REVIEW ARTICLE

What is the evidence of mitochondrial dysfunction in autism spectrum disorders?

Katarina Babinská [1], Gabriela Repiská [1], Anna Gvozdjáková [2], Jarmila Kucharská [2], Lenka Vokálová [1], Mária Vidošovičová [1], Daniela Ostatníková [1]

[1] Comenius University Faculty of Medicine in Bratislava, Institute of Physiology, Academic Research Centre for Autism, Bratislava, Slovakia; [2] Comenius University Faculty of Medicine in Bratislava, Pharmacobiochemical Laboratory of Third Department of Internal Medicine, Bratislava, Slovakia.

Correspondence to: Katarína Babinská, Institute of Physiology, Comenius University, Sasinkova 2, 813 72 Bratislava, Slovakia; e-mail: katarina.babinska@fmed.uniba.sk

Submitted: 2017-06-02 Accepted: 2017-08-15 Published online: 2017-11-15

Key words: **autism spectrum disorders; mitochondrial dysfunction**

Act Nerv Super Rediviva 2017; **59**(2): 39–44 ANSR590217R01

Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by impairments in social interaction and communication, combined with restricted and stereotyped patterns of behaviour and interests. There is an array of comorbid conditions associated with ASD. It has been suggested that ASD may be a systemic disorder, rather than only a dysfunction of the central nervous system.

Mitochondria are organelles found in almost all cells of the human body; essential for basic cellular functions. Multiple studies have shown mitochondrial dysfunction (MD) in the brain, also in the peripheral tissues of individuals with ASD. It has been estimated that the prevalence of classic mitochondrial disease in ASD population is 5%; however, about 50% of children with ASD may present some abnormalities in markers of mitochondrial functions. Interactions between mitochondrial dysfunction and other underlying pathology of ASD were found, such as oxidative stress or immune dysregulation.

Existing data indicate that mitochondrial dysfunction is implicated in pathology of ASD, at least in a subgroup of the patients. The evidence, however, is still only patchy. Advances in understanding of the involvement of mitochondrial dysfunction in ASD could be a step towards better understanding of the pathophysiology of ASD, and unravelling the role of mitochondria in ASD could open new prospectives for diagnostics and therapeutic interventions. There is a need to find a valid biomarker of mitochondrial dysfunction in ASD and to develop standardized procedures to identify the individuals with ASD and MD, who might mostly profit from an intervention.

Introduction

Autism spectrum disorders (ASD) are lifelong pervasive neurodevelopmental conditions characterized by the core symptoms that include persistent deficits in social interaction, impairments in verbal and non-verbal communication, combined with restricted and stereotyped patterns of behaviour and interests (American Psychiatric Association 2013). Currently, ASD are the fastest growing neurodevelopmental disability with estimated prevalence 1 in 132 persons worldwide (Baxter *et al* 2015). The causes of ASD are poorly understood, current understanding of their pathophysiology takes into account genetic factors

along with the environmental factors (Tordjman *et al* 2014). At present time, no ASD – defining biomarker is known, that could help in the diagnosis.

There is an array of other symptoms not considered as "core", which affect a substantial proportion of individuals with ASD such as psychiatric or neurological problems, gastrointestinal disturbances, immune disorders, also genetic or metabolic abnormalities. The reported co-occurrence rate of one or more diagnoses is reported to be as high as over 90% (Mannion & Leader 2013).

A broad spectrum of changes in the brain anatomy and physiology have been described in ASD (Ecker *et al* 2015), and the cognitive and behavioural impairments seem to result from the central nervous system dysfunction (Rossignol & Frye 2014). Considering the diversity of associated comorbid conditions, it has been proposed that ASD may involve also systemic abnormalities rather than being an exclusively a CNS disorder, at least in a subgroup of individuals (Cheng *et al* 2017; Rossignol & Frye 2014). Mitochondrial dysfunction is a promising candidate for a basic cellular abnormality that could cause impairments in a wide range of organ systems, as mitochondria are organelles found basically in all eukaryotic cells, essential for a variety of basic cellular functions (Frye & Rossignol 2011).

Mitochondria – their function and dysfunction

Mitochondria are almost unmistakable double-membrane structures with the inner membrane folded to cristae. In the most traditional view, except playing an essential role in metabolism, mitochondria are known as the cellular "powerhouses" in which nutrients are utilized to generate energy in form of adenosine triphosphate (ATP), and the synthesized ATP then fuels energy-dependent intracellular processes (Valenti *et al* 2014). Most of the ATP is generated in a cascade of processes culminating in oxidative phosphorylation (OXPHOS) via the mitochondrial electron transport chain, that includes five enzyme complexes I-V. In recent years many new functions of mitochondria have been discovered, highlighting their role in several crucial cellular processes, including calcium homeostasis, cell signalling, control of cell cycle, or apoptosis (Hollis *et al* 2017).

