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Review Article

Metabolic disorders and abnormalities associated with autism spectrum disorder

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Abstract. Recent research has implicated systematic physiological and metabolic abnormalities, such as immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and other metabolic disorders that transcend organ specific dysfunction in ASD. In this context, ASD may arise from, or at least involve, systemic physiological abnormalities. In this review, common, and not so common, metabolic abnormalities associated with ASD are outlined. Mitochondrial dysfunction and cerebral folate abnormalities are the most prevalent metabolic disorders affecting children with ASD. Other potentially important metabolic disorders that have been reported in ASD include urea cycle disorders, succinic semialdehyde dehydrogenase deficiency, adenylosuccinate lyase deficiency, phenylketonuria, creatine deficiency syndromes, pyridoxine dependent and responsive seizures, biotinidase deficiency and Smith-Lemli-Opitz syndrome. Other metabolic abnormalities that appear to be associated with ASD are disorders of general cholesterol metabolism and tetrahydrobiopterin metabolism. Although detailed cases of children with ASD and these latter two metabolic abnormalities have not been formally described, there is evidence that some children with ASD may manifest these metabolic abnormalities. Lastly, an algorithm for systematically approaching the diagnosis of metabolic disease in ASD is provided.

Keywords: Autism spectrum disorder, mitochondrial disease, cerebral folate deficiency, folate receptor autoantibodies, metabolic disease

1. Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders that are behaviorally defined by documenting impairments in communication and social interaction along with restrictive and repetitive behaviors [1]. An estimated 1 out of 88 individuals in the United States is currently affected with an ASD [2]. The etiology of ASD is unclear at this time. Although several genetic syndromes such as Fragile X and Rett syndromes have been associated with ASD, empirical studies have estimated that genetic syndromes only account for a minority of ASD cases [3]. Therefore, the majority of ASD cases are not due to a simple single gene or chromosomal disorder.

Although many of the cognitive and behavioral features of ASD are thought to arise from dysfunction of the central nervous system (CNS), evidence from many fields of medicine has documented multiple systemic physiological abnormalities are associated with ASD [4–7], suggesting that, in some individuals, ASD arises from systemic, rather than organ specific, abnormalities. In recent decades, research and clinical studies in ASD have implicated physiological and metabolic systems that transcend organ specific dysfunction, such as immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and other metabolic disorders [8,9]. In this context, ASD may arise from, or at least involve, systemic physiological abnormalities

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Table 1
Metabolic diseases associated with autism spectrum disorder

Disorder	Clinical features	Diagnostic testing
Cerebral folate deficiency	Ataxia, pyramidal signs, acquired microcephaly (not always observed), dyskinesias, visual and hearing loss	Folate receptor alpha autoantibody Cerebrospinal fluid 5-methyltetrahydrofolate
Mitochondrial disease	Developmental regression; gross motor delay; fatigability; ataxia; gastrointestinal abnormalities	Fasting serum lactate, pyruvate, acyl-carnitine, amino acids and urine organic acids
Urea cycle disorder	Protein intolerance, temperature instability, ataxia, episodic somnolence and lethargy, cyclic vomiting, psychosis, intractable seizures	Plasma ammonia and amino acids Urinary orotic acid
Succinic semialdehyde dehydrogenase deficiency	Global developmental delay, myoclonus, hallucinations, ataxia, choreoathetosis and dystonia	Urine gamma-hydroxybutyric acid
Adenylosuccinate lyase deficiency	Global developmental delay, microcephaly, distinct facies, growth retardation, mental retardation, cerebellar vermis hypoplasia, brain atrophy, excessive laughter, very happy disposition	Urine and/or cerebrospinal fluid succinyladenosine
Phenylketonuria	Global developmental delay, mental retardation, microcephaly, spasticity, ataxia, poor growth, poor skin pigmentation, aggressive behavior	Serum phenylalanine
Creatine metabolism disorder	Developmental regression, mental retardation, dyskinesia, family history of x-linked mental retardation	Magnetic resonance spectroscopy Urine and serum creatine and guanidinoacetic acid
Pyridoxine dependent and responsive seizures	Intractable seizures, mental retardation, breath-holding, aerophagia, self-injurious behavior	Pyridoxine trial Plasma and cerebrospinal fluid pipecolic acid Urine α-aminoadipic semialdehyde ALDH7A1 sequencing
Biotin deficiency	Seizures, developmental delays, skin rash, seborrheic dermatitis, alopecia, feeding difficulties, vomiting, diarrhea	Urine Organic Acids Ammonia Enzyme Activity
Smith-Lemli-Opitz	Low birth weight, failure to thrive, poor feeding, eczema, seizures, hydrocephalus, frontal lobe hypoplasia, periventricular gray matter heterotopias, congenital abnormalities including micrognathia, bitemporal narrowing, low-set ears, posteriorly rotated ears, anteverted nares, broad, flat nasal bridge, heart defects, pyloric stenosis, genital, renal, hip, thumb, finger and toe abnormalities	Cholesterol 7-dehydrocholesterol Δ-7-dehydrocholesterol reductase sequencing
Low cholesterol	General ASD phenotype	Serum Cholesterol
Tetrahydrobiopterin deficiency	General ASD phenotype	Citrulline-to-methionine ratio Cerebrospinal fluid tetrahydrobiopterin concentration

