci*., **2007**, *27*, 10695-10702.

[27] Meldrum, S.J.; Strunk, T.; Currie, A.; Prescott, S.L.; Simmer, K.; Whitehouse, A.J. Autism spectrum disorder in children born preterm-role of exposure to perinatal inflammation. *Front. Neurosci.,* **2013**, *7*, 123.

[28] Ehninger, D.; Silva, A.J. Rapamycin for treating tuberous sclerosis complex and autism spectrum disorders. *Trends Mol. Med*., **2011**, *17*, 78-87.

[29] Sawicka, K., Zukin, R.S. Dysregulation of mTOR signaling in neuropsychiatric disorders: therapeutic implications. *Neuropsychopharmacology*, **2012**, *37*, 305-306.

[30] Kim, D.H.; Sarbassov, D.D.; Ali, S.M.; King, J.E.; Latek, R.R.; Erdjument-Bromage, H.; Tempst, P.; Sabatini, D.M. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*, **2002**, *110,* 163-175.

[31] Hara, K.; Maruki, Y.; Long, X.; Yoshino, K.; Oshiro, N.; Hidayat, S.; Tokugaga, C.; Avruch, J.; Yonezawa, K. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell,* **2002**, *110*, 177-789.

[32] van Slegtenhorst, M.; de Hoogt, R.; Hermans, C.; Nellist, M.; Janssen, B.; Verhoef, S.; Lindhout, D.; van den Ouweland, A.; Halley, D.; Young, J.; Burley, M.; Jeremiah, S.; Woodward, K.; Nahmias, J.; Fox, M.; Ekong, R.; Osborne, J.; Wolfe, J.; Povey, S.; Snell, R.G.; Cheadle, J.P.; Jones, A.C.; Tachataki, M.; Ravine, D.; Sampson, J.R.; Reeve, M.P.; Richardson, P.; Wilmer, F.; Munro, C.; Hawkins, T.L.; Sepp, T.; Ali, J.B.; Ward, S.; Green, A.J.; Yates, J.R.; Kwiatkowska, J.; Henske, E.P.; Short, M.P.; Haines, J.H.; Jozwiak, S.; Kwiatkowski, D.J. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science*, **1997**, *277*, 805-808.

[33] European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell,* **1993**, *75*, 1305-1315.

[34] Northrup, H.; Krueger, D.A. International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 Iinternational Tuberous Sclerosis Complex Consensus Conference. *Pediatr. Neurol*., **2013**, *49*, 243-254.

[35] Krueger, D.A.; Northrup, H. International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex surveillance and management: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. *Pediatr. Neurol*., **2013**, *49*, 255-265.

[36] Lewis, J.C.; Thomas, H.V.; Murphy, K.C.; Sampson, J.R. Genotype and psychological phenotype in tuberous sclerosis. *J. Med. Genet*., **2004**, *41*(3), 203-207.

[37] Caglayan, A.O. Genetic causes of syndromic and non-syndromic autism. *Dev. Med. Child Neurol.*, **2010**, *52*(2), 130-138.

[38] Numis A.L.; Major, P.; Montenegro, M.A.; Muzykewicz, D.A.; Pulsifer, M.B.; Thiele, E.A. Identification of risk factors for autism spectrum disorders in tuberous sclerosis complex. *Neurology*, **2011**, *76*, 981-987.

[39] Kobayashi, T.; Minowa, O.; Kuno, J.; Mitani, H.; Hino, O.; Noda, T. Renal carcinogenesis, hepatic hemangiomatosis, and embryonic lethality caused by a germ-line Tsc2 mutation in mice. *Cancer Res*., **1999**, *59*, 1206-1211.

[40] Kobayashi, T.; Minowa, O.; Sugitani, Y.; Takai, S.; Mitani, H.; Kobayashi, E.; Noda, T.; Hino, O. A germ-line Tsc1 mutation causes tumor development and embryonic lethality that are similar, but not identical to, those caused by Tsc2 mutation in mice. *Natl. Acad. Sci. U.S.A*., **2001**, *98*, 8762-8767.

[41] Kobayashi, T.; Hirayama, Y.; Kobayashi, E.; Kubo, Y.; Hino, O. A germline insertion in the tuberous sclerosis (Tsc2) gene gives rise to the Eker rat model of dominantly inherited cancer. *Nat. Genet*., **1995**, *9*, 70-74.

[42] Mizuguchi, M.; Takashima, S.; Yamanouchi, H.; Nakazato, Y.; Mitani, H.; Hino, O. Novel cerebral lesions in the Eker rat model of tuberous sclerosis: cortical tuber and anaplastic ganglioglioma. *J. Neuropathol. Exp. Neurol*., **2000**, *59*, 188-196.

[43] Goorden, S.M.; van Woerden, G.M.; van der Weerd, L.; Cheadle, J.P.: Elgersma, Y. Cognitive deficits in Tsc1+/- mice in the absence of cerebral lesions and seizures. *Ann. Neurol*., **2007**, *62*, 648-655.

