Mitochondrial physiology and autism spectrum disorder

Frye*, D Rossignol

Abstract

Introduction

Recent studies have suggested that mitochondrial dysfunction is relatively common in many children with autism spectrum disorder (ASD) and appears to be the most prevalent metabolic disorder associated with ASD. However, the exact prevalence of mitochondrial disease in ASD is not clear as the classic criteria for diagnosing mitochondrial disease in ASD appear to underestimate the true prevalence. Indeed, the prevalence of biomarkers of mitochondrial dysfunction and abnormal electron transport chain function appears to be high in ASD. In addition, recent studies have uncovered novel forms of mitochondrial dysfunction in ASD that may not be readily recognized by classic diagnostic criteria. This critical review provides a brief overview of mitochondrial function and its influence on other cellular systems in the context of ASD. The mitochondria’s role in producing energy is complex and linked to other metabolic systems. Mitochondrial energy production is a result of the tricarboxylic acid cycle, fatty-acid oxidation and the electron transport chain working in concert. The unique architecture of the mitochondria facilitates the function of these systems. The function and architecture of the mitochondria for producing energy with a special reference to the source of biomarkers of mitochondrial dysfunction is reviewed. Non-energy functions of the mitochondria including calcium buffering, heat production and apoptosis are outlined. Interactions with other systems, including the porphyrin pathway, urea cycle and glutathione metabolism, are also outlined, followed by a discussion of mitochondrial control and regulation. Finally, the recommended algorithm for the diagnosis of mitochondrial disorders in children with ASD is discussed.

Conclusion

The mitochondrial is critically important in understanding the physiological abnormalities associated with ASD. By providing details regarding mitochondrial function, this critical review aims to provide a better understanding of the importance of mitochondria as it is related to ASD.

Discussion

Mitochondrial medicine

Mitochondrial medicine is a relatively new and evolving field. Although the clinical and histological features of mitochondrial disease (MD) were recognized in the 1960s, it was not until 1988 that specific MD could be linked to a causative genetic mutation. From that point on, MDs have primarily been described in reference to their associated genetic alterations. However, as the wide variation in the phenotypic presentation of MD is being appreciated, it is becoming clear that many cases of MD cannot be simply linked to a single specific causative genetic abnormality.

MD and ASD

Recent studies have suggested that mitochondria are not functioning properly in many children with ASD and in genetic diseases associated with ASD. Of the many metabolic disorders associated with ASD, mitochondrial dysfunction appears to be the most prevalent. Table 2 lists the

Table 1

<table>
<thead>
<tr>
<th>Important aspects of mitochondrial function</th>
<th>Mitochondrial functions</th>
<th>Metabolic system that interact with mitochondria</th>
<th>Mitochondrial control and regulation systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Carbohydrate oxidation</td>
<td>• Porphyrin pathway</td>
<td>• mtDNA</td>
<td></td>
</tr>
<tr>
<td>• FAO</td>
<td>• Urea cycle</td>
<td>• nDNA</td>
<td></td>
</tr>
<tr>
<td>• ATP production</td>
<td>• Glutathione metabolism</td>
<td>• Epigenetics</td>
<td></td>
</tr>
<tr>
<td>• Calcium buffering</td>
<td></td>
<td>• Membrane potential regulation</td>
<td></td>
</tr>
<tr>
<td>• Apoptosis</td>
<td></td>
<td>• Redox regulation</td>
<td></td>
</tr>
<tr>
<td>• Heat production</td>
<td></td>
<td>• Regeneration</td>
<td></td>
</tr>
<tr>
<td>• Inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Competing interests: none declared. Conflict of interests: none declared. All authors contributed to conception, design, execution, interpretation, and approved the final manuscript. All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