rather than purely CNS dysfunction, at least in a subset of individuals with ASD [10]. This new view of ASD is important as it provides a pathway for systematically approaching diagnosis and treatments for individuals with ASD.

This review will examine one aspect of systemic abnormalities that affect children with ASD. We will review the common, and not so common, metabolic abnormalities associated with ASD and outline an algorithm for systematically approaching the diagnosis of metabolic disease in ASD. As an overview, we have provided Table 1 which lists the metabolic diseases associated with ASD, characteristics of these particular diseases, if known, and the diagnostic testing that can be utilized. Below we describe the various metabolic disorders beginning with the most prevalent disorders in ASD. We then discuss potential diagnostic algorithms and treatments.

2. Characteristics of specific metabolic disorders

2.1. Cerebral folate deficiency and folate receptor autoantibody

About a decade ago, Ramaekers et al. [11] described five patients who developed normally until about six months of age at which time they developed pro-

gressive neurological symptoms, including irritability, psychomotor retardation, ataxia, dyskinesias, pyramidal signs, visual loss, seizures and acquired microcephaly. The concentration of 5-methyltetrahydrofolate (5MTHF) was found to be normal in the serum and red blood cells but was low in the cerebrospinal fluid (CSF). This new disorder was named cerebral folate deficiency (CFD) to describe the lack of folate specifically in the CNS.

Children with ASD were reported in two of the early case series of children with CFD. In one series, 7 of the 20 children were reported to have ASD [12] while in another series, 5 of the 28 patients were described as having low-functioning ASD with neurological features [13]. Further case reports [14,15] and series [16–18] have expanded the description of CFD in children with ASD.

The major causes of CFD are through two biological mechanisms: autoantibodies to the folate receptor alpha and mitochondrial disease. In 2005, Ramaekers et al. [13] identified high-affinity blocking autoantibodies against the folate receptor alpha (FRα), a receptor that is necessary for the transportation of folate across the blood brain barrier, in the serum of children with CFD. Recently Molloy et al. [19] described a binding FRα autoantibody which has yet to be associated with any pathological disease. In 2006, CFD was associated with Kearns-Sayre syndrome [20], a mitochondrial disease. Further reports expanded this association to other mitochondrial disorders in both children and adults [21], including complex I deficiency [22], Alpers disease [23] and complex IV over-activity [14].