[44] Waltereit, R.; Japs, B.; Schneider, M.; de Vries, P.J.; Bartsch, D. Epilepsy and Tsc2 haploinsufficiency lead to autistic-like social deficit behaviors in rats. *Behav. Genet.,* **2011**, *41*(3), 364-372.

[45] Sato, A.; Kasai, S.; Kobayashi, T.; Takamatsu, Y.; Hino, O.; Ikeda, K.; Mizuguchi, M. Rapamycin reverses impaired social interaction in mouse models of tuberous sclerosis complex. *Nat. Commun*., **2012**, *3*, 1292.

[46] Ehninger, D.; Han, S.; Shilyansky, C.; Zhou, Y.; L,i W.; Kwiatkowski, D.J.; Ramesh, V.; Silva, A.J. Reversal of learning deficits in a Tsc2+/- mouse model of tuberous sclerosis. *Nat. Med*., **2008**, *14*, 843-848.

[47] Potter, W.B.; Basu, T.; O'Riordan, K.J.; Kirchner, A.; Rutecki, P.; Burger, C.; Roopra, A. Reduced juvenile long-term depression in tuberous sclerosis complex is mitigated in adults by compensatory recruitment of mGluR5 and Erk signaling. *PLoS Biol*., **2013**, *11*(8), e1001627

[48] Tsai, P.T.; Hull, C.; Chu, Y.; Greene-Colozzi, E.; Sadowski, A.R.; Leech, J.M.; Steinberg, J.; Crawley, J.N.; Regehr, W.G.; Sahin, M. Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature*, **2012**, *488,* 647-651.

[49] Zeng, L.H.; Rensing, N.R.; Zhan, B.; Gutmann, D.H.; Gambello, M.J.; Wong, M. Tsc2 gene inactivation causes a more severe epilepsy phenotype than Tsc1 inactivation in a mouse model of tuberous sclerosis complex. *Hum. Mol. Genet*., **2011**, *20*, 445-454.

[50] Krueger, D.A.; Wilfong, A.A.; Holland-Bouley, K.; Anderson, A.E.; Agricola, K.; Tudor, C.; Mays, M.; Lopez, C.M.; Kim, M.O.; Franz, D.N. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Ann. Neuro*l., **2013**, *74*, 679-687.

[51] Marsh, D.J.; Kum, J.B.; Lunetta, K.L.; Bennett, M.J.; Gorlin, R.J.; Ahmed, S.F.; Bodurtha, J.; Crowe, C.; Curtis, M.A.; Dasouki, M.; Dunn, T.; Feit, H.; Geraghty, M.T.; Graham, J.M.Jr.; Hodgson, S.V.; Hunter, A.; Korf, B.R.; Manchester, D.; Miesfeldt, S.; Murday, V.A.; Nathanson, K.L.; Parisi, M.; Pober, B.; Romano, C.; Eng, C. PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. *Hum. Mol. Genet*., **1999**, *8*, 1461-1472.

[52] Zhou, X.P.; Marsh, D.J.; Morrison, C.D.; Chaudhury, A.R.; Maxwell, M.; Reifenberger, G.; Eng, C. Germline inactivation of PTEN and dysregulation of the phosphoinositol-3-kinase/Akt pathway cause human Lhermitte-Duclos disease in adults. *Am. J. Hum. Genet*., **2003**, *73*, 1191-1198.

[53] Butler, M.G.; Dasouki, M.J.; Zhou, X.P.; Talebizadeh, Z.; Brown, M.; Takahashi, T.N.; Miles, J.H.; Wang, C.H.; Stratton, R.; Pilarski, R.; Eng, C. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. *J. Med. Genet*., **2005**, *42*, 318-321.

[54] Varga, E.A.; Pastore, M.; Prior, T.; Herman, G.E.; McBride, K.L. The prevalence of PTEN mutations in a clinical pediatric cohort with autism spectrum disorders, developmental delay, and macrocephaly. *Genet. Med*., **2009**, *11*, 111-117.

[55] McBride, K.L.; Varga, E.A.; Pastore, M.T.; Prior, T.W.; Manickam, K.; Atkin, J.F.; Herman, G.E. Confirmation study of PTEN mutations among individuals with autism or developmental delays/mental retardation and macrocephaly. *Autism Res*., **2010**, *3*, 137-41.

[56] Di Cristofano, A.; Pesce, B.; Cordon-Cardo, C.; Pandolfi, P.P. Pten is essential for embryonic development and tumour suppression. *Nat. Genet*., **1998**, *19*, 348-355.

[57] Page, D.T.; Kuti, O.J.; Prestia, C.; Sur, M. Haploinsufficiency for Pten and Serotonin transporter cooperatively influences brain size and social behavior. *Proc. Natl. Acad. Sci. U.S.A*., **2009**, *106,* 1989-1994.