forms of mitochondrial dysfunction that have been associated with ASD. The suggestion that abnormalities in carbohydrate metabolism could be involved in ASD was first proposed over 25 years ago when an unusual high rate of lactic acidosis (a biomarker of MD) was noticed in a case series of children with ASD. Since then, several other notable reports have associated MD with ASD. In 2002, the HEADD syndrome, an association of hypotonia, epilepsy, autism and developmental delay, was described in a series of ASD children with respiratory chain disorders. Although this association appears common in children with ASD and MD, the HEADD acronym has never really been commonly applied clinically or in the research literature. More recently, the association of MD and ASD with vaccines gained controversial attention. In one case that received considerable notoriety, a girl with underlying MD underwent rapid regression into an ASD diagnosis following a febrile episode induced by multiple vaccinations given on the same day. This connection between infectious/inflammatory triggers with ASD and MD was further substantiated by a case series of 28 children with ASD and MD. Shoffner et al. found that 71% of the children in this case series who manifested regression into ASD regressed within 2 weeks of an episode of fever greater than 101°F. Interestingly in 33% of these cases, the fever was associated with routine childhood vaccinations. This should not be completely surprising as it is widely believed that infections can result in metabolic decompensation in some children with underlying metabolic disease such as MD, and studies have verified this association in children with an underlying MD. However, the true prevalence of this association is unknown. Many practitioners suggest vaccination as a way to prevent severe illnesses and point to the fact that, for the large majority of individuals with MD, vaccination does not cause neurodegenerative events. The ability to assess this potential relationship is further obscured by the lack of the differentiation between true MD and mitochondrial dysfunction (refer to the next paragraph) in ASD. Since the association between an inflammatory/infectious trigger and neurodevelopmental regression has only been demonstrated in children with MD, the same association may not hold true for mitochondrial dysfunction.

Despite this interesting connection between ASD and classically defined MD, the proportion of children with ASD and classically defined MD appears to be low. A recent meta-analysis found that 5% of children with ASD met strict criteria for classic MD. One of the major studies that this prevalence estimate was based on was a population-based prospective study conducted in Portugal and the Azores, making the validity of this estimate particularly strong. The classic criteria for diagnosing MD relies heavily on diagnosing genetic abnormalities, but genetic abnormalities are only found in the minority of cases of children with MD and ASD, suggesting that the classic criteria for diagnosing MD may underestimate the number of ASD children with mitochondrial dysfunction. Studies that have examined biomarkers for MD suggest that the percentage of children with ASD who have abnormal mitochondrial function may be as high as 30% to 50%. Furthermore, one study found that 90% of ASD children demonstrated lower than normal electron transport chain (ETC) function in lymphocytes. Interestingly, several studies have pointed to novel forms of mitochondrial dysfunction in ASD, including ETC overactivity rather than deficiencies, abnormalities in supercomplex assembly and unique elevations in acyl-carnitine abnormalities (short- and long- but not medium-chain elevations).

### Table 2 Mitochondrial disorders reported in ASD

<table>
<thead>
<tr>
<th>Functional abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETC deficiency in complex I, II, III, IV and V</td>
</tr>
<tr>
<td>Co-enzyme Q deficiency</td>
</tr>
<tr>
<td>FAO</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mitochondrial DNA abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3243A&gt;G: mitochondrial encephalopathy with lactic acidosis and seizures (MELAS) syndrome</td>
</tr>
<tr>
<td>3397A&gt;G: complex I</td>
</tr>
<tr>
<td>8363G&gt;A, 10406G&gt;A, 4295A&gt;G: tRNA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Novel mitochondrial abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I overactivity</td>
</tr>
<tr>
<td>Complex IV overactivity</td>
</tr>
<tr>
<td>Abnormal supercomplex assembly</td>
</tr>
<tr>
<td>Unique acyl-carnitine abnormalities (short- and long- but not medium-chain elevations)</td>
</tr>
</tbody>
</table>

---

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

of mitochondrial dysfunction that could be secondary to other, possibly amenable, factors. Using a broader criterion for diagnosing MD, such as the Morava et al. criteria (refer to the Conclusion section), could allow for the diagnosis of these novel forms of mitochondrial dysfunction as MD but could also blur the line between an important distinction of different types of MD.

Mitochondrial structure
The double membrane of the mitochondrion creates two separate compartments, each of which has distinct structure and functions.

Outer membrane
The outer membrane encloses the entire mitochondria and is made of a phospholipid bilayer with integral proteins called porins. Porins are large channels that are permeable to molecules of about 5,000 Da, allowing ions, nutrients and other important small molecules to pass through the membrane.

