

# Identification and Treatment of Pathophysiological Comorbidities of Autism Spectrum Disorder to Achieve Optimal Outcomes

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**ABSTRACT:** Despite the fact that the prevalence of autism spectrum disorder (ASD) continues to rise, no effective medical treatments have become standard of care. In this paper we review some of the pathophysiological abnormalities associated with ASD and their potential associated treatments. Overall, there is evidence for some children with ASD being affected by seizure and epilepsy, neurotransmitter dysfunction, sleep disorders, metabolic abnormalities, including abnormalities in folate, cobalamin, tetrahydrobiopterin, carnitine, redox and mitochondrial metabolism, and immune and gastrointestinal disorders. Although evidence for an association between these pathophysiological abnormalities and ASD exists, the exact relationship to the etiology of ASD and its associated symptoms remains to be further defined in many cases. Despite these limitations, treatments targeting some of these pathophysiological abnormalities have been studied in some cases with high-quality studies, whereas treatments for other pathophysiological abnormalities have not been well studied in many cases. There are some areas of more promising treatments specific for ASD including neurotransmitter abnormalities, particularly imbalances in glutamate and acetylcholine, sleep onset disorder (with behavioral therapy and melatonin), and metabolic abnormalities in folate, cobalamin, tetrahydrobiopterin, carnitine, and redox pathways. There is some evidence for treatments of epilepsy and seizures, mitochondrial and immune disorders, and gastrointestinal abnormalities, particularly imbalances in the enteric microbiome, but further clinical studies are needed in these areas to better define treatments specific to children with ASD. Clearly, there are some promising areas of ASD research that could lead to novel treatments that could become standard of care in the future, but more research is needed to better define subgroups of children with ASD who are affected by specific pathophysiological abnormalities and the optimal treatments for these abnormalities.

**KEYWORDS:** autism spectrum disorders, carnitine, cobalamin, epilepsy, folate, genetic disorders, mitochondrial dysfunction, review

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## Introduction

Autism spectrum disorder (ASD) is behaviorally defined but is associated with significant pathophysiology.<sup>1</sup> ASD is estimated to affect 1 in 45 individuals in the United States<sup>2</sup> with a dramatic increase in prevalence over the past two decades. Although significant debate surrounds the reasons for this increase, the fact remains that approximately 2% of children are affected. Although standard of care behavioral therapy can be helpful, to be optimally effective, behavioral therapy requires full-time engagement of a one-on-one therapist over many years, but outcomes are suboptimal in many cases. Recent reviews have pointed out that controlled studies are lacking on behavioral therapies.<sup>3</sup> Thus, medical therapies that can augment the effects of behavioral therapy are urgently needed.

A majority of ASD research have focused on the genetic causes of ASD.<sup>1</sup> This has been driven by the fact that ASD appears to have a highly heritable component. However, despite the apparent heritability, inherited single gene and

chromosomal defects only account for a minority of ASD cases.<sup>4</sup> Other studies have demonstrated that ASD cases may be associated with mutations that affect neuronal function. However, for the most part, these mutations are rare de novo mutations, suggesting that they are acquired, not inherited, mutations that have probably resulted secondary to errors in DNA maintenance and/or the result of DNA damage due to exposure to extrinsic and/or intrinsic stressors.<sup>5,6</sup> These findings indicate that the etiology of ASD is complex and that the heritability component may not be fully accounted for by genetics.<sup>7,8</sup> For example, one common factor shared with siblings is the prenatal maternal environment, which is increasingly being recognized as important for developmental outcomes.<sup>9</sup>

It is becoming clear that ASD arises from complicated interactions between genetic predisposition and environmental factors,<sup>10,11</sup> and that there are numerous environmental agents that may be involved in the etiology of ASD.<sup>12</sup> In fact, genome-wide searches across large sample sizes have primarily



identified rare de novo mutations in ASD, thereby pointing toward acquired mutations that could be the result of environmental factors.<sup>5,6</sup> Since genetic defects cannot be readily treated, we have concentrated on the pathophysiological abnormalities associated with ASD that may be amendable to medical intervention. Recent studies have associated impairments in basic physiological processes such as redox<sup>13</sup> and mitochondrial<sup>1,14,15</sup> metabolism, essential metabolites such as folate,<sup>16</sup> tetrahydrobiopterin<sup>17–19</sup> and carnitine,<sup>20–23</sup> and immune dysfunction.<sup>1</sup> Interestingly, several of these physiological abnormalities are also observed in genetic syndromes associated with ASD such as Rett syndrome,<sup>24–26</sup> PTEN mutations,<sup>27</sup> Phelan-McDermid syndrome,<sup>28,29</sup> 15q11-q13 duplication syndrome,<sup>30,31</sup> Angelman syndrome,<sup>32</sup> Septo-optic dysplasia,<sup>33</sup> and Down syndrome.<sup>34,35</sup>

Identification of pathophysiological abnormalities associated with ASD in individual patients can lead to a better understanding of the basic biological dysfunction affecting the individual patient and lead toward an individualized treatment plan. Thus, some of the more common pathophysiological abnormalities associated with ASD are reviewed, followed by a discussion of the evidence for specific targeted treatments that might be effective. Through a comprehensive understanding of the biological underpinnings of an individual with ASD, in the future, it may be possible to formulate a detailed, personalized, and precise treatment plan in order to achieve optimal outcomes.

