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Mitochondrial Dysfunction's Role in the Pathogenesis of Autism Spectrum Disorder: A Proposed Pathway

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There are many contributing factors to the pathogenesis of Autism Spectrum Disorder (ASD). There are several findings related to dysfunctions in ASD; the most common are mitochondrial dysfunction, overactivation of the glia in the brain's immune system, and reduced synaptic pruning (Petrelli, Pucci, & Bezzi, 2016; Yanuck, 2019). This literature review analyzes the research associating oxidative stress with the increase in cytokine and chemokine levels and how that pathway leads to the activation of the immune system. Immune system activation during brain development is the factor in ASD's pathology that has the most evidence supporting its role in the development of ASD.

1. Introduction

Autism Spectrum Disorder (ASD) is one of the most common neurodevelopmental disorders. One in fifty-four children is diagnosed with ASD in the United States (McPartland et al., 2012). A diagnosis of ASD requires meeting all of the social-communicative criteria and two of the four restrictive and repetitive behaviors criteria (APA, 2013). Frequently, people with ASD have difficulty with their executive function-related tasks (Blijd-Hoogewys et al., 2014). Specifically, the deficits are often in cognitive flexibility and planning/organization. Other common characteristics of people with ASD are difficulties taking another person's perspective, understanding social reciprocity, taking the initiative, adapting to change (rigid and pervasive behaviors), and using self-regulation (Blijd-Hoogewys et al., 2014). People typically diagnosed with autism also commonly have sensory sensitivities; strong scents and loud sounds can cause severe distress. A less widely known symptom is a deficit in episodic memory. The characteristics of ASD are widely varied, which makes studying and researching the disorder more difficult.

The exact etiology of ASD is unknown. It is most likely caused by a combination of genetic and environmental factors. The most basic causal understanding is that there are brain structure and function abnormalities in people with ASD. The commonly seen cause of ASD is a lack of synaptic pruning during brain development (Tye et al., 2019). Current research is focused on how synaptic pruning deficits occur. There are many factors that could contribute to deficits in synaptic pruning; the potential pathway examined in this paper is how mitochondria dysfunction can lead to oxidative stress leading to increases in chemo/cytokines and an overly activated immune system (as represented by increased levels of cytokines and chemokines). An overly activated immune system during brain development has connections to synaptic pruning deficits (Tang et al., 2014; Nicolini et al., 2015).

Figure 1. Pathway overview

1.1 Mitochondrial homeostasis linked to ASD

Mitochondrial dysfunction has been linked to ASD (Giulivi et al., 2010; Goodenowe & Pastural, 2011). Genetic mutations targeting mitochondrial function account for more than 10% of all cases, which is the largest genetic contribution (Goodenowe & Pastural, 2011). A Portuguese study showed that 7% of autistic cases are correlated with mitochondrial respiratory chain disorders, meaning this could be one of the most common disorders associated with autism (Olivieria et al., 2005). Not every child in the Oliviera study was tested for a mitochondrial disorder; therefore the actual prevalence is most likely higher. Mitochondria dysfunction is most likely a large part of the pathogenesis of ASD.

One of the most likely ways that mitochondrial dysfunction can contribute to the pathogenesis of ASD is through producing reactive oxygen species (ROS). A disturbance in the mitochondrial respiratory chain can lead to assembly deficits and structural changes in the oxidative phosphorylation complexes (Morán et al., 2012). Those changes lead to decreased ATP production, electron leakage, accumulation of ROS, and release of apoptotic inducing factors (Morán et al., 2012). Complexes I and III are the primary sites that produce oxygen radicals (Morán et al., 2012). Without a functioning mitochondrial respiratory system, more oxygen radicals are produced, and the build-up can lead to oxidative stress (Morán et al., 2012).