A recent study reported a high prevalence (75%) of the FRα autoantibodies in non-syndromic children with ASD who did not have prominent neurological manifestations [24]. The concentration of blocking FRα autoantibody was significantly correlated with the CSF 5MTHF concentrations which, in each case, were below the mean level found in typically developing (TD) children, but was not below the lower limit of normal for TD children. Children with FRα autoantibodies who were treated with oral leucovorin (folinic acid) in an open-label controlled manner demonstrated significant improvement in ratings of verbal communication, receptive and expressive language, attention and stereotypical behavior. This study suggested that ASD children with borderline low CSF levels of 5MTHF, which are not necessarily below the lower limit of normal, may benefit from treatments that help CFD.

2.2. Mitochondrial disease and dysfunction

A recent meta-analysis by Rossignol and Frye found that 5% of children with ASD met criteria for classic mitochondrial disease (MD) and that children diagnosed with ASD and MD (ASD/MD) have clinical characteristics distinct from the general ASD population, thereby confirming the existence of an ASD/MD subgroup [25]. This meta-analysis also found that approximately 30% of children in the general ASD population exhibited biomarkers consistent with MD even though the prevalence of classic MD is much lower (i.e., 5%) [25]. Since these biomarker studies did not investigate whether or not the children met criteria for classic MD, it is not known whether this high prevalence of abnormal mitochondrial biomarkers translate to a high rate of MD in ASD. However, a recent study has confirmed that abnormal biomarkers of mitochondrial dysfunction can be confirmed approximately half of the time in children with ASD when repeat testing is performed, and that these repeated elevations in biomarkers values are valid markers for mitochondrial disease in ASD [26]. Furthermore, it appears that these biomarkers may identify different subpopulations of children with mitochondrial disorders [26]. Thus, these findings suggested that the prevalence of abnormal biomarker values of MD remains high in the ASD population [26]. Rather than using standard clinical biomarkers to diagnose MD in children with ASD, a recent study compared electron transport chain (ETC) function in lymphocytes between ASD and TD controls to determine if children with ASD manifested mitochondrial 'dysfunction' [27]. This study found that 80% of the children with ASD clearly demonstrated lower than normal mitochondrial function, or in other words, mitochondrial 'dysfunction.'

The ability to define the proportion of children with ASD/MD as part of the ASD population is complicated by the fact that there are multiple criteria used to define MD. The majority of studies that have looked at the prevalence of ASD/MD in the general ASD population have predominantly used one biomarker of mitochondrial dysfunction, lactic acid, and the modified Walker criterion to define MD [28]. However, there are certain significant limitations to this criterion. Specifically, the modified Walker criteria heavily relies on significant reductions in single ETC complex function and known mitochondrial DNA mutations [28]. However, a review of all of the known published ASD/MD cases demonstrates that only 23% of children with ASD/MD have been found to have a known mitochondrial DNA

mutation and several reports have noted that some children with ASD/MD have complex over-activity rather than deficiencies [14,29]. Clearly, such cases would be missed with the modified Walker criteria. Rather than using the modified Walker criterion, Frye and Rossignol [30] suggesting using the Morava et al. criterion [31], another commonly used criterion that considers a wide variety of findings to diagnose MD, including clinical, metabolic, imaging and morphological findings. This criterion allows the clinician to calculate a MD criteria score that can be used to rate MD disease as: not likely, possible, probable, or definite. Notably, for both the Morava et al. and the modified Walker criteria, an invasive muscle biopsy procedure is required in order to obtain tissue for scoring some of the data to be considered as part of the criterion. Unlike the modified Walker criterion, the Morava et al. criterion [31] provides a guideline for the number of findings required to perform a further workup, including a muscle biopsy, in order to confirm the diagnosis of MD.

2.3. *Urea cycle disorders*

Two cases of children with urea cycle disorders have been reported to have ASD. A 4-year-old girl with ASD and multifocal epileptic discharges on EEG was found to have ornithine transcarbamylase deficiency and arginase deficiency [32]. A boy with language regression at 3 years of age with a normal MRI and EEG was diagnosed with ASD and tic disorder at 4 $\frac{1}{2}$ years of age. Carbamyl phosphate synthetase deficiency was diagnosed in this child and language and social skills substantially improved after 6 months of treatment [33].