[58] Kwon, C.H.; Luikart, B.W.; Powell, C.M.; Zhou, J.; Matheny, S.A.; Zhang, W.; Li, Y.; Baker, S.J.; Parada, L.F. Pten regulates neuronal arborization and social interaction in mice. *Neuron*, **2006**, *50*, 377-388.

[59] Zhou, J.; Blundell, J.; Ogawa, S.; Kwon, C.H.; Zhang, W.; Sinton, C.; Powell, C.M.; Parada, L.F. Pharmacological inhibition of

mTORC1 suppresses anatomical, cellular, and behavioral abnormalities in neural-specific Pten knock-out mice. *J. Neurosci*., **2009**, *29*, 1773-1783.

[60] Devys, D.; Lutz, Y.; Rouyer, N.; Bellocq, J.P.; Mandel, J.L. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nat. Genet.*, **1993**, *4*, 335-340.

[61] Tranfaglia, M.R. The psychiatric presentation of fragile x: evolution of the diagnosis and treatment of the psychiatric comorbidities of fragile X syndrome. *Dev. Neurosci*., **2011**, *33*, 337-348.

[62] Bear, M.F.; Huber, K.M.; Warren, S.T. The mGluR theory of fragile X mental retardation. *Trends Neurosci*., **2004**, *27*, 370-377.

[63] Dölen, G.; Osterweil, E.; Rao, B.S.; Smith, G.B.; Auerbach, B.D.; Chattarji, S.; Bear, M.F. Correction of fragile X syndrome in mice. *Neuron*, **2007**, *56*, 955-962.

[64] Sharma, A.; Hoeffer, C.A.; Takayasu, Y.; Miyawaki, T.; McBride, S.M.; Klann, E.; Zukin, R.S. Dysregulation of mTOR signaling in fragile X syndrome in fragile X syndrome. *J. Neurosci*., **2010**, *30*, 694-702.

[65] Bhattacharya, A.; Kaphzan, H.; Alvarez-Dieppa, A.C.; Murphy, J.P.; Pierre, P.; Klann, E. Genetic removal of p70 S6 kinase 1 corrects molecular, synaptic, and behavioral phenotypes in fragile X syndrome mice. *Neuron*, **2012**, *76*, 325-337.

[66] Viskochil, D.; Buchberg, A.M.; Xu, G.; Cawthon, R.M.; Stevens, J.; Wolff, R.K.; Culver, M.; Carey, J.C.; Copeland, N.G.; Jenkins, N.A. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell*, **1990**, *62*, 187-192.

[67] Cawthon, R.M.; Weiss, R.; Xu, G.F.; Viskochil, D.; Culver, M.; Stevens, J.; Robertson, M.; Dunn, D.; Gesteland, R.; O'Connell, P. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. *Cell*, **1990**, *62*, 193-201.

[68] Wallace, M.R; Marchuk, D.A.; Andersen, L.B.; Letcher, R.; Odeh, H.M.; Saulino, A.M.; Fountain, J.W.; Brereton, A.; Nicholson, J.; Mitchell, A.L. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science*, **1990**, *249*, 181-186.

[69] Hyman, S.L.; Shores, A.; North, K.N. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. *Neurology*, **2005**, *65*, 1037-1044.

[70] Costa, R.M.; Federov, N.B.; Kogan, J.H.; Murph, G.G.; Stern, J.; Ohno, M.; Kucherlapati, R.; Jacks, T.; Silva, A.J. Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. *Nature*, **2002**, *415*, 526-30.

[71] Donovan, S.; See, W.; Bonifas, J.; Stokoe, D.; Shannon, K.M. Hyperactivation of protein kinase B and ERK have discrete effects on survival, proliferation, and cytokine expression in Nf1-deficient myeloid cells. *Cancer Cell*, **2002**, *2*, 507-514.

[72] Johannessen, C.M.; Reczek, E.E.; James, M.F.; Brems, H.; Legius, E.; Cichowski, K. The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc. Natl. Acad. Sci. U. S. A*., 2**005**, *102*, 8573-8578.

[73] Li, W.; Cui, Y.; Kushner, S.A.; Brown, R.A.; Jentsch, J.D.; Frankland, P.W.; Cannon, T.D.; Silva, A.J. The HMG-CoA reductase inhibitor lovastatin reverses the learning and attention deficits in a mouse model of neurofibromatosis type 1. *Curr. Biol*., **2005**, *15*, 1961-1967.

[74] Krab, L.C.; de Goede-Bolder, A.; Aarsen, F.K.; Pluijm, S.M.; Bouman, M.J.; van der Geest, J.N.; Lequin, M.; Catsman, C.E.; Arts, W.F.; Kushner, S.A.; Silva, A.J.; de Zeeuw, C.I.; Moll, H.A.; Elgersma, Y. Effect of simvastatin on cognitive functioning in children with neurofibromatosis type 1: a randomized controlled trial. *J.A.M.A*., **2008**, *300*, 287-294.

[75] Mbarek, O.; Marouillat, S.; Martineau, J.; Barthélémy, C.; Müh, J.P.; Andres, C. Association study of the NF1 gene and autistic disorder. *Am. J. Med. Genet*., **1999**, *88*, 729-732.