Inner membrane
The inner membrane is a specialized membrane that is relatively impermeable to most molecules. This facilitates the ability of the ETC to create an electrochemical proton gradient across the inner membrane. Many proteins reside in the inner membrane such as the ETC and specialized transport proteins. The inner membrane has a very high protein-to-phospholipid ratio and unique phospholipids, such as cardiolipin, which help make the inner membrane impermeable.

Cristae
The inner membrane is folded into lamellae called the cristae. These folds greatly increase the inner membrane surface area, allowing a greater number of ETC complexes to exist in the mitochondria and decreasing the distance between the ETC complexes and important enzymes that reside in the matrix.

Matrix
The matrix contains the majority of the enzymes that are responsible for primary functions of the mitochondria including TCA cycle and fatty-acid oxidation (FAO) enzymes as well as mitochondrial DNA (mtDNA) and the enzymes and machinery necessary to transcribe and translate mtDNA into proteins.

Mitochondrial functions
Although mitochondria are the primarily powerhouse of the cell, they serve several other important functions such as calcium buffering and mediating programmed cell death (also known as apoptosis). The important functions of the mitochondria will be reviewed here.

Energy production
The pathways for energy production are outlined in Figure 1 and are discussed in detail below.

Electron carriers between mitochondrial metabolic reactions
Electrons are derived from the oxidation of carbohydrates and fatty acids...
through the TCA cycle and FAO, respectively. These electrons are transported to the ETC, the final common pathway for producing energy, using two electron carriers, the reduced form of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH$_2$).

Carbohydrate oxidation
One of the main functions of the mitochondria is the oxidation of glucose, the main carbohydrate energy source of the cells. Glucose is initially metabolized by glycolysis to form pyruvate.

Pyruvate
If the mitochondria are functioning adequately, pyruvate crosses the mitochondrial membranes into the matrix where it is metabolized into acetyl-CoA through a complex of three enzymes called the pyruvate dehydrogenase complex. Abnormalities in pyruvate dehydrogenase complex function are known to cause childhood MD$^{21}$ but, to date, have not been reported in ASD. Pyruvate is an important biomarker for diagnosing MD. If the mitochondria are dysfunctional, the TCA cycle will slow or shut down, resulting in a build-up of pyruvate. Two metabolites of pyruvate, namely lactate and alanine, also build up if pyruvate is elevated. These are important biomarkers of MD.

Tricarboxylic acid cycle
The TCA cycle combines the two carbon acetyl-CoA (derived from pyruvate) with the four carbon oxaloacetate (a metabolite in the TCA cycle). Through a series of reactions, a total of two carbons are removed and converted to CO$_2$. The electrons from these reactions are transferred to two electron carriers, NADH and FADH$_2$. The TCA cycle comes full circle to reproduce oxaloacetate, which is used in the next cycle. Dysfunction of the mitochondria can slow down or stop the TCA cycle. In such a case, the metabolites of the TCA cycle can build up in the form of organic acids that are biomarkers of MD. One case series has specifically reported dysfunction in the TCA cycle in ASD$^{18}$.

FAO
Fatty acids are oxidized in the mitochondrial matrix. While short-chain and medium-chain fatty acids can freely diffuse across the mitochondrial membranes, long-chain fatty acids (greater than 10 carbons in length) require the carnitine shuttle. Very long-chain fatty acids (greater than 22 carbons in length) are initially oxidized in peroxisomes rather than the mitochondria until they are shortened to long-chain fatty acids. One case series$^{18}$ and one case report$^{7}$ has specifically reported potential abnormalities in FAO pathways in ASD.

Carnitine shuttle
Three enzymes facilitate the transportation of fatty acids into the matrix. Carnitine palmitoyltransferase I (CPT I), which sits on the outer mitochondrial membrane, transfers an acyl group (the fatty acid) from acyl-CoA to Carnitine, producing an acyl-carnitine molecule. The acyl-carnitine crosses the inner mitochondrial membrane using the transporter carnitine-acylcarnitine translocase. Inside the mitochondrial matrix, the acyl is transferred back to a CoA by CPT II. Total carnitine and specific acyl-carnitines are helpful biomarkers of mitochondrial dysfunction. Abnormalities in total carnitine as well as acyl-carnitines have been reported in ASD$^{18}$.