1.2 Oxidative Stress

Oxidative stress is caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products (Pizzino et al., 2017). ROS play a role in cell signaling (Pizzino et al., 2017). They are typically generated as by-products of oxygen metabolism (Pizzino et al., 2017). ROS are usually produced in the mitochondria (Pizzino et al., 2017). During oxidative stress, there is an excess amount of free radicals and oxidants (Pizzino et al., 2017). Oxidative stress can be responsible for the induction of diseases, both chronic and degenerative (Pizzino et al., 2017). Hyperoxia can upregulate the production of TNF and IL-1 (Ghezzi et al. 1991). ROS positively regulate the production of cytokines (Sozzani et al., 2005). Oxidative stress influences many cellular processes.

The focus of this review will be ROS's effects on the immune system. In the immune system, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) can stimulate ROS production through stimulation of the mitochondria (Yang

et al., 2013). ROS can lead to inflammasome activation, which stimulates caspase-1 and the maturation of cytokines (Yang et al., 2013).

1.3 Cytokines and Chemokines

The immune system produces cytokines, chemokines, and other humoral factors to protect the host when threatened (Cerami, 1992). This system usually restores normal homeostasis, but the overproduction of immunoregulatory mediators can prove deleterious to the host (Cerami, 1992). Cytokines and chemokines are signaling molecules for the immune system that can tag cells for destruction by leukocytes (Nathan & Sporn, 1991).

Cytokines and chemokines have been implicated in the pathogenesis of ASD. A mouse model study found that ASD-like behavior in offspring may be caused by altered levels of maternal cytokines (TNFα, IL1β, IL-2, IL-6, and IL-10) (Petrelli, Pucci, & Bezzi, 2016; Jones et al., 2017). Maternal inflammatory cytokines crossing the placental barrier is the potential mechanism by which maternal immune dysregulation increases the risk for ASD. Proinflammatory cytokines in the developing brain lead to neuroinflammation and gliosis. Researchers hypothesize that the inflammatory response contributes to the pathogenesis of ASD (Jones et al., 2017; Estes & McAllister, 2015; Keil et al., 2010; Meyer, Ingersoll, & Hambrick, 2011).

2. Dysfunctions of the mitochondria found in ASD

Several studies have shown that mitochondrial dysfunction is common in people with ASD (Giulivi et al., 2010; Goodenowe & Pastural, 2011; Haas, 2010; Oliveira et al., 2005). Giulivi and colleagues (2010) examined mitochondria dysfunction in people with ASD by measuring oxidative phosphorylation capacity, mtDNA copy number and deletions, mitochondrial rate of hydrogen peroxide production, and plasma lactate and pyruvate. The

Giulivi et al. study found that mitochondrial oxygen consumption was impaired in peripheral lymphocytes in children with ASD. Giulivi and colleagues' study also discovered higher plasma pyruvate levels and higher rates of hydrogen peroxide production in children with ASD. However, this study was purely correlational, so the evidence needs to be further supported by other research. A study by Haas (2010) found that the most common mtDNA mutation is the MELAS A3243G mutation. Oliveira and colleagues (2005) performed a population study with a population of Portuguese children. They assessed the metabolic dysfunction in autism hypothesis using lactate and pyruvate levels for their analysis. Plasma lactate levels were elevated in 20.3% of the participants (Oliveria, 2005). This study also found a defect in oxidative phosphorylation associated with ASD. However, these results are not as trustworthy as other findings because there were no follow-up studies and their analyses were not well thought out. Therefore, their findings would need to be confirmed by other studies. Overall, there are strong correlations between mitochondrial dysfunction and ASD using several markers of mitochondrial dysfunction. Consequently, it is reasonable to conclude that mitochondrial dysfunction plays a role in the pathogenesis of ASD.