### Pathophysiology of ASD

**Seizures and epilepsy.** Individuals with ASD have a higher prevalence of epilepsy compared to typically developing individuals,<sup>36–40</sup> particularly epilepsy that is refractory to standard treatments.<sup>41,42</sup> Epilepsy is associated with higher rates of intellectual disability,<sup>36,43</sup> more severe ASD symptoms,<sup>44</sup> and higher rates of mortality,<sup>45,46</sup> especially if it continues into adulthood.<sup>39,40</sup> The underlying causes of epilepsy are often related to underlying genetic<sup>47,48</sup> and/or metabolic abnormalities.<sup>14,49,50</sup>

Particularly novel to children with ASD are subclinical electrical discharges (SEDs) that have a prevalence as high as 61%<sup>51</sup> and 100%<sup>52</sup> in studies that have used long-term electroencephalogram monitoring and magnetoencephalography, respectively. Studies have suggested that children with ASD who manifest SEDs have a unique cognitive phenotype.<sup>53–56</sup> Children with SEDs and ASD appear to be similar to children with epilepsy and ASD in several ways. Both are more likely to experience regression and have an abnormal neurological examination and cerebral lesions as compared to children with ASD without epilepsy.<sup>53</sup> However, children with epilepsy and ASD are more likely to have intellectual impairment and higher levels of problems with socialization and hyperactivity as compared to children with ASD and SEDs.<sup>54,57</sup>

**Neurotransmitter disorders.** Multiple neurotransmitter deficiencies have been reported in ASD. These include

monoamine (ie, dopamine, norepinephrine, and serotonin),<sup>17–19</sup> amino acid [ie, glutamate, gamma-aminobutyric acid (GABA)],<sup>58,59</sup> and cholinergic (ie, acetylcholine) neurotransmitters.<sup>58</sup> The etiologies of these neurotransmitter disorders are not always known, but studies using animal models of ASD have pointed to genetic mutations that disrupt neurotransmission.<sup>60,61</sup> Metabolic disturbances, some of which are reviewed below, can also disrupt neurotransmission production. For example, central deficiencies in cofactors important for the production of monoamine neurotransmitters, such as folate<sup>16,62</sup> and tetrahydrobiopterin,<sup>17–19</sup> could disrupt monoamine neurotransmitter production. Abnormalities in redox metabolism found in the brain of children with ASD<sup>63,64</sup> can disrupt glutamate metabolism.<sup>13,65</sup> In addition, specific metabolic disorders associated with ASD can directly disrupt neurotransmitter function. For example, succinic semialdehyde dehydrogenase deficiency directly affects GABA,<sup>49</sup> and mitochondrial disorders can influence both GABA and acetylcholine neurotransmission.<sup>14</sup> One neurotransmitter that is becoming increasingly recognized to have a role in ASD is oxytocin, particularly in its role in social impairments.<sup>66</sup> In addition, autoantibodies, which are discussed below, are sometime found in children with ASD, which can disrupt neurotransmission function.

**Sleep disorders.** Sleep abnormalities associated with ASD are extremely common, and include prolonged sleep onset latency, frequent nighttime awakenings, reduced sleep duration, parasomnias, sleep disordered breathing, and daytime sleepiness.<sup>67–69</sup> Sleep abnormalities have been associated with reduced daytime functioning, including impaired social interaction, communication, behavior, anxiety, and aggression<sup>68–71</sup> as well as poor quality of life for the child and their family.<sup>72</sup>

The concentration and circadian rhythm of melatonin, the endogenous neurohormone best known for regulating sleep onset, is often disrupted in ASD.<sup>68,69</sup> 6-Sulfatoxymelatonin, the major metabolite of melatonin, is reduced in some children with ASD suggesting decreased melatonin production<sup>73,74</sup> and concentrations are inversely correlated with the quantity of deep (non-rapid eye movement) sleep.<sup>75</sup> A recent study has suggested that the *N*-acetylserotonin, the metabolite preceding melatonin in the biosynthesis pathway, is increased in ASD and inversely correlated with melatonin concentrations.<sup>76</sup> These findings are consistent with studies that demonstrate that polymorphisms in acetylserotonin methyltransferase, the enzyme that converts *N*-acetylserotonin to melatonin, are associated with disrupted circadian melatonin rhythms and lower melatonin concentrations in children with ASD and the parent(s) from which the polymorphism was inherited.<sup>77</sup> Thus, data seem to point to metabolic abnormalities in melatonin synthesis underlying some of the sleep problems associated with ASD.

**Metabolic disorders.** Several metabolic disorders have been described in individuals with ASD with many having associated treatments.<sup>13,14,16,49,50,62,78</sup> Below we review some



of the metabolic disorders that appear to affect at least a significant portion of children with ASD.

**Folate metabolism.** Folate is essential for many metabolic processes, including redox metabolism and methylation.<sup>79</sup> ASD has been associated with polymorphisms that can decrease the production of 5-methyltetrahydrofolate and/or impair folate transport across the blood–brain barrier and into neurons. Polymorphisms associated with ASD have been found in genes that code for methylenetetrahydrofolate reductase,<sup>80–89</sup> dihydrofolate reductase,<sup>90</sup> and the reduced folate carrier.<sup>88</sup> Also significant is the impairment in folate transport across the blood–brain barrier due to dysfunction of the folate receptor alpha (FR $\alpha$ ).<sup>16</sup> FR $\alpha$  dysfunction can be caused by autoantibodies and by mitochondrial dysfunction.<sup>91</sup> ASD is associated with a high prevalence of FR $\alpha$  autoantibodies<sup>16,92</sup> and mitochondrial dysfunction.<sup>14,15</sup> Dysfunction of the FR $\alpha$ , when severe, causes cerebral folate deficiency, a disorder that causes epilepsy, neurodevelopmental regression, autism, and neurological abnormalities such as spasticity and movement disorders.<sup>16,92–95</sup> Although the FR $\alpha$  autoantibodies are not believed to cause mitochondrial dysfunction, folate is integral for mitochondrial function.<sup>96</sup> Thus, reduced folate availability in the brain could result in central nervous system (CNS) mitochondrial dysfunction.