[76] Walsh, K.S.; Vélez, J.I.; Kardel, P.G.; Imas, D.M.; Muenke, M.; Packer, R.J.; Castellanos, F.X.; Acosta, M.T. Symptomatology of autism spectrum disorder in a population with neurofibromatosis type 1. *Dev. Med. Child Neurol*., **2013**, *55*, 131-138.

[77] Garg, S.; Lehtonen, A.; Huson, S.M.; Emsley, R.; Trump, D.; Evans, D.G.; Green, J. Autism and other psychiatric comorbidity in neurofibromatosis type 1: evidence from a population-based study. *Dev. Med. Child Neurol*., **2013**, *55*, 139-145.

[78] Molosh, A.I.; Johnson, P.L.; Spence, J.P.; Arendt, D.; Federici, L.M.; Bernabe, C.; Janasik, S.P.; Segu, Z.M.; Khanna, R.; Goswami, C.; Zhu, W.; Park, S.J.; Li, L.; Mechref, Y.S.; Clapp, D.W.; Shekhar, A. Social learning and amygdala disruptions in Nf1 mice are rescued by blocking p21-activated kinase. *Nat. Neurosci*., **2014**, doi: 10.1038/nn.3822. [Epub ahead of print]

[79] Antion, M.D.; Merhav, M.; Hoeffer, C.A.; Reis, G.; Kozma, S.C.; Thomas, G.; Schuman, E.M.; Rosenblum, K.; Klann, E. Removal of S6K1 and S6K2 leads to divergent alterations in learning, memory, and synaptic plasticity. *Learn. Mem*., **2008**, *15*, 29-38.

[80] Pause, A.; Belsham, G.J.; Gingras, A.C.; Donzé, O.; Lin, T.A.; Lawrence, J.C.Jr.; Sonenberg, N. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. *Nature*. **1994**, *371*,762-767.

[81] Gingras, A.C.; Raught, B.; Gygi, S.P.; Niedzwiecka, A.; Miron, M.; Burley, S.K.; Polakiewicz, R.D.; Wyslouch-Cieszynska, A.; Aebersold, R.; Sonenberg, N. Hierarchical phosphorylation of the translation inhibitor 4E- BP1. *Genes. Dev*., **2001**, *15*, 2852-2864.

[82] Moerke, N.J.; Aktas, H.; Chen, H.; Cantel, S.; Reibarkh, M.Y.; Fahmy, A.; Gross, J.D.; Degterev, A.; Yuan, J.; Chorev, M.; Halperin, J.A.; Wagner, G. Small-molecule inhibition of the interaction between the translation initiation factors eIF4E and eIF4G. *Cell*, **2007**, *128*, 257-267.

[83] Gkogkas, C.G.; Khoutorsky, A.; Ran, I.; Rampakakis, E.; Nevarko, T.; Weatherill, D.B.; Vasuta, C.; Yee, S.; Truitt, M.; Dallaire, P.; Major, F.; Lasko, P.; Ruggero, D.; Nader, K.; Lacaille, J.C.; Sonenberg, N. Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature*, **2013**, *493*, 371-377.

[84] Santini, E.; Huynh, T.N.; MacAskill, A.F.; Carter, A.G.; Pierre, P.; Ruggero, D.; Kaphzan, H.; Klann, E. Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature*, **2013**, *493*, 411-415.

[85] Auerbach, B.D.; Osterweil, E.K.; Bear, M.F. Mutations causing syndromic autism define an axis of synaptic pathophysiology. *Nature*, **2011**, *480*, 63-68.

[86] Moy, S.S.; Nadler, J.J.; Young, N.B.; Perez, A.; Holloway, L.P.; Barbaro, R.P.; Barbaro, J.R.; Wilson, L.M.; Threadgill, D.W.; Lauder, J.M.; Magnuson, T.R.; Crawley, J.N. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. *Behav. Brain Res*., **2007**, *176*, 4-20.

[87] Silverman, J.L.; Smith, D.G.; Rizzo, S.J.; Karras, M.N.; Turner, S.M.; Tolu, S.S.; Bryce, D.K.; Smith, D.L.; Fonseca, K.; Ring, R.H.; Crawley, J.N. Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. *Sci. Transl. Med*., **2012**, *4*, 131ra51.

[88] Burket, J.A.; Benson, A.D.; Tang, A.H.; Deutsch, S.I. Rapamycin improves sociability in the BTBR T(+)Itpr3(tf)/J mouse model of autism spectrum disorders. *Brain Res. Bull*., **2014**, *100*, 70-75.

[89] Chugani, D.C.; Sundram, B.S.; Behen, M.; Lee, M.L.; Moor, G.J. Evidence of altered energy metabolism in autistic children. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **1999**, *23*, 635-641

[90] Al-Mosalem, O.A.; El-Ansary, A.; Attas, O.; Al-Ayadhi, L. Metabolic biomarkers related to energy metabolism in Saudi autistic children. *Clin. Biochem*., **2009**, *42*, 949-957.