3. Mitochondrial dysfunction increasing oxidative stress levels

The mitochondria consumes ~85-95% of all oxygen inhaled during respiration. Most of the oxygen that passes through the mitochondria is reduced to water in the final step of the respiratory chain (Shigenaga, Hagen, & Ames, 1994). Even when the respiratory chain is functioning normally 0.1-4% of the electrons flowing through leak out and cause single-electron reduction of oxygen and thus forming a free radical superoxide anion ($O_2^{\bullet-}$; Murphy, 2009). Complexes I and III are thought to be the primary sites that leak electrons and form $O_2^{\bullet-}$ (Morán et al., 2010). $O_2^{\bullet-}$ is eliminated by the enzyme superoxide dismutase, the enzyme dismutases $O_2^{\bullet-}$

into H_2O_2 (Morán et al., 2012). When the mitochondria is dysfunctional and the respiratory chain is damaged $O_2^{\bullet-}$ is increased and can favor the accumulation of ROS (Morán et al., 2012).

The dysfunction of the mitochondria that is relevant to ASD is mitochondrial disorders. In primary mitochondrial diseases, the respiratory chain is dysfunctional (Morán et al., 2012). The dysfunction in the respiratory chain leads to disturbances in the electron transport and proton pumping across the membrane (Morán et al., 2012). The main mitochondrial dysfunction seen in ASD is decreased levels of complexes in the electron transport chain (Siddiqui, Elwell, & Johnson, 2016; Tang et al., 2013; Gu et al., 2013). Siddiqui, Elwell, & Johnson (2016) found reduced levels of complexes III and V. A study by Tang and colleagues (2013) found reduced levels of all ETC complexes except II in children with ASD. Gu and colleagues (2013) found decreased pyruvate dehydrogenase activity in post-mortem frontal cortex tissue of people with ASD. Reduced levels of pyruvate dehydrogenase would result in insufficient removal of pyruvate and lactate for the TCA cycle leading to insufficient ATP generation (Gu et al., 2013). The mitochondrial dysfunction seen in ASD leads to an increase in mitochondrial ROS (mtROS) (Manivasagam et al., 2020).
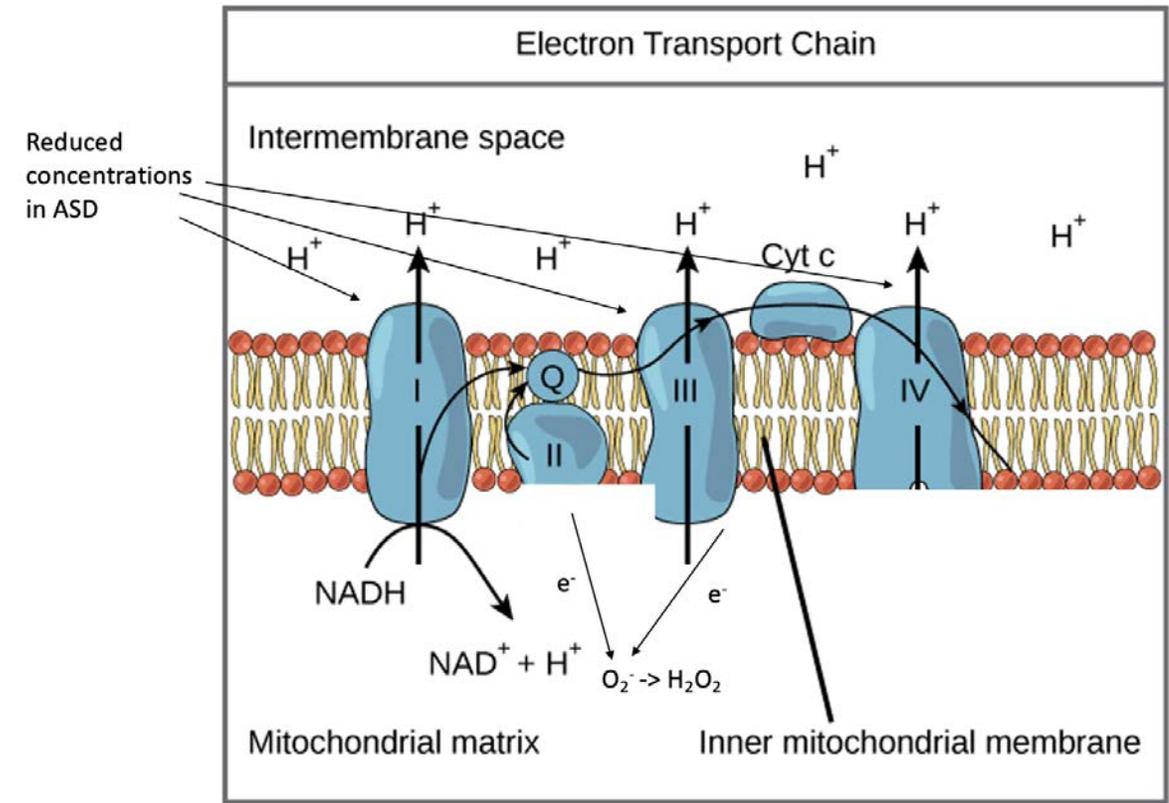
Figure 2. Reduced concentrations of ETC complexes and electron leakage points.