**Cobalamin metabolism.** Abnormalities in cobalamin-dependent metabolism have been associated with ASD,<sup>88</sup> although blood cobalamin concentrations have been inconsistently reported to be both decreased and increased in the ASD population.<sup>80</sup> However, a recent study reported lower cobalamin concentrations in postmortem brain samples from individuals with ASD compared to controls, suggesting that a cerebral cobalamin deficit is associated with ASD.<sup>97</sup> Metabolites in the methylation and glutathione pathways that are partially dependent on cobalamin, such as methionine, homocysteine, and cysteine, have been reported to be abnormal in some children with ASD.<sup>80</sup> In addition, studies have pointed to polymorphism in cobalamin-associated genes including genes that code for methionine synthase, a cobalamin-dependent enzyme, and transcobalamin, the cobalamin transporter.<sup>88</sup> In one recent study, methionine synthase activity was significantly lower in brain samples from individuals with autism compared to age-matched controls.<sup>97</sup>

**Tetrahydrobiopterin metabolism.** Tetrahydrobiopterin (BH<sub>4</sub>) is a naturally occurring molecule that is essential for critical metabolic pathways, including those responsible for the production of monoamine neurotransmitters, the breakdown of phenylalanine, and the production of nitric oxide.<sup>18</sup> BH<sub>4</sub> is readily oxidized by reactive species, leading to its destruction when oxidative stress is prominent, such as in ASD.<sup>17</sup> The cerebrospinal fluid concentration of BH<sub>4</sub> has been reported to be depressed in some individuals with ASD.<sup>17,18</sup> Thus, decreased CNS BH<sub>4</sub> concentrations could account for CNS monoamine neurotransmitter and nitric oxide disturbances associated with ASD.

**Carnitine metabolism.** Carnitine deficiency may be common in ASD but those children with ASD and carnitine deficiency have not been well characterized.<sup>22</sup> Recently, a defect in the TMLHE gene, a gene that codes for the first enzyme in the carnitine biosynthesis pathway, was reported in seven children with ASD.<sup>23</sup> This genetic defect was more common in probands from male–male multiplex ASD families but was not more common in ASD children overall, suggesting that this was not a causative mutation but a factor that interacts with other genetic and/or environmental factors. Disorders of fatty acid metabolism, which may involve carnitine metabolism, have also been reported in individuals with ASD.<sup>21,98</sup> Carnitine metabolism is important in the transportation of short-chain fatty acids produced by the microbiome, so a deficiency in carnitine could result from imbalances in the interactions between the host and the enteric microbiome.<sup>21,99</sup> Given that mitochondrial disease (MD) and dysfunction can disrupt carnitine metabolism, carnitine deficiency could also be secondary to mitochondrial dysfunction.<sup>14,100</sup>

**Redox metabolism.** Several lines of evidence support the notion that some children with ASD have abnormal redox metabolism.<sup>13,64,80</sup> Several case–control studies report abnormal glutathione metabolism in plasma,<sup>101–103</sup> peripheral immune cells,<sup>101</sup> lymphoblastoid cell lines,<sup>104,105</sup> mitochondria,<sup>105</sup> and brain tissue<sup>63</sup> derived from children with ASD. Oxidative damage to proteins has been documented in the brain,<sup>63,64</sup> plasma,<sup>102,106</sup> and lymphoblastoid cell lines,<sup>104</sup> and oxidative damage to DNA has been found in the plasma<sup>102</sup> derived from children with ASD. Interestingly, redox abnormalities have been linked to mitochondrial dysfunction in children with ASD in plasma<sup>21,106</sup> and brain<sup>63</sup> samples and lymphoblastoid cell lines.<sup>104,107,108</sup>

**Mitochondrial metabolism.** Mitochondrial dysfunction is one of the most prevalent metabolic disorders in ASD.<sup>109</sup> One of the original descriptions of a MD in ASD associated it with epilepsy in the so-called hypotonia, epilepsy, autism and developmental delay (HEADD) syndrome.<sup>110</sup> Since that time, multiple case series and case reports have described individuals with ASD and MD,<sup>14</sup> and one population-based study estimated the prevalence of classical MD in children with ASD at 7.2%<sup>111</sup> while a meta-analysis found it closer to 5.0%.<sup>14</sup> While only about 5% of children with ASD meet strict criteria for a diagnosis of classical MD, over 30% demonstrate abnormal biomarkers of classical MD,<sup>14</sup> suggesting that criteria designed to diagnose classical MD may be too narrow to diagnose mitochondrial dysfunction in children with ASD.<sup>15</sup>

Some studies suggest that children with ASD may manifest unique biomarkers of mitochondrial dysfunction that are not always considered in the classical definition.<sup>21,112,113</sup> In support of this high prevalence of mitochondrial dysfunction in ASD are two studies that found lower than normal electron transport chain function in immune cells from 80% of the children with ASD examined.<sup>114,115</sup>



Moreover, there appears to be unique types of mitochondrial dysfunction associated with ASD. Several studies have demonstrated markedly greater than normal activity of complex I in muscle<sup>116</sup> and complex IV in muscle,<sup>91,117</sup> skin,<sup>21</sup> and brain<sup>118</sup> in children with ASD, and other studies have shown that lymphoblastoid cell lines derived from children with ASD demonstrated elevated respiratory activity as compared to controls.<sup>104,107,108</sup> This increase in mitochondrial function as measured in these studies may reflect a type of compensation for a yet to be defined mitochondrial problem.

**Cholesterol metabolism.** Reports have suggested that children with ASD may have lower cholesterol levels than non-ASD children,<sup>119</sup> although other studies have not been able to verify this finding,<sup>120</sup> and another study suggested that lower cholesterol levels were isolated to the high-density lipoprotein fraction.<sup>121</sup> The interest in cholesterol in ASD is related to a congenital metabolic disorder known as Smith-Lemli-Opitz syndrome in which 50%–75% of these patients manifest ASD symptoms.<sup>122,123</sup> In this disorder, cholesterol production is blocked at the enzyme  $\Delta$ -7-dehydrocholesterol reductase, resulting in reduced cholesterol and increased 7-dehydrocholesterol blood concentrations. However, even in the study in which children with ASD were found to have decreased cholesterol levels, the ASD children did not have an elevation in 7-dehydrocholesterol.<sup>119</sup>

**Immune disorders.** There are multiple lines of evidence, suggesting that the immune system is involved in ASD. For example, classical animal models of ASD use prenatal exposure to lipopolysaccharide that induces a maternal immune response.<sup>124,125</sup> One impressive study demonstrated that symptoms could be significantly attenuated in a mouse model of Rett syndrome when the mice underwent bone marrow transplant from a wild mouse.<sup>126</sup> Here, we will review some of the human studies that point toward evidence of immune abnormalities in ASD.