The symptoms of urea cycle disorders can begin at almost any age depending on the type and severity of the specific disorder. Since the urea cycle's primary function is to dispose of nitrogen waste, abnormalities become apparent when proteins are broken down and processed such as after a high-protein meal or during times of illness or physiological stress. This breakdown of protein leads to rises in blood ammonia levels which is the signature sign of urea cycle disorders.

Symptoms can range from decreased appetite to cyclical vomiting to lethargy, or, in severe cases, coma. In some cases, patients may self-select low-protein diets to minimize symptoms. Delusions, hallucinations and psychosis may occur and seizures and pyramidal signs can develop over time. Coma accompanied by seizures and cerebral edema can lead to death. Treatment for urea cycle disorders is primarily focused on reducing ammonia through a low protein diet and ammonia binders. Some urea cycle disorders also respond to supplementation of specific amino acids and various vitamin supplements [34].

2.4. *Succinic semialdehyde dehydrogenase* (*SSADH*) *deficiency*

First described in 1981, SSADH deficiency is a rare autosomal recessive disorder of gamma aminobutyric acid (GABA) metabolism that results from a defect in the aldehyde dehydrogenase gene (ALDH5A1) located at 6p22 [35]. SSADH is partially responsible for the degradation of GABA. In the absence of SSADH, GABA is degraded by an alternative pathway that uses gamma-hydroxybutyric (GHB) dehydrogenase to form GHB acid. Elevated GHB results in the neurological manifestation of this disorder. Positron emission tomography studies suggest that elevated GABA levels downregulate $GABA_A$ receptors [36].

Age of onset ranges from the neonatal period to early adulthood [37,38], but symptom onset occurs before 12 months of age in the majority of patients [39]. Common presenting symptoms include global developmental delay, hypotonia, hyporeflexia, hallucinations, ASD features, seizures, ataxia, choreoathetosis, dystonia, and myoclonus [39]. Ocular problems, including strabismus, nystagmus, retinitis, disc pallor, and oculomotor apraxia can also occur [40].

2.5. *Adenylosuccinate lyase deficiency*

Adenylosuccinate lyase (ADSL) catalyzes two steps in purine nucleotide metabolism. ADSL deficiency (ADSLD) is a rare autosomal disorder of de novo purine synthesis that results in the accumulation of succinyl purines in body fluids [41,42]. Patients with ADSLD show a variable combination of mental retardation, epilepsy, ASD and cerebellar vermis hypoplasia [41, 42] and show striking variability even within the same family [43]. Two patients with ADSLD have been described with a unique behavioral phenotype including excessive laughter, a very happy disposition, ASD, and stereotyped movements mimicking Angelman syndrome [44].

2.6. *Phenylketonuria*

Phenylketonuria (PKU) is an autosomal recessive inborn error of phenylalanine metabolism resulting from

deficiency of phenylalanine hydroxylase secondary to a mutation in the gene on chromosome 12q23.2. Newborn screening programs identify children with PKU at birth, allowing implementation of specific dietary intervention. With good adherence to diet, children born with PKU can expect to lead relatively normal lives [45]. Children with PKU that goes untreated or who don't adhere to the diet adequately may demonstrate poor growth, poor skin pigmentation, microcephaly, seizures, spasticity, ataxia, aggressive behavior, hyperactivity, ASD features, global developmental delay and/or severe intellectual impairment. For example, Baieli et al. [46] found that no children with classic PKU identified as neonates met criteria for ASD, whereas 6% of those with late diagnosed classic PKU were identified with ASD. The prevalence of seizures and epilepsy is dependent on metabolic control of PKU [47] and those who start treatment later are more likely to have an abnormal EEG, seizures, and mental retardation [48].