Beta-oxidation
Fatty acids are oxidized in the matrix by a series of reactions that incrementally decrease the length of the fatty acid by two carbons. Each time a fatty acid is shortened, one acetyl-CoA and one NADH and one FADH$_2$ are produced. This reaction continues until only one acetyl-CoA remains (for even-chain length fatty acids) or until a propionyl-CoA remains (for odd-chain length fatty acids). Propionyl-CoA is further metabolized to succinyl-CoA so it can enter the TCA cycle.

ATP production
Adenosine triphosphate (ATP) is produced by the ETC. The majority of reported cases of MD in ASD involve dysfunction of the ETC.$^2$

ETC
A series of five enzyme complexes oxidize the electron carriers NADH and FADH$_2$ to create ATP. Complexes I, III and IV (collectively known as the supercomplex) pump protons from the matrix into the intermembrane space. mtDNA mutations potentially linked to abnormal supercomplex assembly have been reported in relation to ASD$^{37}$. The electrochemical gradient produced by pumping protons across the membrane results in a proton motive force, which is utilized by complex V (ATP synthase) to produce ATP from adenosine diphosphate. Complex II transfers electrons to the ETC electron carrier coenzyme Q10 (CoQ), which is further utilized by complex III.

Electron carriers of the ETC
Two electron transfer agents are used to transfer electrons between ETC complexes.

Coenzyme Q and the Q cycle
Coenzyme Q10 carries electrons from complex I and II to complex III. A complicated sequence of oxidation and reduction of CoQ, which is called the Q cycle, occurs at complex III. In brief, a reduced CoQ carrying two electrons and two protons is joined by another reduced CoQ derived from the Q cycle to donate four electrons and four protons to complex III. The protons are pumped into the intermembrane space to increase the proton gradient while two electrons reduce cytochrome b and the other two reduce the Rieske iron-sulphur protein. The reduced cytochrome b is

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

then used to reduce CoQ along with two protons derived from the matrix. In this way, the Q cycle assists complex III to indirectly pump protons across the inner mitochondrial membrane. Lower than normal CoQ (ubiquinone) concentrations have been reported in children with ASD as compared to control children.

**Cytochrome C**
Cytochrome C carries electrons from complex III to complex IV. The reduced Rieske iron-sulphur protein in complex III reduces cytochrome C which is oxidized by complex IV and recycled. Cytochrome C also has an important role in apoptosis.

**Calcium buffering**
Calcium is transported in and out of the intermembranous space by the voltage-dependent anion-selective channel VDAC1. The behaviour of this channel is dependent on the mitochondrial membrane potential. Calcium fluxes into the intermembranous space can stimulate ATP production. Regulation of calcium metabolism by the mitochondria is part of the mitochondria-associated endoplasmic reticulum membrane, a structure in which the mitochondria is tethered to the endoplasmic reticulum by a special protein tethering complex. Calcium is critical for neurotransmitter release at synapses, and many different types of calcium metabolism abnormalities have been reported in ASD.

**Apoptosis**
Apoptosis, or programmed cell death, occurs through non-mitochondrial (extrinsic) and/or mitochondrial (intrinsic) pathways. Two molecules are released from the mitochondria that influence cytosolic apoptosis pathways. First, the mitochondrial apoptosis-induced channel (MAC) in the outer mitochondrial membrane allows cytochrome C to leak into the cytosol where it interacts with cellular regulators of apoptosis. Second, an increase in the permeability of the outer mitochondrial membrane allows small mitochondria-derived activator of caspasases to be released from the mitochondria where it modulates apoptosis.

**Heat production**
Mitochondria, primarily in brown adipose tissue, can produce heat, instead of ATP, by allowing the inner membrane proton gradient to leak through uncoupling protein 1 (UCP1).

**Critical systems that interact with the mitochondria**
Many metabolic systems feed their biochemical products into mitochondrial pathways and/or derive their biochemical substrates from mitochondrial pathways; thus, mitochondrial dysfunction can affect non-energy-producing systems.