**Autoantibodies.** Studies have reported various common biomarkers of autoimmunity associated with ASD. Autoantibodies indicative of autoimmunity have been reported, including antinuclear<sup>127</sup> and antinucleosome<sup>128</sup> antibodies, and C-reactive protein has been shown to be abnormally elevated and correlated with autism severity.<sup>129</sup> However, children with ASD appear to have unique autoantibodies, including autoantibodies to brain elements such as myelin basic protein, serotonin receptors, cerebellar tissue and enzymes such as glutamic acid decarboxylase,<sup>130</sup> and antibodies to mitochondria.<sup>131</sup> Although the clinical significance of some autoantibodies in ASD is not clear, certain autoantibodies have some evidence for being clinically significant, including the FR $\alpha$  autoantibody<sup>16</sup> mentioned above and the brain endothelial autoantibody linked to epilepsy.<sup>132,133</sup> Furthermore, a number of studies have correlated the presence of autoantibodies in ASD individuals with the severity of ASD.<sup>127,134–138</sup>

Recently, the overlap between ASD and Pediatric Autoimmune Neuropsychiatric Disorders Associated with

Streptococcal Infection (PANDAS) and Sydenham chorea has been recognized, leading to the consideration of Sydenham chorea associated and PANDAS-associated autoantibodies in ASD. PANDAS and Sydenham chorea associated autoantibodies can disrupt the function of the CaM kinase II enzyme, a multifunctional enzyme highly concentrated in the brain, which functions to control neurotransmission and neuronal excitability,<sup>139</sup> and is closely associated with regulation of catecholamine<sup>140</sup> and glutamate<sup>141</sup> neurotransmission. Abnormalities in the function of CaM kinase II have been linked to movement disorders and neuropsychiatric disorders in children.<sup>142,143</sup>

Another emerging area of research has identified antibodies to fetal brain in the blood of mothers who have had children with ASD. Specific combinations of these antibodies have high specificity for the development of ASD,<sup>144</sup> and research has shown that injection of these antibodies into pregnant monkeys results in offspring with ASD-like characteristics and abnormal brain growth.<sup>145,146</sup> Children exposed to these antibodies in utero appear to have specific characteristics including a more severe behavioral and cognitive phenotype<sup>147</sup> and brain enlargement.<sup>148</sup>

**Hypogammaglobulinemia.** IgG has been reported as both decreased<sup>149–152</sup> and increased<sup>153–155</sup> in children with ASD compared to controls. The hypogammaglobulinemia reported in some children with ASD does not seem to be due to B-cell dysfunction.<sup>156</sup> One study reported that IgG concentrations in ASD children were inversely correlated with the severity of aberrant behavior.<sup>151</sup> Some studies, using sibling controls, demonstrate higher IgG4 concentrations in ASD children.<sup>154,157</sup> One study examining newborn blood samples in children later diagnosed with ASD found that low IgG was associated with an increased risk of developing ASD.<sup>158</sup>

**Neuroinflammation.** One of the first studies to examine neuroinflammation found microglial and astroglial activation associated with patchy neuronal loss, most prominently in the cerebellum and also in the middle frontal and anterior cingulate gyri, on autopsy.<sup>159</sup> Other studies have reported microglial activation or more microglia in the dorsolateral prefrontal cortex,<sup>160</sup> frontoinsula<sup>161</sup> and multiple brain regions<sup>162</sup> and another study suggest that microglia had a close proximity to neurons in the brain of individuals with ASD.<sup>163</sup>

**Cytokine abnormalities.** There have been many reports of abnormal cytokine concentrations in the blood, brain, and cerebrospinal fluid of individuals with ASD. A recent meta-analysis found that interleukin (IL)-1 $\beta$ , IL-6, IL-8, interferon (INF)- $\lambda$ , and MCP-1 are consistently elevated in children with ASD as compared to healthy controls.<sup>164</sup> Studies have reported increases in IL-8,<sup>159,165</sup> INF- $\lambda$ ,<sup>159,165</sup> monocyte chemoattractant protein (MCP)-1,<sup>159</sup> tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ),<sup>165</sup> IL-6,<sup>165,166</sup> and granulocyte-macrophage colony-stimulating factor<sup>165</sup> in the brain tissue of individuals with ASD. Others have suggested that cytokine abnormalities are related to gastrointestinal (GI) symptoms in children with ASD.<sup>167</sup>





**GI disorders.** A wide range of GI symptoms, which can be debilitating, affect up to 91% of children with ASD.<sup>168,169</sup> For example, a recent report found that children with ASD were more likely to have constipation, diarrhea, or food allergy and/or intolerance as compared to typically developing children and children with developmental delays without ASD.<sup>170</sup> Other studies have linked unique abnormalities in GI function with ASD, including abnormal carbohydrate transportation across enterocytes<sup>171</sup> and nonspecific inflammation.<sup>168,169</sup>

There is a growing body of evidence that the trillions of microbes that inhabit the human digestive tract, known as the enteric microbiome, affect immune and metabolic functions,<sup>172,173</sup> modulate gene expression,<sup>174,175</sup> and play an important role in brain and behavioral development.<sup>176</sup> A landmark mouse study highlighted the potential importance of the microbiome in ASD. In this study, the authors showed that alternation in the enteric microbiome using a probiotic ameliorated ASD-like behaviors.<sup>177</sup>

Several studies suggest that the enteric microbiome is atypical in ASD.<sup>171,178–182</sup> The enteric microbiome of some children with ASD has decreased species diversity and overrepresentation of particular species, including Clostridial species.<sup>183,184</sup> Disruption in the enteric microbiome may be particularly associated with children who present with GI symptoms at the time of, or prior to, the onset of ASD symptoms and in those who have regressive-type ASD.<sup>185,186</sup>

