Gene–Environment Interactions and Epigenetic Pathways in Autism: The Importance of One-Carbon Metabolism

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Abstract

Both genetic and epigenetic factors play important roles in the rate and severity of classic autism and autism spectrum disorders (ASDs). This review focuses on DNA methylation as a key epigenetic mechanism in autism. The critical role that one-carbon (C1) metabolism plays in establishing and maintaining DNA methylation patterns makes it a likely candidate pathway to regulate epigenetic processes in ASDs. This review is the first, to our knowledge, to examine how altering C1 metabolic function through genetic and environmental factors (focusing on diet) may lead to aberrant DNA methylation and increase susceptibility to ASDs. Additionally, the critical time windows for sensitivity to genetic and dietary factors both during the development of cortical networks implicated in ASDs and in regard to potential treatments are discussed. One thing is clear, if C1 metabolism plays a critical role in ASDs, it provides a potential avenue for treatment and perhaps, ultimately, prevention.

Key Words: autism spectrum disorder (ASD); choline; DNA methylation; folate; MTHFR; one-carbon metabolism

Introduction

Autism is a heterogeneous neurological disorder characterized by three core behavior abnormalities—namely, deficits in social interaction, reduced verbal and nonverbal communication, and highly focused stereotyped behaviors that emerge after a period of relatively normal development (American Psychiatric Association 2000). Individuals with a diagnosis of typical or classic autism exhibit symptoms from all three core behavior categories. There is an additional broader category of autism spectrum disorders (ASDs1), including pervasive developmental disorder not otherwise specified, childhood disintegrative disorder, Asperger syndrome, and Rett syndrome (American Psychiatric Association 2000), in which some, but not necessarily all, of these core symptoms are present. ASDs are often accompanied by cognitive and attention deficits and affect predominately males, with an estimated 4:1 ratio between males and females (Yeargin-Allsopp et al. 2003). The prevalence of ASDs is currently 1 in 110 children in the United States, with numbers increasing each year (Centers for Disease Control and Prevention 2009). Although the recent swell in cases of ASDs is due, to a certain extent, both to a greater awareness of the disorder and to more broad clinical definitions (Croen et al. 2002; Smeeht et al. 2004), there are likely additional factors contributing to the swell, including environmental factors. In order to gain a better understanding of the underlying molecular mechanisms of the disorder, a multitude of recent etiologic studies probed the relative importance of genetic and environmental causes in children with ASDs and their families. Despite the differences in severity between classic autism and more mild variants like Asperger syndrome, in general, the risk associated with genetic and environmental factors reviewed herein is similar regardless of clinical diagnosis; therefore, we present data from across the spectrum. In the few instances where environmental factors may be sensitive to clinical diagnosis, specific mention is made.

Early studies suggest a strong genetic component to autism (Bailey et al. 1995); however, mutations in only a relatively small number of genes have been currently identified (State 2010). Known genetic imbalances account for less than 25% of ASD cases (Schaaf and Zoghbi 2011). Growing evidence suggests that these earlier studies may have overestimated the genetic component and underestimated the importance of epigenetic factors (Hallmayer et al. 2011). Epigenetics refers to heritable changes in gene expression that do not alter the DNA sequence (Goldberg et al. 2007). Epigenetic programming occurs through the addition of “marks” on DNA or histones. Depending on the type of mark and its location, gene expression can be up- or downregulated (Nakao 2001), and downstream proteins can be affected in a multitude of fashions. Epigenetic marks

1Abbreviations that appear ≥3x throughout this article: 5-FTHF, 5-formyl-tetrahydrofolate; 5-MTHF, 5-methyl-tetrahydrofolate; 5,10-MTHF, 5,10-methylene-tetrahydrofolate; ASD, autism spectrum disorder; C1, one-carbon; COMT, catechol-O-methyltransferase; CSF, cerebral spinal fluid; DHFR, dihydrofolate reductase; DNMT, DNA methyltransferase; GCPII, glutamate carboxypeptidase II; MeCP2, methyl-CpG-binding protein 2; MBD, methyl-CpG-binding domain protein; MTHFR, methylene tetrahydrofolate reductase; MTRR, methionine synthase reductase; RDA, recommended daily allowance; RFC-1, reduced folate carrier; SAH, S-adenosyl-homocysteine; SAM, S-adenosyl-methionine; SHMT, serine hydroxymethyltransferase; TCN2, transcobalamin II; THF, tetrahydrofolate.
primarily include methylation and acetylation; methylation will be the focus of this review. Although there is some evidence for changes in the methylation patterns on histones in ASDs (Shulha et al. 2011), the majority of current research focuses on abnormal DNA methylation and the dietary requirements and enzymes associated with this process.