**Porphyrin pathway**
Porphyris are aromatic heterocyclic macrocycles, which are best known for their ability to hold metals within their ring. The best known porphyrin complex is heme. Heme is widely used in many critical enzymes including cytochromes a, b and c, which are components of ETC complex III and IV. Porphyrin production occurs both inside and outside the mitochondria and requires a special transporter that is ATP dependent (see Figure 2A). Thus, mitochondrial dysfunction can decrease porphyrin production, which can further lead to inadequate ETC function and a further decrease in porphyrin production, resulting in a vicious cycle. This may be an important area of research as abnormalities in porphyrins appear to be associated with ASD.

---

**Figure 2:** Interaction between the mitochondria and other important metabolic systems. (a) The initial and final steps in the porphyrin pathway are located in the mitochondria. One of the products of the porphyrin pathway, heme, is essential for subunits of ETC complexes. (b) Portions of the urea cycle are located in the mitochondria. Notably, function of the ornithine transporter is dependent on the inner mitochondrial membrane potential. (c) Generation of glutathione de novo requires ATP, which is derived from the mitochondria. Mitochondria are dependent on glutathione for redox regulation.
Urea cycle
The urea cycle maintains the concentration of the toxic ammonium ion in a narrow, tolerable range by converting ammonia into urea, which can be excreted by the kidney. The urea cycle resides partially in the mitochondrial matrix (see Figure 2B). Transportation of ornithine, one of the key amino acids in the urea cycle, across the inner mitochondrial membrane is dependent on the proton gradient. A reduction in the inner mitochondrial membrane potential, as occurs with ETC dysfunction, limits the ability of ornithine to be transported across the inner mitochondrial membrane. Increased ammonia is toxic and has been reported in some individuals with MD and also in individuals with ASD\(^2\). Urea cycle disorders have been reported in only a few cases of ASD\(^4\).

Glutathione metabolism
Glutathione is important for maintaining the normal redox state by eliminating reactive oxygen and nitrogen species. As mitochondria are the source of these toxic species, proper mitochondrial function is dependent on glutathione. Glutathione cannot be made in the mitochondria but is rather transported into the mitochondria from the cytosol through a special carrier. The production of glutathione requires ATP. Thus, mitochondrial dysfunction can reduce the ability of the cell to produce new glutathione, thus leaving the mitochondria vulnerable to reactive species, some of which it produces itself (see Figure 2C). Abnormalities in glutathione metabolism have been frequently reported in children with ASD\(^2\).

Mitochondrial control and regulation
Mitochondrial DNA
Mitochondria have their own DNA, which provides genetic information to produce several ETC subunits; the remaining ETC subunits are coded by nuclear DNA (nDNA) (Table 3). Many MDs, particularly those that are common in adults, have been linked to abnormalities in genes in the mtDNA. A minority of children with ASD and MD have been reported to have abnormalities in mtDNA\(^2\).

The existence of mtDNA has several consequences. First, a separate system for translation and transcription of mtDNA genes into proteins is needed for the mitochondria. Some components of this separate system are also coded for by mtDNA. Because this system is not as robust as the nuclear system, mtDNA is much more vulnerable to intrinsic and extrinsic factors that cause mutations. Second, since mitochondria are inherited through maternal cell lines (specifically the ovum), MD does not follow a pattern of Mendelian inheritance. In addition, only some of the mitochondria inherited may manifest abnormal mtDNA, leading to heteroplasmy. This may result in only one portion of the body or one organ system being affected by MD.

nDNA and epigenetics
In addition to the genetic information in mtDNA, 1500 or more nu-
clear genes control mitochondrial function. These include genes encoding ETC complexes, membrane transporters, FAO and TCA enzymes as well as genes for mtDNA replication and maintenance, mitochondrial quality control and ETC complex assembly. Mitochondria play a critical role in controlling nuclear genes through epigenetics. Products and metabolites derived from mitochondria, including ATP, acetyl-CoA and reducing equivalents, modulate cytosolic and nuclear pathways that control nuclear gene activity. Because nDNA is involved in some portions of mitochondria function, some forms of MD may exhibit Mendelian inheritance.

**Mitochondrial membrane potential**

The inner mitochondrial membrane potential results from the proton gradient created by the ETC. Large electrochemical gradients can be associated with increased oxidative stress. At least two mechanisms are used to reduce this oxidative stress: calcium fluctuations and uncoupling proteins (UCP1, UCP2, UCP3), which allow protons to leak across the inner mitochondrial membrane without going through complex V.