4. mtROS causes dysregulation of cytokines and chemokines

As stated earlier PAMPs and DAMPs can stimulate ROS production by stimulating the mitochondria (Yang et al., 2013). ROS can cause thioredoxin-interacting protein (TXNIP) to dissociate from thioredoxin (TRX), which allows TXNIP to bind to Nod-like receptor pyrin domain-containing 3 (NLRP3), which leads to NLRP3 inflammasome activation (Yang et al., 2013). Then NLRP3 inflammasome then activates caspase-1, which catalyzes the proteolytic maturation of pro-inflammatory cytokines IL-1β and IL-18 (Yang et al., 2013). Mitochondrial ROS (mtROS) can also promote pro-inflammatory cytokine production without using an inflammasome by inactivating MAPK phosphatase, which inhibits pro-inflammatory cytokine gene transcription (Yang et al., 2013). Nakahira et al. (2011) and Zhou et al. (2011) showed that increased mitochondria-derived ROS caused by respiratory chain inhibitors lead to enhanced

NLRP3-dependent caspase-1 activation and IL-1β secretion. ROS from the mitochondria have

been proposed to mediate inflammasome activation (Yang et al., 2013). mtROS mediating the

NLRP3 inflammatory response can lead to increased amounts of inflammatory cytokines
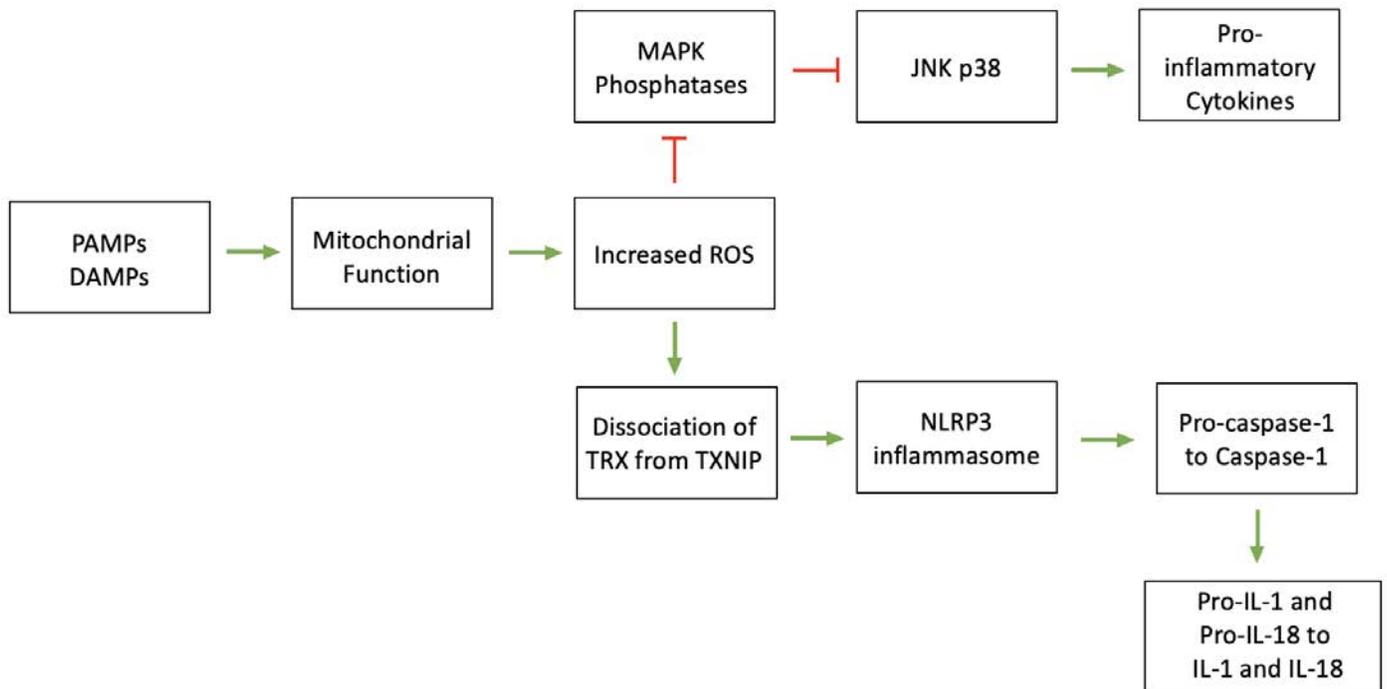
(Nakahira et al., 2011).