DNA methylation involves the addition of methyl groups to cytosine residues in CpG dinucleotides and generally impedes transcription of genes by two mechanisms (reviewed in Vaissiere et al. 2008). First, increased DNA methylation is associated with the tight winding of DNA around histones such that the cellular machinery necessary for gene transcription is blocked from associating with the DNA. Second, methylated CpGs can be bound by methyl-CpG-binding domain proteins (MBDs1) that recruit additional chromatin remodeling proteins, including those that add more epigenetic marks on DNA and histones that cause chromatin to compact further. In contrast, reduced DNA methylation, or hypomethylation, is associated with more loosely wound DNA and increased gene expression. A variety of environmental factors can influence the epigenome, including nutrition, stress, maternal care behavior, or toxins (Faulk and Dolinoy 2011). This review will focus on the mechanisms through which genetic and environmental factors (specifically dietary) can act alone or in combination to alter DNA methylation patterns, and we will review the evidence supporting a critical role for these mechanisms in the etiology of ASDs.

**Critical Periods for Epigenetic Regulation of Gene Expression**

Dynamic changes in DNA methylation patterns occur throughout life to modulate gene expression (Fraga et al. 2005; Yuen et al. 2011). The time in life during which DNA methylation patterns are perturbed may have different impacts on the organism. During early embryonic development, epigenetic programming is essential in regulating processes such as X chromosome inactivation, gene imprinting, and cell differentiation (Feng et al. 2007; Li 2002). DNA methylation patterns are established shortly after egg fertilization and before implantation, at which time the genome undergoes widespread demethylation, except in the case of imprinted genes, to produce pluripotent cells, followed by de novo methylation (Morgan et al. 2005). As development proceeds, subsequent differentiation of cells into tissue subtypes is associated with additional gene-specific changes in methylation patterns (Feng et al. 2007).

In the brain, epigenetic programming appears to be most sensitive to environmental and genetic influences in utero and early in postnatal life (Dominguez-Salas et al. 2011; Faulk and Dolinoy 2011). Methylation reactions are crucial during this time to develop functional neuron networks. Early modifications in DNA methylation that cause cells to deviate from differentiating into their normal lineage can result in significant decreases or expansions of neuron pools that are irreversible (Zeisel 2011). For example, a maternal diet low in choline leads to global DNA hypomethylation and an increase in the expression of genes that turn off cell cycling and promote early cell differentiation during development (Niculescu et al. 2006). Methylation patterns also appear to regulate neuron connectivity. In the cortex, neurons lacking functional methyl-CpG-binding protein 2 (MeCP2), an MBD protein responsible for readout of methylation patterns, have significantly smaller dendritic arbors in both humans (Armstrong et al. 1995) and mice (Kishi and Macklis 2010), which suggests that DNA methylation and the readout of methylation patterns play a role in establishing neuron morphology. Importantly, once adult-like dendritic arbors are stabilized early in postnatal development, large-scale structural plasticity does not appear to be possible (Chen and Nedivi 2010; Romand et al. 2011; Seress and Pokorny 1981). These experiments suggest that epigenetic dysregulation during the period when brain organization develops can lead to a number of neuroanatomical abnormalities, including alterations in brain size and connectivity, that are likely irreversible later in life.