[91] Chauhan, A.; Gu, F.; Essa, M.M.; Wegiel, J.; Kaur, K.; Brown, W.T.; Chauhan, V. Brain region-specific deficit in mitochondrial electron transport chain complexes in children with autism. *J. Neurochem*., **2011**, *117*, 209-220.

[92] Tang, G.; Gudsnuk, K.; Kuo, S.H.; Cotrina, M.L.; Rosoklija, G.; Sosunov, A.; Sonders, M.S.; Kanter, E.; Castagna, C.; Yamamoto, A.; Yue, Z.; Arancio. O.; Peterson, B.S.; Champagne, F.; Dwork, A.J.; Goldman, J.; Sulzer, D. Loss of mTOR-dependent macroautophagy causes autistic-like synsptic pruning deficit. *Neuron*, **2014**, *83*(5), 1131-1143.

[93] Schieke, S.M.; Phillips, D.; McCoy, J.P. Jr.; Aponte, A.M.; Shen, R.F.; Balaban, R.S.; Finkel, T. The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. *J. Biol. Chem*., **2006**, *281*(37), 27643-27652.

[94] Goo, C.K.; Lim, H.Y.; Ho, Q.S.; Too, H.P.; Clement, M.V.; Wong, K.P. PTEN/Akt signaling controls mitochondrial respiratory capacity through 4E-BP1. *PLoS One,* **2012**, *7*(9), e45806.

[95] Di Nardo, A.; Kramvis, I.; Cho, N.; Sadowski, A.; Meikle, L.; Kwiatkowski, D.J.; Sahin, M. Tuberous sclerosis complex activity is required to control neuronal stress responses in an mTOR-dependent manner. *J. Neurosci*., **2009**, *29*, 5926-5937.

[96] James, S.J.; Rose, S.; Melnyk, S.; Jernigan, S.; Blossom, S.; Pavliv, O.; Gaylor, D.W. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. *F.A.S.E.B. J.*, **2009**, *23*, 2374-2383.

[97] Ozcan, U.; Ozcan, L.; Yilmaz, E.; Düvel, K.; Sahin, M.; Manning, B.D.; Hotamisligil, G.S. Loss of the tuberous sclerosis complex tumor suppressors triggers the unfolded protein response to regulate insulin signaling and apoptosis. *Mol. Cell*, **2008**, *29*, 541-551.

[98] Haas, R.H.; Parikh, S.; Falk, M.J.; Saneto, R.P.; Wolf, N.I.; Darin, N.; Cohen, B.H. Mitochondrial disease: a practical approach for primary care physicians. *Pediatrics*, **2007**, *120*(6), 1326-1233.

[99] Goh, S.; Dong, Z.; Zhang, Y.; DiMauro, S.; Peterson, B.S. Mitochondrial dysfunction as a neurobiological subtype of autism spectrum disorder: evidence from brain imaging. *J.A.M.A. Psychiatry,* **2014**, *71*(6), 665-671.

[100] Weissman, J.R.; Kelley, R.I.; Bauman, M.L.; Cohen, B.H.; Murray K.F.; Mitchel, R.L.; Kern, R.L.; Natowicz, M.R. Mitochondrial disease in autism spectrum disorder patients: a cohort analysis. *PLos One,* **2008**, *3*(11), e3815.

[101] Frye, R.E.; Delatorre, R.; Taylor, H.; Slattery, J.; Melnyk, S.; Chowdhury, N.; James, S.J. Redox metabolism abnormalities in autistic children associated with mitochondrial disease. *Transl. Psychiatry*, **2013**, *3*, e273.

[102] Yorbik, O.; Sayal, A.; Akay, C.; Akbiyi, DI.; Sohmen, T. Investigation of antioxidant enzymes in children with autistic disorder. *Prostagland. Leukot. Essent. Fatty Acids*, **2002**, *67*: 341-343.

[103] Sögüt, S.; Zoroğlu, SS.; Özyurt, H.; Yilmaz, H.R.; Özugurlu, F.; Sivasli, E.; Yetkin, O.; Yanik, M.; Tutkun, H.; Savaş, H.A.; Tarakçioğlu, M.; Akyol, O. Changes in nitric oxide levels and antioxidant enzyme activities may hay a role in the pathophysiolgical mechanisms involved in autism. *Clin. Chim. Acta*, **2006**, *331*: 111-117.

[104] Zoroglu, S.S.; Armutcu, F.; Ozen, S.; Gurel, A.; Sivasli, E.; Sivasli, E.; Yetkin, O.; Meram, I. Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. *Eur. Arch. Psychiatry Clin. Neurosci.*, **2004**, *254*, 143-147.

[105] Meguid, N.; Dardir, A.A.; Abdel-Raouf E.R.; Hashish, A. Evaluation of oxidative stress in autism: defective antioxidant enzymes and increased lipid peroxidation. *Biol. Trace Elem. Res.*, **2011**, *143,* 58-65.