2.7. Creatine deficiency syndromes

Creatine and phosphocreatine play important roles in energy storage and transmission of high-energy phosphates. Creatine is primarily synthesized in the liver and is transported from the bloodstream into most non-liver cells against a large concentration gradient by the sodium/chloride dependent transporter known as CrT1. Three inborn disorders of creatine metabolism, collectively known as the creatine deficiency syndromes, have been described since 1994. Two disorders involve deficiencies in two enzymes responsible for creatine production, arginine: glycine amidinotransferase (AGAT) and S-adenosyl-L-methionine: N-guanidinoacetate methyltransferase (GAMT), while the third disorder involves a deficiency in the creatine transporter. The general presentation of children with these disorders includes developmental delay, regression, ASD features, mental retardation, receptive and expressive language disorders, dyskinesia, and seizures [49]. The severity of symptoms depends on the specific disorder. Creatine transporter deficiency is an X-linked recessive disorder, so a family history of X-linked mental retardation is supportive. Individuals with GAMT deficiency are most severely affected, with almost invariable development of ASD and seizures with severe delays in language and abnormalities on MRI. Individuals with creatine transporter deficiency demonstrate an intermediate phenotype, while children with AGAT deficiency demonstrate the mildest phenotype [49].

2.8. Pyridoxine dependent and responsive seizures

Pyridoxine and its primary biologically active form, pyridoxal-5-phosphate (P5P), are cofactors for over 60 enzymes. Pyridoxine dependent seizures (PDS) and pyridoxine responsive seizures (PRS) present as intractable seizures in the first months of life and are defined by their clinical response to pyridoxine therapy [50,51]. The majority of PDS appear to result from a deficiency in the enzyme α-aminoadipic semialdehyde (α-AASA) dehydrogenase associated with mutations in the ALDH7A1 (antiquitin) gene [52,53]. These mutations result in the production of excess Δ^1–piperideine 6-carboxylate, which complexes with and depletes P5P [52]. P5P depletion reduces glutamic acid decarboxylase activity resulting in a reduction in GABA synthesis [52,54,55]. Although early onset intractable tonic-clonic seizures are the usual presentation, late onset seizures [56–58] and other seizure types [59–61] have been described. It has been suggested that PRS may be a clinical entity distinct from PDS [62]. In children with ASD, several studies have reported significant improvement in behavior and cognition attributable to combined therapy with Mg and pyridoxine [63–66], but others have not been able to document such a response [67,68]. There has been one case report of ASD associated with severe mental retardation, aerophagia, breath holding, self-injury and PDS [69]. According to this report, pyridoxine improved seizures but did not improve ASD features.

2.9. Biotinidase deficiency

Biotin, also known as vitamin B7, is an essential cofactor for carboxylase enzymes, particular those involved in fatty acid, isoleucine and valine metabolism as well as gluconeogenesis. Biotin is obtained from the diet and produced by intestinal bacteria. Disorders of biotin metabolism are broadly known as multiple carboxylase deficiency, and include two specific disorders, biotinidase deficiency, a metabolic disorder which affects the ability of biotin to be recycled, and holocarboxylase synthetase deficiency, a metabolic disorder which affects the ability of biotin dependent enzyme to utilize biotin. Individuals with biotin metabolism deficiencies typically demonstrate seizures, developmental delays, skin rash, seborrheic dermatitis, alopecia, feeding difficulties, vomiting, diarrhea, brain atrophy, and ataxia. Metabolic abnormalities include organic aciduria, including elevations in beta-hydroxyisovalerate, lactate,

beta-methylcrotonylglycine, beta-hydroxypropionate, methylcitrate, and mild hyperammonemia. Biotinadase and holocarboxylase synthetase activity can be measured by many commercial laboratories.

One case of partial biotinidase deficiency has been reported in a 4 year old boy with ASD [70]. Treatment with 10 mg of daily biotin did not result in improvement in this case but early treatment of his younger brother who also manifested partial biotinidase deficiency may have prevented the development of ASD as the brother went on to have typical development.