In the adult brain, on the other hand, the effects of altering methylation patterns are more likely reversible. Methylation of DNA and proteins regulates synaptic function in the adult brain. At the synapse, transient changes in DNA methylation patterns regulate gene transcription in response to neuron activity (Lubin et al. 2011; Martinowich et al. 2003), whereas neurotransmitters are directly methylated to alter their availability (Axelrod 1971). DNA methylation also regulates differentiation and elaboration of dendritic arbors in the small populations of neurons that continue to divide (Ma et al. 2009). It appears possible to reverse aberrant neuron function through the use of specifically chosen nutritional, behavioral, or pharmacologic interventions (Nag et al. 2009; Szyf 2009, 2012). However, it remains unclear whether rescuing synaptic signaling in an adult animal is sufficient to rescue behavioral impairments that stem from aberrant neuron connectivity established early in development (Berger-Sweeney 2011). Based on the available evidence, we predict that the most critical periods for epigenetic regulation in ASDs occur prenatally, when cells are proliferating actively and differentiating, and very early postnatally, when methylation patterns are necessary to establish normal neuron networks in the brain.

Numerous alterations in anatomy and connectivity are described in the brains of young children with ASDs that are consistent with altered development during a prenatal and early postnatal critical period. Pathologic hallmarks of ASDs include early overgrowth of specific brain regions (reviewed in Anagnostou and Taylor 2011; Courchesne et al. 2007), such as the prefrontal and temporal cortices necessary for executing high-order social and cognitive processes (Chayer and Freedman 2001), as well as long-distance underconnectivity and short-range overconnectivity (Wass 2011). A number of important processes, which may be regulated through epigenetic mechanisms (Zeisel 2011), occur in the first years of human life to establish neuron networks, including cell death, synaptogenesis, and pruning of inappropriate dendritic arbors and synapses.
studies to date have analyzed gene-specific methylation patterns in ASDs. Global DNA hypomethylation was apparent in autistic children, as well as in the affected child. Following a period of early brain overgrowth, growth stagnates between early and late childhood and may even undergo a period of premature decline following adolescence in individuals with ASDs (Courchesne et al. 2011). These two later phases in the development of autistic brain pathology both may be regulated by different epigenetic abnormalities. This review, therefore, includes a discussion of genetic and environmental interactions in the parents of children with ASDs as well as in the affected child.

The Epigenome in ASDs

A number of recent studies describe changes in the epigenome, referring here to the complex profile of DNA methylation patterns, in ASDs. Global hypomethylation of DNA has been reported in the parents of autistic children (James et al. 2008, 2010) and in children with ASDs (Melnyk et al. 2011; Nguyen et al. 2010). Despite global hypomethylation, an examination of gene-specific DNA methylation patterns in affected children reveals that genes implicated in ASDs were hypermethylated, generally leading to downregulation of gene expression (Vaisiere et al. 2008). For example, methyl-CpG binding protein 2 (MeCP2), an MBD protein responsible for Rett syndrome, was hypermethylated (Nagarajan et al. 2006) as were FMR1 and FMR2, critical gene products in fragile X syndrome, which often presents with clinical symptoms of autism (Grafodatskaya et al. 2010). In addition, the oxytocin receptor, a signaling pathway implicated in social behavior, was hypermethylated in a cohort of children with autism (Gregory et al. 2009). CpG islands of 73 additional genes were also hypermethylated in the autistic twin in studies with discordant monozygotic twins, one of whom was autistic and one of whom was not. These genes include BCL-2 (B-cell lymphoma 2), a regulator of apoptosis, and RORA (retinoid-related orphan receptor-alpha), a nuclear steroid hormone receptor (Nguyen et al. 2010). It is noteworthy that no genes in this study exhibited promoter-specific DNA hypomethylation. Although global DNA hypomethylation was apparent in both affected children and their parents, which suggests that hypomethylation may be inherited (Mirabello et al. 2010), no studies to date have analyzed gene-specific methylation patterns in the parents of children with ASDs.

How might aberrant epigenetic programming occur in ASDs? DNA methylation can be altered due either to changes in the availability of methyl groups or to abnormal function of DNA methyltransferases (DNMTs), which are enzymes required for the addition of methyl groups to DNA. To date, no abnormalities in DNMT expression have been reported in children with ASDs (Voineagu et al. 2011). The presence of global and gene-specific changes in methylation patterns in ASDs have directed a growing number of researchers to examine the primary pathway responsible for methyl group donation—namely, one-carbon (C1) metabolism.