Mitochondrial disease refers to a heterogeneous group of conditions that result from abnormal energy production by mitochondria because of abnormal oxidative phosphorylation. Primary mitochondrial diseases occur due to mutations in genes directly involved in the ATP-generating pathway. Mitochondria are the only cellular organelle possessing own genome, however, their functions depend also on the nuclear genes; thus, mitochondrial disorders may be caused by defects in both nuclear and mitochondrial DNA. Symptoms of impaired mitochondrial function may be elicited by mutations in other genes that are not related to ATP producing oxidative phosphorylation reactions. These cases are referred to as secondary mitochondrial dysfunction. They can be either inherited or acquired due to environmental conditions, such as pollutants, toxicants, nutrient deficiencies, or medications (Niyazov *et al* 2016; Rossignol & Frye 2012).

Mitochondrial disorders present as multisystem diseases that often include symptoms of impaired neuronal functions. Mitochondria seem to be specifically important for the neuronal cells because of their high energy requirements: the brain, while accounting for only 2% of total body weight, consumes 20% of body´s energy needs (Cheng *et al* 2017). Correspondingly, neurons are particularly sensitive to bioenergetic fluctuations, leading to abnormalities in their function. Mitochondria are shown to be involved in fundamental aspects of brain functions, including proliferation and maturation of neural stem cells, process outgrowth, cortical migration; they are closely associated with function of synapses, playing a key role in both the development and the regulation and maintenance of synaptic activity (Cheng *et al* 2017; Hollis *et al* 2017). Several neurodevelopmental, neurodegenerative or psychiatric disorders, such as Parkinson´s disease, Alzheimer´s disease, Down syndrome, schizophrenia, mood disorder or autism spectrum disorders have been shown to be associated with impairments of mitochondrial function (Hollis *et al* 2017; Valenti *et al* 2014).

Evidence linking autism spectrum disorder and mitochondrial dysfunction

Association between mitochondrial dysfunction and ASD was first suggested in 1985 by Coleman and Blass, who hypothesised that individuals with autism can have "abnormal carbohydrate metabolism" (Coleman & Blass 1985). Later, in 1998 the concept of autism as a disorder of atypical mitochondrial function was proposed by Lombard (Lombard 1998). Since that time, a growing number of studies investigated links between mitochondrial dysfunction and ASD. Different methods were used to study a variety of markers of mitochondrial functions, including genetic defects in nuclear and mitochondrial DNA, OXPHOS activity, or metabolite abnormalities, both in brain and in peripheral tissues.

Studies attempting to find explanation of the MD in the ASD patients at the genetic level succeeded to detect both mitochondrial and nuclear DNA gene abnormalities in several brain regions (Legido *et al* 2013). A few surveys focused explicitly on mitochondrial electron transport chain complexes in brain of individuals with ASD, and they consistently showed region-specific differences in protein and activity levels. Brain levels of markers of mitochondrial function were measured, including N-acetyl aspartate, creatine, phosphocreatine, choline or myoinositol in children affected by ASD, and

differences compared to healthy controls were found (Griffiths & Levy 2017; Hollis *et al* 2017; Rossignol & Frye 2012).

Besides brain tissue, studies analyzed also samples from peripheral tissues, such as lymphocytes, muscle biopsies, skin fibroblasts, or buccal swabs of ASD patients. These tissues also exhibited abnormalities in mitochondrial or nuclear DNA, as well as changes in protein and activity levels of the electron transport chain in mitochondria (Griffiths & Levy 2017). A variety of abnormal biochemical markers associated with mitochondrial functions have been found, such as elevated levels of lactate, pyruvate, or alanine in blood and cerebrospinal fluid, or decreased levels of plasma carnitine; abnormal levels of urine organic acids; and impaired mitochondrial fatty acid β-oxidation (Legido *et al* 2013; Rossignol & Frye 2012).

Recent evidence linking mitochondrial perturbations to ASD is discussed in much detail in several review articles (Cheng *et al* 2017; Griffiths & Levy 2017; Hollis *et al* 2017; Valenti *et al* 2014; Legido *et al* 2013; Rossignol & Frye 2012).