2.10. Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz Syndrome (SLOS) is an autosomal recessive disorder involving mutations of the DHCR7 gene, the gene that encodes for the Δ-7-dehydrocholesterol reductase enzyme. Defective function of this enzyme causes a reduction in cholesterol synthesis and accumulation of its substrate, 7-dehydrocholesterol. SLOS was the first metabolic disorder linked to congenital abnormalities. Such abnormalities include heart defects, pyloric stenosis, genital, renal, hip, thumb, finger and toe abnormalities as well as dysmorphology such as micrognathia, bitemporal narrowing, low-set posteriorly rotated ears, anteverted nares and broad flat nasal bridge. Children with SLOS also have low birth weight, failure to thrive, poor feeding, eczema, seizures and anatomic brain abnormalities including hydrocephalus, frontal lobe hypoplasia and periventricular gray matter heterotopias. Although SLOS is relatively uncommon (1 in 20,000–60,000 births), 50%–75% of children with SLOS meet criteria for ASD [71,72]. Treatment with cholesterol supplementation in children with SLOS has been reported to improve ASD behaviors in some children [73].

2.11. Cholesterol deficiency

Reports have suggested that a substantial subset of children with ASD have abnormally low cholesterol without an elevation in 7-dehydrocholesterol [74]. Although no specific characteristics were reported in this subgroup, the authors suggested that chronic diarrhea, fungal overgrowth and other intestinal disorders could be associated with hypocholesterolemia through bile acid and cholesterol malabsorption. Since cholesterol supplementation in SLOS has resulted in fewer ASD features and improved behavior, it has been suggested that cholesterol supplementation might benefit children with ASD and low cholesterol [73]. Further studies to investigate the characteristics, significance and treatment options of children with ASD and low cholesterol are warranted.

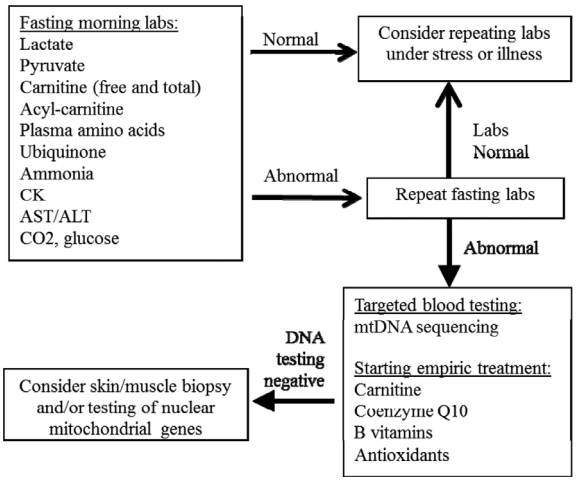

Fig. 1. Algorithms for evaluation of a mitochondrial disorder. Biomarkers of mitochondrial disorders are tested under fasting conditions and if abnormal should be repeated to verify consistent abnormalities. If abnormal biomarkers are verified, standardized treatment can be started while further genetic, enzymatic and pathologic information is pursued.

2.12. Tetrahydrobiopterin deficiency

Tetrahydrobiopterin (BH_4) is a naturally occurring substance that is an essential cofactor for several critical metabolic pathways, including those responsible for the production of monoamine neurotransmitters, the breakdown of phenylalanine, and the production of nitric oxide [75]. BH_4 is also readily oxidized by reactive species [76]. Abnormalities in several of these metabolic pathways or the products of these pathways for which BH_4 is important have been noted in some individuals with ASD and CSF concentration of BH_4 has been reported to be depressed in some individuals with ASD [75–78]. Clinical trials conducted over the past 25 years have shown encouraging results using sapropterin, a synthetic form of BH_4, to treat children with ASD [75]. Three controlled studies and several published open-label studies have documented improvements in communication, cognitive ability, adaptability, social abilities and verbal expression with sapropterin treatment in ASD, especially in children younger than 5 years of age and in those who are relatively higher functioning [75,78–91]. Although no specific ASD cases with BH_4 deficiency have been reported, from the studies reviewed above, it is clear that BH_4 metabolism is important in ASD. Recently Frye has shown that the ratio of serum citrulline to methionine is related to the BH_4 concentration in the CSF and that serum biomarkers of nitric oxide metabolism may predict response to BH_4 supplementation in children