C1 Metabolism and DNA Methylation

C1 metabolism is comprised of three interconnected pathways, each of which mediates critical cellular functions, including de novo nucleotide synthesis for DNA replication and repair (folate cycle), methylation reactions (methionine cycle), and cellular redox balance (transsulfuration pathway) (Figure 1). Collectively, the folate and methionine cycles are referred to as the transmethylation pathway. Not only is methylation status altered in children with ASDs, but there is also evidence for aberrant DNA replication and repair processes (reviewed in Anagnostou and Taylor 2011; Schumann and Nordahl 2011) as well as dysregulation of redox homeostasis (reviewed in Deth et al. 2008; Villagonzalo et al. 2010), which reinforces a critical role for C1 metabolism in the etiology of ASDs.

Methylation reactions occur within the methionine cycle when methyltransferases transfer a methyl group from S-adenosyl-methionine (SAM), the universal methyl group donor, to a methyl group acceptor, including DNA, RNA, proteins, phospholipids, or neurotransmitters (Figure 1) (Chiang et al. 1996). DNMTs specifically catalyze the addition of methyl groups to DNA. During methylation reactions, SAM is converted into S-adenosyl-homocysteine (SAH), a potent inhibitor of further methylation (Crooks et al. 1984). Thus, a lower SAM/SAH ratio is considered an indication of reduced methylation potential. Consistent with global DNA hypomethylation observed in autistic children, the SAM/SAH ratio is also reduced (Adams et al. 2011b; James et al. 2006; Melnyk et al. 2011). Concentrations of SAM and SAH and ultimately DNA methylation can be regulated by a number of genetic and environmental factors that affect the function of the transmethylation pathway. We will review current evidence implicating environment (i.e., dietary factors), genetic, and diet–gene interactions within the C1 metabolic pathway that alter C1 metabolic function and may affect susceptibility to ASDs.

Dietary Factors

A nutrient is a compound acquired from an organism’s environment (or diet) that is necessary for many chemical reactions within the body to maintain life. Several of the essential vitamins and amino acids, which play critical roles in C1
metabolism, are altered both in the mothers and the children affected by ASDs. These nutrition factors fall into two general categories: (1) factors that are substrates for C1 metabolism and (2) vitamin cofactors.

**Substrates**

The folate and methionine cycles are named after the main essential nutrients that act as substrates for the transfer of methyl groups (i.e., naturally occurring folate or synthetic folic acid and their derivatives, as well as methionine). Within the folate cycle, folate is converted into a number of different forms, including dihydrofolate, tetrahydrofolate (THF\(^1\)), 5,10-methylene-THF (5,10-MTHF\(^2\)), and 5-methyl-THF (5-MTHF\(^3\)). An additional folate derivative, 5-formyl-THF (5-FTHF\(^4\)) is used specifically in reactions for DNA synthesis and repair (for an in depth overview of the pathway, see Tibbetts and Appling 2010). Additionally, within the folate cycle, the most abundant contributor of methyl groups to C\(_1\) metabolism is the essential amino acid serine. Within the methionine cycle, either 5-MTHF (from folate cycle) or choline and its oxidized form betaine donate methyl groups for the conversion of homocysteine to methionine; this latter process occurs primarily in the kidneys and liver (McKeever et al. 1991).

![Figure 1](image)

**Figure 1** Folate and methionine cycles in one-carbon (C\(_1\)) metabolism. Shown is a diagram depicting many of the important substrates, vitamin cofactors, and enzymes that participate in the C\(_1\) metabolic pathway. Abnormalities in a number of the metabolites and enzymes within the transmethylation pathway have been reported in children with autism spectrum disorders (ASDs). An overview of the abnormalities reviewed herein are indicated by the color of the letters. Black letters indicate that no significant abnormalities have been noted in that metabolite or enzyme in ASDs. The concentrations of metabolites written in red are frequently low in children with ASDs. The concentrations of metabolites in blue are frequently elevated, whereas the concentrations of metabolites in purple are lower in some studies and elevated in others. For the enzymes, color represents risk for ASDs in individuals carrying polymorphisms in the gene that encodes the enzyme. Specifically, enzymes written in red are associated with increased susceptibility for ASDs. Enzymes in green are protective, whereas risk for ASDs is inconsistent across studies for enzymes in purple. Folate receptor \(\alpha\) (FR\(\alpha\)), reduced folate carrier (RFC-1), and transcobalamin II (TCN2) are transport proteins. Gray arrows point to the vitamin transported into the cell by each protein. Abbreviations: 5-MTHF, 5-methyl tetrahydrofolate; 5,10-MTHF, 5,10-methylene tetrahydrofolate; ASMT, acetylserotonin-O-methyltransferase; COMT, catechol-O-methyltransferase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; DNMTs, DNA methyltransferases; GAMT, guanidinoacetate methyltransferase; MeCP2, methyl-CpG binding protein 2; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; SAH, S-adenosyl-homocysteine; SAM, S-adenosyl-methionine; SHMT-1, serine hydroxylmethyltransferase; THF, tetrahydrofolate.