Interestingly, a rodent model of ASD has shown that a short-chain fatty acid metabolite known as propionic acid (PPA), which is produced by bacteria that are overrepresented in the enteric microbiome of children with ASD (eg, *Clostridia*, *Bacteroides*, and *Desulfovibrio* species), can cause ASD-like behaviors in rats.<sup>99,125,126,187,188</sup> Independent studies have reported that 17%–24% of ASD children demonstrated consistent elevations in short and long chain acyl-carnitines<sup>99,189</sup> which is a unique pattern of biochemical abnormalities reported in the PPA rodent model of ASD.<sup>113</sup> One study reported an elevated urinary PPA metabolite in children with ASD compared to controls.<sup>190</sup>

## Treatments for Disorders Associated with Autism

**Seizure treatments.** Research into the particular treatments that best control seizures in children with ASD is limited. Such information is important since some children with ASD tend to be sensitive to the adverse effects of antiepileptic drugs (AEDs). One study used a national survey to determine which traditional and nontraditional treatments might be most effective and best tolerated in individuals with ASD.<sup>191</sup> Overall, it was found that four AEDs, valproic acid, lamotrigine, levetiracetam, and ethosuximide, were rated as the most effective and best tolerated, while several non-AED treatments were rated as effective and well-tolerated, including the ketogenic diet, modified Atkin's diet (MAD) and gluten-free casein-free (GFCCF) diet. A recent systematic review found that valproic acid, lamotrigine, and levetiracetam had the

most evidence for effectiveness and tolerability in ASD, with valproic acid also having good evidence for also improving core ASD symptoms.<sup>50</sup> The review also highlighted the evidence for the ketogenic diet and MAD, especially in AED-resistant epilepsy and pointed out that metabolic and genetic disorders that may underlie epilepsy in ASD have targeted treatments that can be effective if such underlying disorders are identified. In addition, intravenous immunoglobulin (IVIG), which can sometime improve ASD-related symptoms, may also be effective in drug-resistant epilepsy.<sup>192–194</sup>

The other issue that is often raised is whether SEDs should be treated in children with ASD. Several case studies<sup>195,196</sup> and case series<sup>55</sup> have demonstrated that valproic acid improved core ASD behaviors and language in children with ASD who had SED but no seizures. Other studies have examined the effect of AEDs on children with behavioral problems and epilepsy who manifested SEDs. Two double-blind, placebo controlled (DBPC) trials demonstrated improvements in these children with lamotrigine treatment,<sup>197,198</sup> while a small DBPC crossover trial using valproic acid did not show positive results.<sup>199</sup> However, in this latter trial, valproic acid blood concentrations were supratherapeutic, potentially resulting in adverse cognitive effects.

**Neurotransmitter treatments.** Many of the medications used to manipulate dopamine and norepinephrine neurotransmission are used under the umbrella of attention-deficit hyperactivity disorder treatments. A recent systematic review of these treatments for ASD highlights the complicated nature of treatment due to the diverse nature of children with ASD and associated adverse effects.<sup>200</sup> For example, despite studies showing positive effects of methylphenidate for hyperactivity, adverse effects are more prevalent in individuals with ASD resulting in a limitation of the dose that can be used.<sup>111,200,201</sup> Atomoxetine has been found to be helpful with attention-deficit hyperactivity, but not social interaction, symptoms in two DBPC studies<sup>202–205</sup> and open-label extensions.<sup>206,207</sup> Atypical antipsychotics are very effective for behavioral symptoms associated with ASD, particularly irritability, stereotypy, and hyperactivity.<sup>208</sup> However, they have significant detrimental effects on lipid, cholesterol and glucose metabolism, and body weight that develop quickly (within 12 weeks),<sup>209,210</sup> and they increase the risk of developing type 2 diabetes.<sup>211</sup> Although the incidence is low, tardive dyskinesia, a potentially permanent movement disorder, is an additional risk.<sup>212</sup> For several decades, the effectiveness of selective serotonin reuptake inhibitors has been studied, but a recent Cochrane review found no evidence for the efficacy of these medications in ASD.<sup>213</sup>

Given the evidence for an abnormal inhibitory–excitatory balance in the brain of individuals with ASD, several medications which target GABA and glutamate neurotransmission have been investigated, many based on the Fragile X mouse model of ASD.<sup>61,214</sup> Arbaclofen, a GABA agonist, demonstrated promising results in an open-label trial<sup>215</sup>



but failed to show efficacy in a DBPC trial.<sup>61</sup> Recently, the diuretic bumetanide, a chloride-importer antagonist which modulates GABA neurotransmission, has demonstrated promising results in a DBPC study.<sup>216</sup> In a DBPC study, L-carnosine, a modulator of GABA neurotransmission and an antioxidant, has been reported to improve core ASD symptoms and language.<sup>217</sup> Acamprosate, a glutamate modulator, has demonstrated some positive results in small open-label<sup>218</sup> and single-blind, placebo lead-in<sup>219</sup> studies. Positive results in both large open-label and controlled studies have been reported for memantine, an *N*-methyl-D-aspartate receptor antagonist, with reported improvements in language, social function and behavior.<sup>58</sup> Amantadine, also an *N*-methyl-D-aspartate receptor antagonist, has been shown in open-label and DBPC studies to improve speech, hyperactivity, and irritability in some children with ASD.<sup>220–222</sup> *N*-Acetyl-L-cysteine, which provides the precursor to glutathione and reduces brain glutamate, has been shown to be effective in improving irritability and social functioning in ASD in DBPC studies.<sup>65,223,224</sup> Thus, several agents have been investigated to target GABA and glutamate neurotransmission but, to date, only memantine, amantadine, and *N*-acetyl-L-cysteine have undergone multiple clinical trials. Other agents are promising but larger controlled clinical trials would be useful to confirm their efficacy.