[106] Parellada, M.; Moreno, C.; Mac-Dowell, K.; Leza, J.C.; Giraldez, M.; Bailón, C.; Castro, C.; Miranda-Azpiazu, P.; Fraguas, D.; Arang, C. Plasma antioxidant capacity is reduced in Asperger syndrome. *J. Psychiat. Res.*, **2012**, *46*, 394-401.

[107] Lü, J.M.; Lin, P.H.; Yao, Q.; Chen, C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *J. Cell Mol. Med.*, **2010**, *14*(4), 840-860.

[108] Hartman, M.L.; Shirihai, O.S.; Holbrook, M.; Xu, G.; Kocherla, M.; Shah, A. Relation of mitochondrial oxygen consumption in peripheral blood mononuclear cells to vascular function in type 2 diabetes mellitus. *Vasc. Med.,* **2014**, *19*(1), 67-74.

[109] Kusano, C.; Ferrari, B.; Total antioxidant capacity: a biomarker in biomedical and nutritional studies. *J. Cell Mol. Biol.,* **2008**, *7*(1): 1-15.

[110] Niki, E. Do antioxidants impair signaling by reactive oxygen species and lipid oxidation products? *FEBS Lett.,* **2012**, *586*(21), 3767-3770.

[111] Ribas, V.; García-Ruiz, C.; Fernández-Checa, JC. Glutathione and mitochondria. *Front. Pharmacol.,* **2014**, *5, 151*. doi: 10.3389/ fphar.2014.00151.

[112] Dunning, S.; Ur Rehman, A.; Tiebosch, M.H.; Hannivoort, R.A.; Haijer, F.W.; Woudenberg, J.; van den Heuvel, FA.; Buist-Homan, M.; Faber, K.N.; Moshage, H. Glutathione and antioxidant enzymes serve complementary roles in protecting activated hepatic stellate cells against hydrogen peroxide-induced cell death. *Biochim. Biophys. Acta,* **2013**, *1832*(12), 2027-2034.

[113] Franco, R.; Cidlowski, J.A. Apoptosis and glutathione: beyond an antioxidant. *Cell Death Differ.,* **2009**, *16*(10), 1303-1314.

[114] Greetham, D.; Grant, C.M.; Antioxidant activity of the yeast mitochondrial one-Cys peroxiredoxin is dependent on thioredoxin reductase and glutathione *in vivo*. *Mol. Cell Biol.,* **2009**, *29*(11), 3229-3240.

[115] Dröse, S.; Brandt, U.; Wittig, I. Mitochondrial respiratory chain complexes as sources and targets of thiol-based redox-regulation. *Biochim. Biophys. Acta,* **2014**, *1844*(8), 1344-1354.

[116] Koopman, W.J.; Nijtmans, L.G.; Dieteren, C.E.; Roestenberg, P.; Valsecchi, F.; Smeitink, .JA.; Willems, P.H. Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. *Antioxid. Redox Signal.*, **2010**, *12*(12), 1431-1470.

[117] Sorrentino, G.; Comel, A.; Mantovani, F.; Del Sal, G. Regulation of mitochondrial apoptosis by Pin1 in cancer and neurodegeneration. *Mitochondrion*, **2014**, Pt A:88-96. doi: 10.1016/j.mito.2014.08.003.

[118] Ghanizadeh, A.; Berk, M.; Farrashbandi, H.; Alavi Shoushtari, A.; Villagonzalo, K.A. Targeting the mitochondrial electron transport chain in autism, a systematic review and synthesis of a novel therapeutic approach. *Mitochondrion*, **2013**, *13*(5), 515-519.

[119] Naviaux, R.K. Oxidative shielding or oxidative stress? *J. Pharmacol. Exp. Ther*., **2012**, *342*(3), 608-618.

[120] Nicolson, G.L. Mitochondrial dysfunction and chronic disease: treatment with natural supplements. *Altern. Ther. Health Med.,* **2014**, Suppl 1, 18-25.

[121] Mattman, A.; Sirrs, S.; Mezei, M.M.; Salvarinova-Zivkovic, R.; Alfadhel, M.; Lillquist, Y. Mitochondrial disease clinical manifestations: An overview. *B.C.M. J*., **2011**, *53*(4), 183-187.

[122] Correia, C.; Coutinho, A.M.; Diogo, L.; Grazina, M.; Marques, C.; Miguel, T.; Ataíde, A.; Almeida, J.; Borges, L.; Oliveira, C.; Oliveira, G.; Vicente, A.M. Brief report: High frequency of biochemical markers for mitochondrial dysfunction in autism: no association with the mitochondrial aspartate/glutamate carrier SLC25A12 gene. *J. Autism Dev. Disord*., **2006**, *36*(8), 1137-1140.

[123] Oliveira, G.; Diogo, L.; Grazina, M.; Garcia, P.; Ataíde, A.; Marques, C.; Miguel, T.; Borges, L.; Vicente, A.M.; Oliveira, C.R. Mitochondrial dysfunction in autism spectrum disorders: a population-based study. *Dev. Med. Child Neurol.,* **2005**, *47*(3), 185-189.