Table 2
Diagnostic worksheet for mitochondrial disorder

Section I: Clinical signs and symptoms			
(a) Muscular presentation (points)			
– Ophthalmoplegia (2)	– Facies myopathica (1)	– Abnormal EMG (1)	
– Exercise intolerance (1)	– Rhabdomyolysis (1)	– Muscle weakness (1)	
Total Points I(a):		(Max Score 2 points)	
(b) CNS presentation (points)			
– Developmental delay (1)	– Pyramidal signs (1)	– Brainstem dysfunction (1)	
– Loss of skills/Regression (1)	– Stroke-like episodes (1)	– Cortical blindness (1)	
– Seizures (1)	– Migraine (1)	– Myoclonus (1)	
– Extrapyramidal signs (1)			
Total Points I(b):		(Max Score 2 points)	
(c) Multisystem disease (points)			
– Hematology (1)	– Recurrent/familial (1)	– Vision (1)	
– GI tract (1)	– Heart (1)	– Kidney (1)	
– Endocrine/growth (1)	– Hearing (1)	– Neuropathy (1)	
Total Points I(c):		(Max Score 3 points)	
I(a) + I(b) + I(c) Total Points Section I:		(Max Score 4 points)	
Section II: Metabolic/imaging studies (points)			
– Elevated lactate (2)/alanine (2)	– Elevated CSF lactate (2), protein (1), alanine (2)		
– Elevated lactate/pyruvate ratio (1)	– Stroke-like MRI picture on MRI (1)		
– Urinary tricarbon acid excretion (2)	– Leigh syndrome on MRI (2)		
– Ethylmalonic aciduria (1)	– Elevated lactate on MRS (1)		
Total Points Section II:		(Max Score 4 points)	
Section III: Morphology from muscle biopsy (points)			
– Reduced succinic dehydrogenase staining (1)	– Ragged red/blue fibers (4)		
– Abnormal mitochondria on electron microscopy (2)	– Cox-negative fibers (4)		
– Reduced cytochrome oxidase staining (4)	– Succinic dehydrogenase positive blood vessels (2)		
Total Points Section III:		(Max Score 4 points)	
Total Score (I + II + III):		(Max Score 12 points)	
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 mitochondrial disease. 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 mitochondrial disease.

with ASD, thereby providing potential biomarkers for identifying and treating patients with BH_4 metabolism abnormalities [76,91]. Further reports are needed to clarify the phenotype of children with BH_4 deficiency and ASD.

3. Diagnostic algorithm

Cerebral folate abnormalities and MD are the two most prevalent metabolic disorders associated with ASD. These two disorders should be pursued as a first step of ruling out metabolic disorders in children with ASD.

Rossignol and Frye suggested that a work-up for MD should be performed as part of the standard evaluation for children with ASD [25]. The workup for MD is outlined in Fig. 1. The findings from this workup can be used in association with the Morava et al. criterion [31] to make an MD diagnosis. The Morava et al. criterion is based on several objective clinical, histological, biochemical, molecular, neuroimaging, and enzymatic findings (Table 2). This method recognizes several types of clinical presentations, including primarily muscular, CNS and multisystem presentations, and utilizes metabolic, imaging and morphological data to diagnose MD. The likelihood of MD can be determined by summing the number of points (some symptoms and findings count as two points) to obtain a MD criteria (MDC) score. The MDC score predicts MD as: not likely ($\leqslant 1$), possible (2–4 points), probable (5–7 points), or definite ($\geqslant 8$ points). Notably, some of the laboratory evidence utilized in this criterion requires an invasive muscle biopsy procedure in order to obtain tissue for histology, electron microscopy and/or functional enzymatic assays. Furthermore, Morava et al. [31] outlined a MDC score of $\geqslant 3$ as criterion for performing a further workup, including a muscle biopsy, in order to confirm a diagnosis of MD.