In ASD, several medications that modulate acetylcholine have been investigated, including tacrine, donepezil, rivastigmine, and galantamine.<sup>58</sup> Of these, only galantamine has demonstrated consistent positive results in controlled studies with reported improvements in both language and social functioning.

One area of growing interest is treatment with oxytocin nasal spray.<sup>66</sup> Despite some studies showing promising results, other studies have failed to demonstrate an effect.<sup>225</sup> In addition, the mechanism of action and the long-term effects of oxytocin are not known.<sup>66</sup> Currently, a large multicenter trial is investigating the efficacy of oxytocin in ASD.

Lastly, it should be mentioned that neurotransmitter deficiencies that are secondary to metabolic abnormalities, such as tetrahydrobiopterin and/or folate deficiencies, should be corrected by addressing the metabolic disorder.

**Sleep treatments.** Sleep is extremely important in children with ASD, as studies have demonstrated that improving the quality of sleep often translates to improvements in daytime behavior.<sup>226</sup> According to practice pathway guidelines, the treatment for sleep disorders in children with ASD starts with improving sleep hygiene and bedtime routine using behavioral parent training.<sup>227,228</sup> A number of pharmacological treatments are used to improve sleep in children with neurodevelopmental disorders but only melatonin has good empirical evidence for its effectiveness.<sup>229</sup> Melatonin is perhaps the best studied nutritional supplement in the ASD population, having six DBPC studies confirming its efficacy for improving sleep onset and sleep duration,<sup>68,69,230</sup> particularly when combined with cognitive-behavioral therapy.<sup>230</sup>

A recent open-label, dose escalation study demonstrated that 1–3 mg of melatonin was effective for most children within one week of treatment with the effect maintained over months without adverse effect.<sup>226</sup>

Not all sleep problems can be treated with behavioral training and melatonin. Other sleep disturbances such as nocturnal awakening do not appear to be treated effectively with melatonin and a comprehensive sleep history should be obtained from ASD children who have sleep disturbances in order to ensure that other sleep disorders such as sleep-disordered breathing and/or periodic limb movements during sleep are not present. In addition, since there is growing concern that GI problems also disrupt sleep, investigation and treatment for GI-related problems should also be considered.<sup>67</sup> Since seizures can disrupt sleep in children with ASD, further evaluation with an overnight EEG may be warranted.<sup>231</sup> Finally, clonidine has been shown to improve sleep in children with ASD as well as hyperactivity, social interaction, and ASD behaviors in open-label<sup>232–234</sup> and DBPC<sup>235</sup> studies.

**Metabolic disorders.** *Folate metabolism.* Reduced folates are common treatments of children with ASD. However, specific treatments for folate pathway polymorphisms have not been well studied. Research on treatments has concentrated on folate transport into the CNS. Individuals positive for FR $\alpha$  autoantibodies have been treated with 0.5–2 mg/kg/day of folinic acid, while individuals with MD may require up to 4 mg/kg/day of folinic acid. In addition, a milk-free diet has been shown to reduce serum FR $\alpha$  autoantibody titers in a controlled study.<sup>236</sup> Multiple small and large case series have demonstrated that folinic acid treatment in ASD children who possess FR $\alpha$  autoantibodies can result in partial improvements in communication, social interaction, attention, and stereotypical behavior<sup>16,93,94,236,237</sup> to complete recovery of ASD symptoms and other neurological problems such as seizures.<sup>94,95</sup>

*Cobalamin metabolism.* A recent prospective, open-label study demonstrated that glutathione, but not methylation, metabolism could be normalized in children with ASD following a three-month supplementation with methylcobalamin injections and oral folinic acid.<sup>238</sup> A follow-up study reported significant improvements in adaptive behaviors with this therapy, which correlated with improvements in glutathione metabolism.<sup>239</sup> A small 12-week DBPC pilot study of injected methylcobalamin showed that clinical improvements occurred in participants who demonstrated improvements in glutathione metabolism.<sup>240</sup> A recent eight-week DBPC study of 50 children with ASD found that methylcobalamin injections improved the Clinical Global Impression scale with improvements correlating with improvements in methylation metabolism.<sup>241</sup>

*Tetrahydrobiopterin metabolism.* Clinical trials conducted over the past 25 years have reported encouraging results using sapropterin, a synthetic form of BH<sub>4</sub>, to treat children with ASD.<sup>18</sup> Three controlled<sup>242–245</sup> and several open-label trials have documented improvements in communication, cognitive



ability, adaptability, social abilities, and verbal expression with sapropterin treatment in ASD, especially in children younger than five years of age and in those who are relatively higher functioning at the beginning of the trial.<sup>18</sup>

More recently, an open-label study demonstrated that sapropterin treatment improves redox metabolism and fundamentally alters BH<sub>4</sub> metabolism in children with ASD. Interestingly, serum biomarkers of nitric oxide metabolism were associated with a positive response to sapropterin treatment in children with ASD,<sup>19</sup> thereby suggesting that the therapeutic effect of BH<sub>4</sub> supplementation may primarily involve nitric oxide metabolism. BH<sub>4</sub> supplementation could help stabilize nitric oxide synthase and act as an antioxidant and improve monoamine neurotransmitter production. Further DBPC studies incorporating biomarkers of metabolic pathways related to BH<sub>4</sub> metabolism will be needed to determine which children with ASD will most likely benefit from formulations of BH<sub>4</sub> supplementation such as sapropterin.