Figure 3. Diagram of inflammatory cytokine production dependent and independent of

inflammasome activation.

The Nakahira et al. (2011), Zhou et al. (2011), and Bulua et al. (2011) studies all indicate

that mtROS act as signaling molecules to trigger proinflammatory cytokine production. Bulua

and colleagues designed a cellular experiment to confirm that mitochondria are the cellular

source of excessive ROS. They found that inhibiting mtROS production inhibited MAPK

activation and production of IL-6 and TNF. The findings showed that with less mtROS

production there were fewer inflammatory cytokines in the cells. Therefore, it can reasonably be

concluded that mtROS cause dysregulation in the production of inflammatory cytokines and chemokines. An overproduction of inflammatory cytokines leads to neuroinflammation.

5. Immune system dysregulation causing synaptic pruning deficits

Neuroinflammation is increased in ASD. A study by Takano (2015) found that inflammatory cytokines, a signaling molecule that increases inflammation, are increased in postmortem brain tissue taken from patients with ASD (Takano, 2015). Noreiga and colleagues (2014) also reported altered cytokine profiles in patients diagnosed with ASD. Increased cytokine production of IL-1β, IL-6, and IL-18 were found in the postmortem brains of people with ASD (Noreiga et al., 2014; Takano, 2015). The increased levels of inflammatory cytokines indicate increased microglial activation and expression because microglia are one of the main producers of inflammatory cytokines (Noreiga et al., 2014). The cytokine increase that is found in ASD is a common research finding; since several studies show the same increased cytokine profile in people with ASD, which adds confidence to the findings.

Neuroinflammation is the inflammatory response that occurs within the brain and spinal cord. The inflammation is mediated by cytokines, chemokines, reactive oxygen species, and other secondary messengers (DiSabato, Quan, & Godbout, 2017). These mediators are produced by microglia, astrocytes, endothelial cells, and peripheral immune cells. Common cytokines involved in neuroinflammation are IL-1β, IL-6, and IL-18 (Petrelli, Pucci, & Bezzi, 2016). Neuroinflammation can be damaging to the brain and cause neural circuitry to lose integrity through glial activation.