**Maternal nutrition.** Very few studies examine the relationship between maternal nutritional status and the offspring’s risk for developing ASDs (results summarized in Table 1). A biochemical analysis of the nutritional status was completed in the mothers of affected children in two studies. In the first, low methionine concentrations were not statistically significant (James et al. 2008), whereas in the second, plasma levels of methionine and folate were significantly reduced in mothers of children with ASDs compared with mothers of neurotypical children (James et al. 2010). Although suggestive, blood samples for metabolic analysis in these studies were acquired 3 to 10 years after pregnancy, making it difficult to know the nutritional status during gestation. Two additional studies compared self-reported prenatal vitamin intake during pregnancy in the mother and incidence of autism in the offspring. In one, children were found to be at a higher risk of developing ASDs when their mothers reported not having taken prenatal vitamins during the 3 months prior to and during the first month of pregnancy (Schmidt et al. 2011), which suggests not only the importance of proper nutrition, but also that the time period around conception may be especially important...
### Table 1 Summary of studies reporting levels of nutrients in parents and children with autism spectrum disorders

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Results</th>
<th>Subjects&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Age, years&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Diagnosis</th>
<th>Sex, % male&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sample</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Parental nutrition</strong></td>
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<tr>
<td>B12</td>
<td>↓ 12% (NS)</td>
<td>57, 80</td>
<td>33 ± 6.4, 28 ± 6.6</td>
<td>Autism</td>
<td>Mothers</td>
<td>Plasma</td>
<td>James 2010</td>
</tr>
<tr>
<td>Folate</td>
<td>↓ 11% (&lt;i&gt;p = 0.05&lt;/i&gt;)</td>
<td>57, 80</td>
<td>33 ± 6.4, 28 ± 6.6</td>
<td>Autism</td>
<td>Mothers</td>
<td>Plasma</td>
<td>James 2010</td>
</tr>
<tr>
<td>Methionine</td>
<td>↓ 13% (&lt;i&gt;p &lt; 0.001&lt;/i&gt;)</td>
<td>57, 80</td>
<td>33 ± 6.4, 28 ± 6.6</td>
<td>Autism</td>
<td>Mothers</td>
<td>Plasma</td>
<td>James 2010</td>
</tr>
<tr>
<td>↓ 12% (NS)</td>
<td>46, 200</td>
<td>30 ± 5, 28</td>
<td>Autism</td>
<td>Mothers</td>
<td>Plasma</td>
<td>James 2008</td>
<td></td>
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<tr>
<td>0% (NS)</td>
<td>40, 200</td>
<td>30 ± 5, 28</td>
<td>Autism</td>
<td>Fathers, mothers</td>
<td>Plasma</td>
<td>James 2008</td>
<td></td>
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<tr>
<td><strong>Nutritional status in children with ASDs</strong></td>
<td></td>
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<tr>
<td>B2</td>
<td>↑ 1% (NS)</td>
<td>55, 44</td>
<td>10.0 ± 3.1, 11.0 ± 3.1</td>
<td>ASD</td>
<td>89%, 89%</td>
<td>Blood</td>
<td>Adams 2011</td>
</tr>
<tr>
<td>B6</td>
<td>↑ 54–56% (&lt;i&gt;p &lt; 0.001&lt;/i&gt;)</td>
<td>24, 11, 11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.9 ± 1.4, 7.2 ± 1.4, 7.8 ± 1.2</td>
<td>ASD</td>
<td>89%, 89%</td>
<td>RBC</td>
<td>Adams 2011</td>
</tr>
<tr>
<td>B12</td>
<td>↑ 3% (NS)</td>
<td>40, 40, 54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.3 ± 2.76, 8.3 ± 1.82</td>
<td>Autism</td>