**Carnitine metabolism.** Several studies have reported improvements in core and associated ASD behaviors with L-carnitine treatment,<sup>20,31,245–248</sup> including two DBPC studies.<sup>249,250</sup> One DBPC study demonstrated that increases in serum carnitine levels correlated with improvements in cognitive and behavioral indexes in children with ASD.<sup>249</sup> Carnitine has been suggested to help children with ASD and abnormal PPA metabolism,<sup>113</sup> and a recent genetic disorder associated with ASD and abnormal carnitine metabolism could be responsive to supplemental carnitine.<sup>23</sup>

**Redox metabolism.** Treatments for oxidative stress have been shown to be beneficial for some children with ASD. Studies have demonstrated that glutathione metabolism can be improved in children with ASD with subcutaneously injected methylcobalamin,<sup>238,239</sup> a vitamin, and mineral supplement that includes antioxidants, coenzyme Q10 and B vitamin supplementation,<sup>251</sup> and tetrahydrobiopterin.<sup>19</sup> *N*-Acetyl-L-cysteine, which can improve glutathione and reduce oxidative stress, has been shown to be effective in reducing irritability in several DBPC studies.<sup>65</sup> These treatments have been reported to improve core ASD symptoms,<sup>223,239</sup> hyperactivity, tantruming and parental impression of general functioning,<sup>251</sup> language,<sup>19</sup> and irritability.<sup>223,224</sup> Although there are treatments that improve ASD symptoms which should also improve oxidative stress, such as the phytochemical sulforaphane,<sup>252</sup> flavonoid luteolin,<sup>253</sup> vitamin C<sup>254</sup> and carnosine,<sup>217</sup> the studies on these treatments did not include measures of oxidative stress to examine the biological mechanism of action. This is important as antioxidants can also treat mitochondrial dysfunction,<sup>14</sup> which is common in ASD. Overall, the data suggest that antioxidants may have a role in improving some ASD symptoms, but the exact treatment that is most effective and the targeted symptoms are not completely clear and require more study.

**Mitochondrial metabolism.** Children with ASD who have been diagnosed with classical MD have been given standard

treatments for MD,<sup>255</sup> including carnitine, coenzyme Q10, B vitamins, antioxidants, and vitamins C and E.<sup>14</sup> However, mitochondrial treatments have not undergone critical efficacy or effectiveness studies in children with ASD and mitochondrial dysfunction. On the other hand, some treatments typically used for MD have been shown to improve core and associated symptoms of ASD in controlled studies. Such treatments include L-carnitine in two DBPC studies,<sup>249,250</sup> multivitamins containing B vitamins, antioxidants, vitamin E, and coenzyme Q10 in two DBPC studies,<sup>251,256</sup> *N*-acetyl-L-cysteine in DBPC trials,<sup>65</sup> and ubiquinol in an open-label trial.<sup>257</sup> Many of these treatments may also address other metabolic problems associated with ASD, so in many studies, it is not clear that such treatments specifically address mitochondrial disorders or other metabolic abnormalities, or both. Thus, although it is important to consider these treatments in children with ASD, further studies are needed to determine the biological targets of these treatments and the best biomarkers to identify children with ASD who might benefit from these treatments.

**Cholesterol metabolism.** In children with Smith-Lemli-Opitz syndrome, supplementation with cholesterol has been shown to reduce autistic behaviors as well as irritability, temper tantrums, hyperactivity, aggressive behaviors, self-injurious behaviors, and trichotillomania.<sup>258</sup> Studies using cholesterol as a treatment in children with ASD who also have low cholesterol are needed to confirm these findings.

**Immune disorders.** IVIG has been shown in several studies to improve symptoms of autism, including improvements in aberrant behavior<sup>259</sup> and speech and social interaction<sup>150</sup> and autistic behavior.<sup>260</sup> One study reported significant improvements in children with ASD receiving IVIG in Aberrant Behavior Checklist (ABC) irritability, hyperactivity, and inappropriate speech and eye contact compared to those receiving placebo injections.<sup>261</sup> Another study demonstrated regression with the discontinuation of IVIG treatment.<sup>259</sup> In contrast, other smaller case series did not find improvements in children with ASD.<sup>262,263</sup> IVIG is a potential treatment for children with ASD who show autoantibodies including those associated with PANDAS, as IVIG has been used successfully to treat PANDAS type symptoms in placebo controlled studies,<sup>264</sup> case reports,<sup>265</sup> and case series.<sup>266</sup> Oral immunoglobulin has also been used for the treatment of GI symptoms. One small open-label study suggested a 50% response rate,<sup>267</sup> but a larger DBPC study did not find any significant effects.<sup>268</sup>

Some modulators of inflammation have also been studied. Celecoxib, a nonsteroidal anti-inflammatory drug, has been shown to improve irritability, social withdrawal, and stereotypic behavior, as measured by the ABC, in a DBPC trial when added to risperidone.<sup>269</sup> Pentoxifylline, a drug that is believed to decrease TNF- $\alpha$ , a cytokine that has been shown to be elevated in some children with ASD in the blood and CNS,<sup>270</sup> has been shown to improve all subscales of the ABC when added to risperidone in a DBPC trial.<sup>271</sup> In prospective<sup>272</sup> and





retrospective<sup>273</sup> open-label trials, steroids used in combination with valproic acid in patients with epileptic aphasia demonstrated a marked improvement in language in a majority of the patients. In a prospective open-label pilot study, low partial pressure hyperbaric oxygen was found to improve C-reactive protein, suggesting it provided improvement in inflammatory processes.<sup>274</sup>

**GI treatments.** Although many standard GI treatments are applied to GI symptoms in children with ASD, many children are refractory to such treatments, leading to the use of many novel GI treatments aimed at eliminating specific foods that the child may be sensitive to, or treatments that address microbiome imbalances.<sup>168,169,275</sup> A consensus statement developed by a multidisciplinary panel suggested that children with ASD deserve the same standard of care GI workup as non-ASD children.<sup>169</sup> This group also pointed out that children with ASD might present with behavioral symptoms rather than more typical GI symptoms as manifestations of GI disease.<sup>168</sup> For example, children with ASD and esophagitis sometimes present with aggressive or self-injurious behaviors.<sup>286</sup> This group also developed guidelines based on expert opinion for the workup and treatment of children with ASD.<sup>168</sup> Overall, they suggested that treatments should be targeted at symptomology and findings from a clinical evaluation. The report concentrated on diarrhea, constipation, and gastroesophageal reflux disease, all very common problems in children with ASD. For the most part, the group recommended standard of care GI therapy based on the differential diagnosis.