**Mitochondrial quality control**

Mitochondria can reproduce, regenerate and terminate through the processes of fission, fusion and autophagy. These processes are important for controlling the quality of mitochondrial function. For example, mitochondria with abnormal mtDNA can combine with mitochondria without such abnormalities to reduce the influence of such abnormalities.

**Diagnosing and treating MD**

The workup for MD can be quite complex. One recent review has outlined an evaluation algorithm for children with ASD (Figure 3). The findings from this workup can be used in association with the Morava et al. criterion. The Morava et al. criterion is based on several objective clinical, histological, biochemical, molecular, neuroimaging and enzymatic findings (Table 4). The likelihood of an MD can be determined by the MD criteria (MDC) score. An MDC score predicts MD as: not likely (≤1), possible (2–4 points), probable (5–7 points) or definite (≥8 points). Morava et al. suggest an MDC score ≥3 as criterion for performing a further workup, including a muscle biopsy, in order to confirm a diagnosis of MD.

It is believed that there are no clear cures for MD but with supportive treatment, the usual downhill course associated with MD may be mitigated, and it is possible that recovery from the neurodevelopmental effects of MD may be somewhat

**Table 3** Origin of genes for ETC complex subunits

<table>
<thead>
<tr>
<th>ETC complex</th>
<th>nDNA subunits</th>
<th>mtDNA subunits</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>38</td>
<td>7</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>V</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>

**Figure 3:** Recommended algorithm for the metabolic workup of patients with ASD. First, patients are screened with biomarkers of abnormal mitochondrial function in the fasting state. Abnormalities may need to be verified with repeat testing. For patients with biomarkers that point to an FAO defect, other disorders of fatty-acid metabolism may need to be ruled out before further workup for a mitochondrial disorder is conducted. Patients with consistent biomarkers for mitochondrial dysfunction may initially be investigated for genetic causes before a muscle and/or skin biopsy is considered.

Table 4 Diagnostic worksheet for MD

<table>
<thead>
<tr>
<th>Section I: Clinical signs and symptoms</th>
<th>(a) Muscular presentation (points)</th>
<th>(b) CNS presentation (points)</th>
<th>(c) Multisystem disease (points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Ophthalmoplegia (2)</td>
<td>• Facies myopathica (1)</td>
<td>• Pyramidal signs (1)</td>
<td>• Haematology (1)</td>
</tr>
<tr>
<td>• Exercise intolerance (1)</td>
<td>• Rhabdomyolysis (1)</td>
<td>• Stroke-like episodes (1)</td>
<td>• Gl tract (1)</td>
</tr>
<tr>
<td>• Abnormal EMG (1)</td>
<td>• Muscle weakness (1)</td>
<td>• Migraine (1)</td>
<td>• Endocrine/growth (1)</td>
</tr>
<tr>
<td><strong>Total Points I(a): (maximum score 2 points)</strong></td>
<td><strong>Total Points I(b): (maximum score 2 points)</strong></td>
<td><strong>Total Points I(c): (maximum score 3 points)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>I(a) + I(b) + I(c) Total points Section I: (maximum score 4 points)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Section II: Metabolic/imaging studies (points)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Elevated lactate (2)/alanine (2)</td>
<td>• Elevated CSF lactate (2), protein (1), alanine (2)</td>
<td></td>
</tr>
<tr>
<td>• Elevated lactate/pyruvate ratio (1)</td>
<td>• Stroke-like MRI picture on MRI (1)</td>
<td></td>
</tr>
<tr>
<td>• Urinary tricarbon acid excretion (2)</td>
<td>• Leigh syndrome on MRI (2)</td>
<td></td>
</tr>
<tr>
<td>• Ethylmalonic aciduria (1)</td>
<td>• Elevated lactate on MRS (1)</td>
<td></td>
</tr>
<tr>
<td><strong>Total points Section II: (maximum score 4 points)</strong></td>
<td><strong>Total points Section III: (maximum score 4 points)</strong></td>
<td><strong>Total score (I + II + III): (maximum score 12 points)</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Section III: Morphology from muscle biopsy (points)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Reduced succinic dehydrogenase staining (1)</td>
<td>• Reduced cytochrome oxidase staining (4)</td>
<td></td>
</tr>
<tr>
<td>• Abnormal mitochondria on electron microscopy (2)</td>
<td>• Ragged red/blue fibres (4)</td>
<td></td>
</tr>
<tr>
<td>• Reduced cytochrome oxidase staining (4)</td>
<td>• Cox-negative fibres (4)</td>
<td></td>
</tr>
<tr>
<td>• Ethylmalonic aciduria-positive blood vessels (2)</td>
<td>• Succinic dehydrogenase-positive blood vessels (2)</td>
<td></td>
</tr>
<tr>
<td><strong>Total points Section III: (maximum score 4 points)</strong></td>
<td><strong>Total points Section II: (maximum score 4 points)</strong></td>
<td><strong>Total score (I + II + III): (maximum score 12 points)</strong></td>
</tr>
</tbody>
</table>