<td>89%, 89%</td>
<td>Plasma</td>
<td>Melnyk 2011</td>
</tr>
<tr>
<td>↓ Suboptimal in 7 of 12</td>
<td>12, 9</td>
<td>8.3 ± 2.76, 8.3 ± 1.82</td>
<td>Autism</td>
<td>75%, 67%</td>
<td>Plasma</td>
<td>Pasca 2010</td>
<td></td>
</tr>
<tr>
<td>↑ 22% (NS)</td>
<td>17, 16</td>
<td>10.0 ± 3.1, 11.0 ± 3.1</td>
<td>ASD</td>
<td>89%, 89%</td>
<td>RBC</td>
<td>Adams 2007</td>
<td></td>
</tr>
<tr>
<td>↑ 3% (NS)</td>
<td>55, 44</td>
<td>10.0 ± 3.1, 11.0 ± 3.1</td>
<td>ASD</td>
<td>89%, 89%</td>
<td>Plasma</td>
<td>Adams 2011</td>
<td></td>
</tr>
<tr>
<td>↓ 1% (NS)</td>
<td>5, 8</td>
<td>9.23 ± 1.82, 10.22 ± 1.05</td>
<td>Asperger’s</td>
<td>100%, 100%</td>
<td>Serum</td>
<td>Pasca 2009</td>
<td></td>
</tr>
<tr>
<td>Total choline</td>
<td>↑ 17% (&lt;i&gt;p &lt; 0.0001&lt;/i&gt;)</td>
<td>55, 44</td>
<td>10.0 ± 3.1, 11.0 ± 3.1</td>
<td>ASD</td>
<td>89%, 89%</td>
<td>RBC</td>
<td>Adams 2011</td>
</tr>
<tr>
<td>Folate</td>
<td>↑ 29% (NS)</td>
<td>15, 13</td>
<td>5.1 ± 0.45, 5.89 ± 0.61</td>
<td>Autism</td>
<td>86.7%, 61.5%</td>
<td>Serum</td>
<td>Pasca 2009</td>
</tr>
<tr>
<td>↓ 8% (NS)</td>
<td>40, 40, 54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.8 ± 2.1, 5.6 ± 2.3, 6.3 ± 2.1</td>
<td>Autism</td>
<td>85%, 45%, 48%</td>
<td>Plasma</td>
<td>Melnyk 2011</td>
<td></td>
</tr>
<tr>
<td>↓ 2% (NS)</td>
<td>25, 25</td>
<td>6.88, 6.76</td>
<td>Autism</td>
<td>72%, 56%</td>
<td>Serum</td>
<td>Ramaekers 2007</td>
<td></td>
</tr>
<tr>
<td>↓ 4% (NS)</td>
<td>17, 16</td>
<td>2–16, not reported</td>
<td>Autism</td>
<td>72%, 56%</td>
<td>Serum</td>
<td>Ramaekers 2007</td>
<td></td>
</tr>
<tr>
<td>↑ 33% (&lt;i&gt;p = 0.085&lt;/i&gt;)</td>
<td>17, 16</td>
<td>2–16, not reported</td>
<td>ASD</td>
<td>89%, 89%</td>
<td>Serum</td>
<td>Adams 2007</td>
<td></td>
</tr>
<tr>
<td>↓ 5% (NS)</td>
<td>55, 44</td>
<td>10.0 ± 3.1, 11.0 ± 3.1</td>
<td>ASD</td>
<td>89%, 89%</td>
<td>Serum</td>
<td>Adams 2011</td>
<td></td>
</tr>
<tr>
<td>↓ 165% (&lt;i&gt;p = 0.06&lt;/i&gt;)</td>
<td>5, 8</td>
<td>9.23 ± 1.82, 10.22 ± 1.05</td>
<td>Asperger’s</td>
<td>100%, 100%</td>
<td>Serum</td>
<td>Pasca 2009</td>
<td></td>
</tr>
<tr>
<td>5-MTHF</td>
<td>↓ 77% (&lt;i&gt;p &lt; 0.001&lt;/i&gt;)</td>
<td>25, 25</td>
<td>6.88, 6.77</td>
<td>Autism</td>
<td>72%, 56%</td>
<td>CSF</td>
<td>Ramaekers 2007</td>
</tr>
<tr>
<td>↓ 20–37% (40–120 normal)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7, 0</td>
<td>2–15</td>
<td>ASD, Rett’s, Angelman’s</td>
<td>29%</td>
<td>CSF</td>
<td>Moretti 2008</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>↓ 16% (&lt;i&gt;p = 0.09&lt;/i&gt;)</td>
<td>15, 13</td>
<td>5.1 ± 0.45, 5.89 ± 0.61</td>
<td>Autism</td>
<td>86.7%, 61.5%</td>
<td>Plasma</td>
<td>Pasca 2009</td>
</tr>
<tr>
<td>↓ 4% (NS)</td>
<td>40, 46</td>
<td>12.3, 11.17</td>
<td>Autism</td>
<td>68%, 59%</td>
<td>Serum</td>
<td>D’Eufemia 1995</td>
<td></td>
</tr>
<tr>
<td>↓ 2% (NS)</td>
<td>55, 44</td>
<td>10.0 ± 3.1, 11.0 ± 3.1</td>
<td>ASD</td>
<td>89%, 89%</td>
<td>Serum</td>
<td>Adams 2011</td>
<td></td>
</tr>
<tr>
<td>↓ 16% (NS)</td>
<td>5, 8</td>
<td>9.23 ± 1.82, 10.22 ± 1.05</td>
<td>Asperger’s</td>
<td>100%, 100%</td>
<td>Plasma</td>
<td>Pasca 2009</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>↓ 26% (&lt;i&gt;p &lt; 0.0001&lt;/i&gt;)</td>
<td>80, 73</td>
<td>7.3 ± 3.2, 10.8 ± 4.1</td>
<td>Autism</td>
<td>89%, not reported</td>
<td>Plasma</td>
<td>James 2006</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