In their meta-analysis, Rossignol and Frye have demonstrated that classic mitochondrial disease was found in approximately 5% of children with ASD (Rossignol & Frye 2012). This is several times more than in general population, in which the prevalence is estimated to be about 0.02% (Paraskevaidi *et al* 2017). In addition, the prevalence of abnormal metabolic biomarkers is even higher in children with ASD; suggesting that about 30–50% of patients may experience metabolic abnormalities resulting from impaired function of mitochondria. The higher co-existence of mitochondrial abnormalities in patients with ASD than in the general population indicates a possible a role of MD in ASD (Frye *et al* 2013). In comparison to general population of children with ASD, the prevalence of developmental regression and comorbidities, such as seizures, motor disabilities, gastrointestinal problems, or metabolic abnormalities was significantly higher in cohorts with ASD and concomitant MD. Actually, the frequency of many of the above mentioned impairments was similar as in the population of children with MD, suggesting that ASD/MD may be a specific subgroup of children with MD (Rossignol & Frye 2012).

Males are about 4.5 times more commonly affected by ASD than the females (Baxter *et al* 2015). Animal studies resulted in an interesting observation of sex-related differences in respiratory functions and morphology of brain mitochondria with a male predominance. Abnormalities in electron transport chain resulting in increased levels of reactive oxygen species have been reported as significant factor in the pathophysiology of several male biased neurological conditions (Khalifa *et al* 2017).

Epilepsy is a significant comorbidity in ASD. It has been observed, that epileptic seizures occur in many of individuals with mitochondrial disease, suggesting common links between ASD and MD. Also, gastrointestinal dysfunction, which is another frequent comorbid problem in ASD, is also highly prevalent in mitochondrial disease (Cheng *et al* 2017; Rossignol & Frye 2012; Frye *et al* 2015).

The biological basis for mitochondrial dysfunction in ASD is only partially explained. Both genetic and environmental factors seem to play a role. It was found that several genes known to regulate mitochondrial function are at the same time autism-risk genes. Similarly, evidence suggests that environmental exposures that have been implicated in the development of ASD such as toxins, heavy metals, some drugs, immune activation, or metabolic disturbances, may also result in mitochondrial dysfunction (Frye & Rossignol 2011). In a meta-analysis and systemic review of ASD and MD it was found that most ASD/MD cases (79%) were not associated with genetic abnormalities, raising the possibility of secondary mitochondrial dysfunction (Rossignol & Frye 2012).

Severely altered energy production can lead to higher susceptibility of the cell to insults, such as oxidative stress, or inflammation, which can eventually cause malfunction or cell death. There is evidence that both oxidative stress and inflammation are other plausible mechanisms involved in pathophysiology of ASD (Rossignol & Frye 2014; Frye & Rossignol 2011; Griffiths & Levy 2017).

Mitochondria are a major producer of free reactive oxygen species (ROS). At the same time, they are a target for damage mediated by the ROS. Impaired function of the mitochondrial electron transport chain can cause excessive production of ROS and oxidative stress, which then further adversely affects mitochondrial function, and leads to even higher production of ROS and a vicious circle ensues (Legido *et al* 2013). It has been shown that several environmental risk factors of ASD may trigger also oxidative stress, at the same time, they can promote mitochondrial dysfunction, especially in the case if mitochondria are genetically susceptible to it (Griffiths & Levy, 2017; Rossignol & Frye 2014).

There is evidence of immune dysfunction and abnormal immune markers in subjects with ASD (Depino 2013). Scientific data show that mitochondria, are directly involved, or even required for activation of the inflammatory response. At the same time, mitochondria may be the target of inflammatory mediators that activate intracellular signalling pathways, resulting in impairments to mitochondrial metabolism, followed by alterations of mitochondrial function that might ultimately result in cell death. Severely impaired mitochondria may cause additional amplification of the inflammatory process. Interestingly, mitochondrial DNA is such a mitochondria-derived component, which is known to elicit immune response (Gilkerson & Materon 2014).

The interactions between redox homeostasis, inflammation and mitochondrial function are vital to maintaining healthy cell physiology and their abnormalities may play role in disease initiation or progression. There is a need to clarify if one of these metabolic abnormalities is a primary cause of the other abnormalities or whether they constitute separate pathways in ASD (Frye *et al* 2013).