Interpretation: 1: Unlikely 2–4: Possible 5–7: Probable 8–12: Definite

This worksheet, which is based on the Morava et al. criterion, can be used to diagnose MD. The points from Section I: Clinical signs and symptoms, Section II: Metabolic/imaging studies and Section III: Morphology from muscle biopsy are added together; the total provides a probability of a diagnosis of MD.

Critical review

In some cases, the diet can be modified to optimize mitochondrial function. These include carnitine, CoQ, B vitamins, vitamin C and vitamin E as well as antioxidants. Third, in some cases, the diet can be modified to optimize mitochondrial function. Some have found that diets such as a ketogenic diet or a modified Atkins diet can be helpful. Last, common secondary effects of MD such as gastrointestinal disturbances, thyroid dysfunction, cerebral folate alleviated. Treatment for MD is focused on four strategies. First, precautions must be implemented to prevent metabolic decompensation and further developmental regression. This involves avoiding environmental triggers (such as environmental toxicants) and medications that are known to be harmful to the mitochondria as well as avoiding illness, dehydration and fever. Second, supplementation with specific vitamins can help support mitochondrial function. These include carnitine, CoQ, B vitamins, vitamin C and vitamin E as well as antioxidants. Third, in some cases, the diet can be modified to optimize mitochondrial function. Some have found that diets such as a ketogenic diet or a modified Atkins diet can be helpful. Last, common secondary effects of MD such as gastrointestinal disturbances, thyroid dysfunction, cerebral folate...
deficiency and seizures should be addressed to optimize health.

Conclusion
Mitochondrial dysfunction and ASD
It appears that mitochondrial dysfunction, rather than classic MD, is more prevalent in children with ASD. Children with mitochondrial dysfunction may have a different developmental trajectory than children with classic MD, especially if the mitochondrial dysfunction is secondary to an amendable factor. For example, the mitochondrion is an adaptable organelle which can modify its function depending on the intracellular and extracellular environment. Specific conditions could be inhibiting the function of the mitochondria in some children with ASD, causing them to be dysfunctional. If this is the case, changing the intracellular and extracellular environment may restore mitochondrial function, at least theoretically. In addition, the mitochondrion is capable of repair and regeneration. In the absence of a genetic defect, it is possible that the function of the mitochondria could be restored by these repair mechanisms, and it might be possible in the future to enhance these repair mechanisms. Although we are just beginning to understand the significance of mitochondrial dysfunction and MD in children with ASD, this new understanding of the molecular mechanisms associated with ASD provides a pathway for discovering new treatments. In fact, several studies have now reported clinical improvements in children with ASD using certain treatments that may target the mitochondria such as certain antioxidants, vitamins and minerals2,11,30.

Abbreviations list
ASD, autism spectrum disorder; ATP, adenosine triphosphate; CoQ, coenzyme Q10; CPT, carnitine palmitoyltransferase; ETC, electron transport chain; FADH2, flavin adenine dinucleotide; FAQ, fatty-acid oxidation; HEADD, hypotonia, epilepsy, autism and developmental delay; MAC, mitochondrial apoptosis-induced channel; MD, mitochondrial disease; MDC, MD criteria; mtDNA, mitochondrial DNA; NADH, nicotinamide adenine dinucleotide; nDNA, nuclear DNA; TCA, tricarboxylic acid; UCP, uncoupling protein

References