CFD and FRα autoantibodies should be considered in all children with ASD. It is reasonable to offer empirical treatment to children with ASD who have positive FRα autoantibodies even if a lumbar puncture to measure the 5MTHF CSF concentration is deferred [24]. In addition, a lumbar puncture is indicated in children with classical MD to determine CNS involvement of mitochondrial dysfunction and to rule out CFD because these children may have CFD in the absence of the FRα autoantibody.

If these aforementioned disorders have not been found, further workup for less prevalent disorders can be pursued, especially if the child has symptoms consistent with one of these disorders. Most of these disorders have only been reported to be associated with ASD in case reports, but it is very possible that many cases are missed since investigation of these disorders are rarely performed in children with ASD.

Many of the symptoms of urea cycle disorders are caused by ammonia elevation, so plasma ammonia is the screening test of choice and is most sensitive during symptomatic episodes and after a high-protein meal. In fact, if a urea cycle disorder is suspected, serum ammonia and amino acids as well as urinary orotic acid should be collected one to two hours after high-protein meal. Some suggest also obtaining these laboratories prior to the meal to provide baseline values for postprandial comparisons.

A suspected diagnosis of SSADH is supported by an elevation in 4-hydroxybutyric acid in urine, serum, or CSF, but this highly volatile compound can be difficult to measure on routine organic acid testing. The diagnosis can be confirmed by examining enzyme activity in leukocytes and/or by sequencing the ALDH5A1 gene. Despite early onset of this disorder in most children, diagnosis is often delayed for many years [39].

ADSLD can be diagnosed using the Bratton-Marshall assay to measure succinylamino-imidazole carboxamide riboside and succinyladenosine concentration in the urine or CSF. These metabolites are included in urine of CSF purine analysis from many laboratories.

In most developed countries, PKU is screened for at birth, but in countries where neonatal screening is not standard or the child did not undergo such screening, PKU should be suspected as a possibility. Serum or plasma amino acids will demonstrate elevations in phenylalanine.

The three disorders of creatine metabolism can be diagnosed and differentiated by measuring urinary and serum creatine and guanidinoacetic acid. These tests can be variable and the diagnosis can be unequivocally made by demonstrating an absence of a creatine peak on a magnetic resonance spectroscopy of the brain.

PDS can be diagnosed by measuring pipecolic acid in the plasma or CSF or α-aminoadipic semialdehyde in the urine. The disorder can be confirmed by sequencing the *ALDH7A1 gene*. Instead of undergoing these metabolic and genetic tests, some practitioners attempt a trial of pyridoxine in order to determine if there is a response.

Disorder of biotin metabolism should be especially suspected in ASD children with seizures and skin abnormalities especially when brain atrophy is present. Biotinidase and holocarboxylase synthetase function can be measured by most commercial laboratories.

Preliminary studies suggest that general disorders of cholesterol metabolism may be prominent in children with ASD, so screening for low cholesterol may be reasonable in many children with ASD. Of course, any child identified with low cholesterol should have SLOS ruled-out, particularly if congenital abnormalities are present.

Lastly, there appears to be evidence for disorders of tetrahydrobiopterin metabolism in children with ASD. Although clinical characteristics that can specifically differentiate these ASD children from the general ASD population are unclear at this time, preliminary biomarkers that may be able to identify these children are under development.

4. Conclusions

There are several metabolic disorders that have been reported in children with ASD. Two of these disorders, MD and cerebral folate abnormalities, appear to have a relatively high prevalence in ASD. Other disorders have only been reported as rare cases but since many children with ASD are not routinely evaluated for these disorders, it is possible that many of these disorders are under-diagnosed. We have outlined these disorders as well as a diagnostic algorithm for working up children with ASD. The significance of diagnosing these disorders should not be unstated as treatments which address these underlying disorders have the capability of reducing symptoms and improving function in children with ASD.

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