[124] Chinnery, P.F.; Elliott, H.R.; Hudson, G.; Samuels, D.C.; Relton, C.L. Epigenetics, epidemiology and mitochondrial DNA diseases. *Int. J. Epidemiol*., **2012**, *41*(1), 177-187.

[125] Shoffner, J.; Hyams, L.; Langley, G.N.; Cossette, S.; Mylacraine, L.; Dale, J.; Ollis, L.; Kuoch, S.; Bennett, K.; Aliberti, A.; Hyland, K. Fever plus mitochondrial disease could be risk factors for autistic regression. *J. Child. Neurol*., **2010**, *25*(4), 429-434.

[126] Palmieri, L.; Persico, A.M. Mitochondrial dysfunction in autism spectrum disorders: cause or effect? *Biochim. Biophys. Acta*, **2010**, *1797*(6-7), 1130-1137.

[127] Essa, M.M.; Guillemin, G.J.; Waly, M.I.; Al-Sharbati, M.M.; Al-Farsi, Y.M.; Hakkim, F.L.; Ali, A.; Al Shafaee, M.S. Increased markers of oxidative stress in autistic children of the Sultanate of Oman. *Biol. Trace Elem. Res*., **2012**, *147*(1-3), 25-27.

[128] Rossignol, D.A.; Frye, R.E. Evidence linking oxidative stress, mitochondrial dysfunction, and inflammation in the brain of individuals with autism. *Front. Physiol.,* **2014**, *150,* doi: 10.3389/fphys.2014.00150.

[129] Lemire, J.; Auger, C.; Mailloux, R.; Appanna, V.D. Mitochondrial lactate metabolism is involved in antioxidative defense in human astrocytoma cells. *J. Neurosci. Res*., **2014**, *92*(4), 464-75.

[130] Vanni, S.; Socci, F.; Pepe, G.; Nazerian, P.; Viviani, G.; Baioni, M.; Conti, A.; Grifoni, S. High plasma lactate levels are associated with increased risk of in-hospital mortality in patients with pulmonary embolism. *Acad. Emerg. Med*., **2011**, *18*(8), 830-835.

[131] Schell, J.C.; Rutter, J. The long and winding road to the mitochondrial pyruvate carrier. *Cancer Metab*., **2013**, *1*(1), 6.

[132] Nielsen, T.H.; Olsen, N.V.; Toft, P.; Nordström, C.H.; Cerebral energy metabolism during mitochondrial dysfunction induced by cyanide in piglets. *Acta Anaesthesiol. Scand*., **2013**, *57*(6), 793-801.

[133] Lowes, D.A;, Webster, N.R.; Murphy, M.P.; Galley, H.F.; Antioxidants that protect mitochondria reduce interleukin-6 and oxidative stress, improve mitochondrial function, and reduce biochemical markers of organ dysfunction in a rat model of acute sepsis. *Br. J. Anaesth*., **2013**, *110*(3), 472-480.

[134] Sadli, N.; Barrow, C. J.; McGee, S.; Suphioglu, C.; Effect of DHA and coenzymeQ10 against Aβ- and zinc-induced mitochondrial dysfunction in human neuronal cells. *Cell Physiol. Biochem.,* **2013**, *32*(2), 243-252.

[135] Sandhir, R.; Mehrotra, A.; Quercetin supplementation is effective in improving mitochondrial dysfunctions induced by 3-nitropropionic acid: implications in Huntington's disease. *Biochim. Biophys. Acta,* **2013**, *1832*(3), 421-430.

[136] Ferretta, A.; Gaballo, A.; Tanzarella, P.; Piccoli, C.; Capitanio, N.; Nico, B.; Annese, T., Di Paola, M.; Dell'aquila, C.; De Mari, M.; Ferranini, E.; Bonifati, V.; Pacelli, C.; Cocco, T. Effect of resveratrol on mitochondrial function: implications in parkin-associated familiar Parkinson's disease. *Biochim. Biophys. Acta*, **2014**, *1842*(7), 902-915.

[137] Wu, G.; Fang, Y.Z.; Yang, S.; Lupton, J.R.; Turner, N.D. Glutathione metabolism and its implications for health. *J. Nutr.,* **2004**, *134*(3), 489-492.

[138] Main, P.A.; Angley, M.T.; O'Doherty, C.E.; Thomas, P.; Fenech, M. The potential role of the antioxidant and detoxification properties of glutathione in autism spectrum disorders: a systematic review and meta-analysis. *Nutr. Metab (London)*., **2012**, *9*,35. doi: 10.1186/1743-7075-9-3.

[139] James, S. J.; Rose, S.; Melnyk, S.; Jernigan,S.; Blossom,S.; Pavliv, O.; Gaylor, D.W. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. *F.A.S.E.B.J.,* **2009**, *23*(8): 2374-2383.