Recently, epigenetic mechanisms exerting changes in gene expression without changing the DNA sequence have been identified as a potential contributor to the pathophysiology of several neurodevelopmental disorders, including ASD (Tordjman *et al* 2014). MicroRNAs (miRNAs) have recently emerged as prominent epigenetic factor (Fregeac *et al* 2016). They are small noncoding RNAs composed of about 20–22 nucleotides transcribed from mitochondrial, but also from nuclear genomes. MiRNAs may localize and act in the cytosol, and also in the mitochondria (Hicks & Middleton 2016), where they regulate mitochondrial genes and functions (Valenti *et al* 2014). The ability of miRNAs to regulate broad molecular pathways in response to environmental stimuli makes them an intriguing player in ASD, being a disorder characterized by genetic predisposition with environmental triggers. A number of miRNAs have been found deregulated in different brain regions or peripheral tissue types derived from ASD patients, and 27 miRNAs, were consistently associated with ASD. It is noteworthy, that studies have revealed that their targets included many genes regulating the immune responses, thus supporting the links between mitochondrial abnormalities and immune dysregulation in ASD (Fregeac *et al* 2016; Hicks & Middleton 2016).

Targeting mitochondrial dysfunction in ASD

At present time, there is no known cure for ASD, and mainly behavioural interventions are used to reduce the symptoms of ASD. Accumulating data about mitochondrial dysfunction in ASD suggest potential mechanisms of disease and indicate a novel candidate target for therapeutic intervention.

Different experimental approaches to treat MD have been studied in ASD patients with the aim to improve the mitochondrial function and the clinical symptoms (Legido *et al* 2013; Griffiths & Levy 2017). They included administration of dietary supplements typically used for the treatment of other mitochondrial disorders. Some of the interventions, including the use of L-carnitine, were shown to improve the core and associated ASD symptoms, similarly, supplementation with N-acetyl-L-cysteine, ascorbic acid, α-tocopherol, methylcobalamin, and carnosine also improved behavioural symptoms in ASD patients. Also, multivitamins combined with minerals improved plasma biomarkers and resulted in positive changes challenging of behaviour and language in individuals with ASD (Delhey *et al* 2017; Griffiths & Levy 2017).

In ASD patients, the existing supplementation studies provide suggestive evidence, however despite a major progress in mitochondrial medicine, generally no effective treatments for mitochondrial disorders are known, and further larger-scale trials are needed to confirm the efficacy of the interventions (Paraskevaidi *et al* 2017; Frye & Rossignol 2014).

The absence of effective treatment in MD is perhaps one of reasons why mitochondrial dysfunction is not routinely diagnosed in patients with ASD, unless a mitochondrial disease is not suspected. It also needs to be added, that testing for MD is very complex, and it includes examinations of blood and urine, different imaging techniques; often a muscle biopsy or metabolic screening of cerebrospinal fluid is needed, as well as sequencing of mitochondrial and nuclear DNA for potential mutations. In addition, diagnosis of MD is more difficult in children than in the adults, since they carry mostly nuclear DNA mutations, where the "classic" symptoms of a mitochondrial disease are not present (Paraskevaidi *et al* 2017). It is needed to develop new biomarkers of mitochondrial dysfunction in order to identify individuals with this impairment and to develop personalized treatment for them (Legido *et al* 2013).

Conclusion

The existing data document impairments of mitochondrial function both in the brain and peripheral tissues of subjects with ASD, suggesting possible links between abnormalities in mitochondrial function and ASD. There is accumulating evidence that the mitochondrial dysfunction might be the factor that connects numerous etiological factors of ASD, as well as the seemingly disparate clinical symptoms associated with ASD, at least in part of the individuals (Frye & Rossignol 2011). Evidence, however, is still patchy, and a complete theory of the key mechanisms of MD in ASD that go from cause to outcome is only limited.

Advances in understanding of the mechanisms of mitochondrial dysfunction in ASD could be a step towards better understanding of the pathophysiology of ASD. Unravelling the role of mitochondria in ASD is expected to open new prospective for the diagnostics and therapeutic interventions. There is also a need to find a valid biomarker of mitochondrial dysfunction in ASD and to develop standardized procedures in order to identify the individuals with concomitant ASD and mitochondrial dysfunction, who might benefit from an intervention.

Acknowledgement

Supported by grants APVV 15-0085, APVV 15-0045, APVV 0254-11.