Proinflammatory cytokines in the brain can lead to neuroinflammation and gliosis by activating the brain's immune system. Researchers hypothesis that the inflammatory response contributes to the pathogenesis of ASD (Jones et al., 2017; Estes & McAllister, 2015; Keil et al.,

2010; Meyer, Ingersoll, & Hambrick, 2011). Specifically, that reactive gliosis might disturb microglia's ability to modulate the maturation, elimination, and functioning of synaptogenesis (Petrelli, Pucci, & Bezzi, 2016; Malkova et al., 2012; Knuesel et al., 2014). Microglia are essential in monitoring and maintaining synapses in the brain by regulating synaptogenesis and neuronal maturation/activity (Paolicelli et al., 2011). They do this by engulfing synaptic material. During reactive gliosis, microglia do not engulf synaptic material which leads to a lessening of synaptic pruning. Decreased synaptic pruning is the largest hallmark of ASD and is thought to be one of the main contributing factors to the symptoms and pathology seen in people with ASD (Petrelli, Pucci, & Bezzi, 2016; Estes & McAllister, 2015).

6. Conclusion

As demonstrated throughout this paper mitochondrial dysfunction starts a pathway leading to reactive gliosis and decreased synaptic pruning. Mitochondrial dysfunction is one of the most common secondary disorders found in people with ASD (Goodenowe & Pastoral, 2011). Mitochondrial dysfunction produces superoxide with then transforms into hydrogen peroxide which is a reactive oxygen species (Siddiqui, Elwell, & Johnson, 2016; Tang et al., 2013). ROS are DAMPs that cause an increase of ROS in the system which then leads to the production of inflammatory cytokines and reactive gliosis (Petrelli, Pucci, & Bezzi, 2016). During reactive gliosis normal synaptic pruning does not occur which leads to a higher amount of synapses than are present in a neurotypical brain (Paolicelli et al., 2011). Decreased synaptic pruning and an overabundance of synapses is the hallmark of brains with ASD (Blijd-Hoogewys et al., 2014). While the pathway discussed has not been confirmed by other research. Its logic aligns with what research has shown. The next research step would be to confirm the connections suggested in this pathway.

7. Works Cited

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). Arlington, VA: Author.

Blijd-Hoogewys, E. M. A., Bezemer, M. L. & van Geert, P. L. C. Executive functioning in children with ASD: an analysis of the BRIEF. J. Autism Dev. Disord. 44, 3089–3100 (2014).

Bulua, A. C. et al. Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). J. Exp. Med. 208, 519–533 (2011).

Cerami, A. Inflammatory cytokines. Clin. Immunol. Immunopathol. 62, S3–S10 (1992).

DiSabato, D. J., Quan, N. & Godbout, J. P. Neuroinflammation: the devil is in the details. J. Neurochem. 139 Suppl 2, 136–153 (2016).

Estes, M. L. & McAllister, A. K. Immune mediators in the brain and peripheral tissues in autism spectrum disorder. Nat. Rev. Neurosci. 16, 469–486 (2015).

Ghezzi, P. et al. Hypoxia increases production of interleukin-1 and tumor necrosis factor by human mononuclear cells. Cytokine 3, 189–194 (1991).

Giulivi, C. et al. Mitochondrial dysfunction in autism. JAMA 304, 2389–2396 (2010).

Goodenowe, D. & Pastural, E. The biochemical basis of autistic behavior and pathology. in Autism - A Neurodevelopmental Journey from Genes to Behaviour (ed. Eapen, V.) (InTech, 2011). doi:10.5772/18571.

Gu, F. et al. Alterations in mitochondrial DNA copy number and the activities of electron transport chain complexes and pyruvate dehydrogenase in the frontal cortex from subjects with autism. Transl. Psychiatry 3, e299 (2013).

Haas, R. H. Autism and mitochondrial disease. Dev. Disabil. Res. Rev. 16, 144–153 (2010).

Jones, K. L. et al. Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. Mol. Psychiatry 22, 273–279 (2017).

Keil, A. et al. Parental autoimmune diseases associated with autism spectrum disorders in offspring. Epidemiology 21, 805–808 (2010).