[140] Gu, F.; Chauhan, V.; Chauhan, A. Impaired synthesis and antioxidant defense of glutathione in the cerebellum of autistic subjects: alterations in the activities and protein expression of glutathione-related enzymes. *Free Radic. Biol. Med.*, **2013**, *65*, 488-496.

[141] Rose, S.; Melnyk, S.; Pavliv, O.; Bai, S; Nick, T.G.; Frye, R.E.; James, S.J. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. *Transl. Psychiatry*, **2012**, *2*:e134. doi: 10.1038/tp.2012.61.

[142] Frye, R.E.; Melnyk, S.; Fuchs, G.; Reid, T.; Jernigan, S.; Pavliv, O.; Hubanks, A.; Gaylor, D.W.; Walters, L.; James, S.J. Effectiveness of methylcobalamin and folinic Acid treatment on adaptive behavior in children with autistic disorder is related to glutathione redox status. *Autism Res. Treat*., **2013**, 609705.

[143] James, S.J.; Cutler, P.; Melnyk, S.; Jernigan, S.; Janak, L.; Gaylor, D.W.; Neubrander, J.A. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am. J. Clin. Nutr.,* **2004**, *80*(6), 1611-1617.

[144] James, S.J.; Melnyk, S.; Fuchs, G.; Reid, T.; Jernigan S. Pavliv, O.; Hubanks, A.; Gaylor, D.W. Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism. *Am. J. Clin. Nutr.*, **2009**, *89*(1), 425-430.

[145] Alabdali, A.; Al-Ayadhi, L.; El-Ansary, A. key role for an impaired detoxification mechanism in the etiology and severity of autism spectrum disorders**.** *B.B.F.,* **2014**, *10*, 14.

[146] Mazefsky, C. A.; McPartland, J. C.; Gastgeb, H. Z.; Minshew, N. J. Brief report: comparability of DSM-IV and DSM-5 ASD research samples. *J. Autism Dev. Disord.,* **2013**, *43*(5), 1236-1242.

[147] Frye, R.E.; James, S.J. Metabolic pathology of autism in relation to redox metabolism. *Biomark. Med.*, **2014**, *8*(3), 321-330.

[148] Jheng, H.F.; Tsai, P.J.; Guo, S.M.; Kuo, L.H.; Chang, C.S.; Su, I.J.; Chang, C.R.; Tsai, Y.S. Mitochondrial fission contributes to mitochondrial dysfunction and insulin resistance in skeletal muscle. *Mol. Cell Biol*., **2012**, *32*(2), 309-319.

[149] Kim, J.A.; Wei, Y.; Sowers, J.R. Role of mitochondrial dysfunction in insulin resistance. *Circ. Res.,* **2008**, *102*(4), 401-414.

[150] Lu, S.C. Regulation of glutathione synthesis. *Mol. Aspects Med.,* **2009**, *30*(1-2), 42-59.

[151] Chauhan, A.; Chauhan, V.; Brown, WT.; Cohen, I. Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin-the antioxidant proteins. *Life Sci.*, **2004**, *75*, 2539-2549.

[152] Tórsdóttir, G.; Hreidarsson, S.; Kristinsson, J.; Snaedal, J.; Jóhannesson, T. Ceruloplasmin, superoxide dismutase and copper in autistic patients. *Basic Clin. Pharmacol. Toxicol.*, **2005**, *96,* 146-148.

[153] Lauritsen, M.B. Autism spectrum disorders. *Eur. Child Adolesc. Psychiatry*, **2013**, *Suppl 1*, S37-42.

[154] Kotan, V.O.; Sarandol, E.; Kirhan, E.; Ozkaya, G.; Kirli, S. Effects of long-term antidepressant treatment on oxidative status in major depressive disorder: a 24-week follow-up study. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2011**, *35*(5), 1284-1290.

[155] Anes A.B.; Fetoui, H.; Bchir, S.; ben Nasr, H.; Chahdoura, H.; Chabchoub, E.; Yacoub, S.; Garrouch, A.; Benzarti, M.; Tabka, Z.; Chahed, K. Increased oxidative stress and altered levels of nitric oxide and peroxynitrite in tunisian patients with chronic obstructive pulmonary disease: correlation with disease severity and airflow obstruction. *Biol. Trace Elem. Res.*, **2014**, *161*(1), 20-31.

[156] Damodaran, L.P.; Arumugam, G. Urinary oxidative stress markers in children with autism. *Redox Rep.*, **2011**, *16*(5), 216-222.

[157] Ranjbar, A.; Rashedi, V.; Rezaei, M. Comparison of urinary oxidative biomarkers in Iranian children with autism. *Res. Dev. Disa.,* **2014**, *35*(11), 2751-2755. doi: 10.1016/j.ridd.2014.07.010.

[158] Rai, K.; Hegde, A.M.; Jose, N. Salivary antioxidants and oral health in children with autism. *Arch. Oral Biol.,* **2012**, *57*(8), 1116-11.

Received: ?????????, 2014 Revised: ?????????, 2014 Accepted: ?????????, 2014