Disclosure: *The authors report no conflicts of interest in this work.*

REFERENCES

1 American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association). 5th ed. Washington DC: American Psychiatric Publishing.
2 Baxter AJ, Brugha TS, Erskine HE, Scheurer RW, Vos T, Scott JG (2015). The epidemiology and global burden of autism spectrum disorders. *Psychol Med.* **45**: 601–613.
3 Cheng N, Rho JM, Masino SA (2017). Metabolic dysfunction underlying autism spectrum disorder and potential treatment approaches. *Front Mol Neurosci.* **10**: 34
4 Coleman M & Blass JP (1985). Autism and lactic acidosis. *J Autism Dev Disord*. **15**: 1–8.
5 Delhey L, Nur Kilinc E, Yin L, Slattery J, Tippett M, Rose S, *et al* (2017). The effect of mitochondrial supplements on mitochondrial activity in children with autism spectrum disorder. *J Clin Med*. **6**: 18.
6 Depino AM (2013). Peripheral and central inflammation in autism spectrum disorders. *Mol Cell Neurosci*. **53**: 69–76.
7 Ecker C, Bookheimer SY, Murphy, DGM (2015). Neuroimaging in autism spectrum disorder: brain structure and function across the lifespan. *Lancet Neurol*. **14**: 1121–1134.
8 Fregeac J, Colleaux L, Nguyen LS (2016). The emerging roles of MicroRNAs in autism spectrum disorders. *Neurosci Biobehav Rev*. **71**: 729–738.
9 Frye RE & Rossignol DA (2011). Mitochondrial dysfunction can connect the diverse medical symptoms associated with autism spectrum disorders. *Pediatr Res*. **69**: 41R–47R.
10 Frye RE & Rossignol DA (2014). Treatments for biomedical abnormalities associated with autism spectrum disorder. *Front Pediatr*. **2**: 66.
11 Frye RE, DeLaTorre R, Taylor H, Slattery J, Melnyk S, Chowdhury N, James SJ (2013). Redox metabolism abnormalities in autistic children associated with mitochondrial disease. *Transl Psychiatry.* **3**: e273.
12 Frye RE, Rose S, Slattery J, MacFabe DF (2015). Gastrointestinal dysfunction in autism spectrum disorder: the role of the mitochondria and the enteric microbiome. *Microb Ecol Health Dis.* **26**: 10.
13 Gilkerson R & Materon L (2014). Two roads converging: Mitochondria and inflammatory signaling. *J Clin Immunol Immunother.* **125**: 807–815.
14 Griffiths KK & Levy RJ (2017). Evidence of mitochondrial dysfunction in autism: biochemical links, genetic-based associations, and non-energy-related mechanisms. *Oxid Med Cell Longev.* **2017**: 1–12.
15 Hicks SD & Middleton FA (2016). A Comparative review of microRNA expression patterns in autism spectrum disorder. *Front Psychiatry.* **7**: 176.
16 Hollis F, Kanellopoulos AK, Bagni C (2017). Mitochondrial dysfunction in Autism Spectrum Disorder: clinical features and perspectives. *Curr Opin Neurobiol*. **45**: 178–187.
17 Khalifa ARM, Abdel Rahman EA, Mahmoud AM, Ali MH, Noureldin M, Saber SH, *et al* (2017). Sex-specific differences in mitochondria biogenesis, morphology, respiratory function, and ROS homeostasis in young mouse heart and brain. *Physiol Rep*. **5**: e13125.
18 Legido A, Jethva R, Goldenthal MJ (2013). Mitochondrial dysfunction in autism. *Semin Pediatr Neurol*. **20**: 163–175.
19 Lombard J (1998). Autism: a mitochondrial disorder? *Med Hypotheses.* **50**: 497–500.
20 Mannion A & Leader G (2013). Comorbidity in autism spectrum disorder: a literature review. *Res Autism Spectr Disord*. **7**: 1595–1616.
21 Niyazov DM, Kahler SG, Frye RE (2016). Primary mitochondrial disease and secondary mitochondrial dysfunction: importance of distinction for diagnosis and treatment. *Mol Syndromol*. **7**: 122–137.
22 Paraskevaidi M, Martin-Hirsch PL, Kyrgiou M, Marti FL (2017). Underlying role of mitochondrial mutagenesis in the pathogenesis of a disease and current approaches for translational research. *Mutagenesis*. **32**: 335–342.
23 Rossignol DA & Frye RE (2012). Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol Psychiatry.* **17**: 290–314.
24 Rossignol DA & Frye RE (2014). Evidence linking oxidative stress, mitochondrial dysfunction, and inflammation in the brain of individuals with autism. *Front Physiol.* **5**: 150.
25 Tordjman S, Somogyi E, Coulon N, Kermarrec S, Cohen D, Bronsard G, *et al* (2014). Gene – environment interactions in autism spectrum disorders: role of epigenetic mechanisms. *Front. Psychiatry*. **5**: 53.
26 Valenti D, de Bari L, De Filippis B, Henrion-Caude A, Vacca RA (2014). Mitochondrial dysfunction as a central actor in intellectual disability-related diseases: An overview of Down syndrome, autism, Fragile X and Rett syndrome. *Neurosci Biobehav Rev.* **46**: 202–217.