Many novel therapies targeting GI processes are commonly provided to children with ASD. Similar to standard treatments for GI symptoms, these treatments have a limited evidence base, although some have been evaluated in clinical trials.

It is common for children with ASD to be treated with various dietary interventions. The more common dietary treatments include the GFCF diet, specific carbohydrate diet, or the MAD. Several studies have examined the effectiveness of the GFCF diet. Although small studies have been inconsistent,<sup>277,278</sup> one larger single-blind study has been more promising.<sup>279</sup> One study suggested that the GFCF diet was most effective in children with GI symptoms, food allergies, or sensitivities and/or those whose caretakers could implement it consistently.<sup>280</sup> Several groups believe that the MAD or ketogenic diet may be helpful in children with ASD,<sup>281</sup> especially those with epilepsy.<sup>50</sup> Studying the efficacy of dietary interventions in children with ASD can be particularly difficult as children with ASD sometimes self-restrict what they eat, and dietary changes need to be coordinated with other caretakers such as school officials. In addition, behavioral and biological changes resulting from dietary changes may require several months or more to observe.

Since some children with ASD have been shown to have deficits in enzymes responsible for carbohydrate digestion,<sup>171</sup> several studies have examined the effect of digestive enzyme

supplementation. While an open-label study that provided a comprehensive enzyme that helped with both carbohydrate and protein digestion at every meal reported promising results,<sup>283</sup> a DBPC crossover study, which used a digestive enzyme, which only helped with protein digestion given only one meal per day did not show any effect, except for a small statistically significant improvement in food variety scores.<sup>283</sup> However, a more recent three-month DBPC of 101 children with ASD reported significant improvements in autistic behaviors, emotional response, and GI symptoms with digestive enzyme treatment compared to placebo.<sup>284</sup>

Still other treatments have focused on altering imbalances in the microbiome.<sup>275</sup> Temporary improvements in ASD symptoms were found in a partially blinded clinical trial of oral vancomycin, an antibiotic that targets *Clostridium* genus and is not absorbed systematically, conducted on children with ASD who had GI symptoms and irritability.<sup>186</sup> One open-label study using probiotics in children with ASD reported improvements in concentration and following directions,<sup>285</sup> and a DBPC crossover study documented improvements in stool consistency and ASD behaviors as well as a reduction in *Clostridium* species using a probiotic containing lactobacillus compared to placebo.<sup>286</sup> A summary of the findings of a working group that discussed this topic was recently published. This working group included clinicians, research scientists, and parents of children with ASD and was an extension of the First International Symposium on the Microbiome in Health and Disease with a Special Focus on Autism ([www.microbiome-autism.com](http://www.microbiome-autism.com)).<sup>275</sup>

## Conclusions

This manuscript reviews pathophysiological abnormalities that have been found to be consistently associated with ASD in multiple studies. Overall, we found there is evidence that ASD is associated with seizure and epilepsy, neurotransmitter disorders, sleep abnormalities, metabolic imbalances including abnormalities in folate, cobalamin, tetrahydrobiopterin, carnitine, redox and mitochondrial metabolism, and immune and GI disorders. Although evidence for an association between these pathophysiological abnormalities and ASD exist, further research is needed to better define the exact relationship between these identified pathophysiological abnormalities and ASD. For example, the relationship between the etiology of ASD and these pathophysiological abnormalities is not clear. It is possible that some of these abnormalities could cause brain dysfunction that could result in core or associated ASD symptoms, while it is also possible that processes that result in brain dysfunction could also cause these associated pathophysiological abnormalities. Since, at this point, these pathophysiological abnormalities are identified and studied after the diagnosis of ASD, the temporal relationship between these abnormalities and the processes that cause ASD is uncertain.





Evidence for the significance of these pathophysiological abnormalities can be derived from determining whether or not treatments that target specific pathophysiological abnormalities are effective at improving core and/or associated ASD symptoms. Studies examining treatments vary in quality with many preliminary studies. Targeted treatments specific for ASD-associated pathophysiological abnormalities that have been studied in high-quality clinical trials including treatments for neurotransmitter abnormalities, particularly imbalances in glutamate and acetylcholine, sleep onset disorder with behavioral therapy and melatonin, and metabolic abnormalities in folate, cobalamin, tetrahydrobiopterin, carnitine, and redox abnormalities. Clinical studies have also examined specific treatments for epilepsy and seizures, mitochondrial and immune disorders, and GI abnormalities, particularly imbalances in the enteric microbiome, but the studies are overall preliminary and require further clinical trials.

Clearly, there are some promising areas of ASD research that could lead to novel treatments, but more research is needed to better study these treatments. One particular challenge when studying children with ASD is the fact that there is significant heterogeneity in this population and that certain pathophysiological abnormalities probably only affect specific subgroups of children with ASD. Thus, first defining these subgroups before applying targeted treatments may be the best approach, but reliable biomarkers have not been developed for many of these pathophysiological abnormalities.

Until further information is available on many of these treatments, it is reasonable to empirically treat children with ASD who are identified with specifically pathophysiological abnormalities if careful follow-up is maintained, and the medical professional is knowledgeable regarding the treatment, particularly for treatments that have highly favorable adverse effect profiles and for patients that are unresponsive to standard behavioral therapy. As more information becomes available, treatment guidelines can be developed. Overall, the development of treatments that target specific pathophysiological disorders has the potential to provide significant improvement in the lives of children with ASD and their families.

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### Author Contributions

Conceived and designed the experiments: REF, DAR. Analyzed the data: REF, DAR. Wrote the first draft of the manuscript: REF. Contributed to the writing of the manuscript: REF, DAR. Agree with manuscript results and conclusions: REF, DAR. Jointly developed the structure and arguments for the paper: REF, DAR. Made critical revisions and approved

final version: REF, DAR. Both authors reviewed and approved of the final manuscript.

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