Knuesel, I. et al. Maternal immune activation and abnormal brain development across CNS disorders. Nat. Rev. Neurol. 10, 643–660 (2014).

Malkova, N. V., Yu, C. Z., Hsiao, E. Y., Moore, M. J. & Patterson, P. H. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain Behav. Immun. 26, 607–616 (2012).

Manivasagam, T. et al. Role of oxidative stress and antioxidants in autism. Adv. Neurobiol. 24, 193–206 (2020).

Meyer, K. A., Ingersoll, B. & Hambrick, D. Z. Factors influencing adjustment in siblings of children with autism spectrum disorders. Res. Autism Spectr. Disord. 5, 1413–1420 (2011)

Morán, M. et al. Mitochondrial bioenergetics and dynamics interplay in complex I-deficient fibroblasts. Biochim. Biophys. Acta 1802, 443–453 (2010).

Morán, M. et al. Mitochondrial respiratory chain dysfunction: implications in neurodegeneration. Free Radic. Biol. Med. 53, 595–609 (2012).

Murphy, M. P. How mitochondria produce reactive oxygen species. Biochem. J. 417, 1–13 (2009).

Nathan, C. & Sporn, M. Cytokines in context. J. Cell Biol. 113, 981–986 (1991).

Nakahira, K. et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat. Immunol. 12, 222–230 (2011).

Nicolini, C., Ahn, Y., Michalski, B., Rho, J. M. & Fahnestock, M. Decreased mTOR signaling pathway in human idiopathic autism and in rats exposed to valproic acid. Acta Neuropathol. Commun. 3, 3 (2015).

Noriega, D. B. & Savelkoul, H. F. J. Immune dysregulation in autism spectrum disorder. Eur. J. Pediatr. 173, 33–43 (2014).

Oliveira, G. et al. Mitochondrial dysfunction in autism spectrum disorders: a population-based study. Dev. Med. Child Neurol. 47, 185–189 (2005).

Paolicelli, R. C. et al. Synaptic pruning by microglia is necessary for normal brain development. Science 333, 1456–1458 (2011).

Petrelli, F., Pucci, L. & Bezzi, P. Astrocytes and Microglia and Their Potential Link with Autism Spectrum Disorders. Front. Cell. Neurosci. 10, 21 (2016).

Pizzino, G. et al. Oxidative stress: harms and benefits for human health. Oxid. Med. Cell. Longev. 2017, 8416763 (2017).

Shigenaga, M. K., Hagen, T. M. & Ames, B. N. Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci USA 91, 10771–10778 (1994).

Siddiqui, M. F., Elwell, C. & Johnson, M. H. Mitochondrial dysfunction in autism spectrum disorders. Autism Open Access 6, (2016).

Sozzani, S., Bosisio, D., Mantovani, A. & Ghezzi, P. Linking stress, oxidation and the chemokine system. Eur. J. Immunol. 35, 3095–3098 (2005).

Takano, T. Role of microglia in autism: recent advances. Dev. Neurosci. 37, 195–202 (2015).

Tang, G. et al. Mitochondrial abnormalities in temporal lobe of autistic brain. Neurobiol. Dis. 54, 349–361 (2013).

Tye, C., Runicles, A. K., Whitehouse, A. J. O. & Alvares, G. A. Characterizing the interplay between autism spectrum disorder and comorbid medical conditions: an integrative review. Front. Psychiatry 9, 751 (2018).

Yang, Y., Bazhin, A. V., Werner, J. & Karakhanova, S. Reactive oxygen species in the immune system. Int. Rev. Immunol. 32, 249–270 (2013).

Yanuck, S. F. Microglial phagocytosis of neurons: diminishing neuronal loss in traumatic, infectious, inflammatory, and autoimmune CNS disorders. Front. Psychiatry 10, 712 (2019).

Zhou, R., Yazdi, A. S., Menu, P. & Tschopp, J. A role for mitochondria in NLRP3 inflammasome activation. Nature 469, 221–225 (2011).