| 25% (*p = 0.01) | 15, 13 | 5.1 ± 0.45, 5.89 ± 0.61 | Autism | 86.7%, 61.5% | Plasma | Pasca 2009 |
| 11% (*p < 0.001) | 40, 40, 54c | 5.8 ± 2.1, 5.6 ± 2.3, 6.3 ± 2.1 | Autism | 85%, 45%, 48% | Plasma | Melnyk 2011 |
| 4% (NS) | 40, 46 | 12.3, 11.17 | ASD | 68%, 59% | Serum | D'Eufemia 1995 |
| 16% (p < 0.02) | 26, 10, 24d | <5 | ASD | Not reported | Plasma | Arnold 2003 |
| 5% (NS) | 55, 44 | 10.0 ± 3.1, 11.0 ± 3.1 | ASD | 89%, 89% | Serum | Adams 2011 |
| 4% (NS) | 31, 11 | 4.17 ± 1.3, 6.9 ± 1.6 | ASD | 87%, 82% | Plasma | Suh 2008 |
| 16% (NS) | 5, 8 | 9.23 ± 1.82, 10.22 ± 1.05 | Asperger’s | 100%, 100% | Plasma | Pasca 2009 |

A diagnosis of autism spectrum disorder (ASD) refers to studies that combined individuals with typical autism, pervasive developmental disorder not otherwise specified, and Asperger syndrome in the analysis. Values in bold are statistically significant or are reported as significant trends. CSF, cerebral spinal fluid; NS, not significant; RBC, red blood cell.

Numbers given as cases, controls.

Subjects include previous study cases, present study cases, present study controls.

Subjects include case, sibling control, control.

Subjects include case, case on restricted diets, controls (diagnosed with developmental delay).

Serine

| 21% (*p = 0.08) | 15, 13 | 5.1 ± 0.45, 5.89 ± 0.61 | Autism | 86.7%, 61.5% | Plasma | Pasca 2009 |
| 3% (NS) | 40, 46 | 12.3, 11.17 | ASD | 68%, 59% | Serum | D'Eufemia 1995 |
| 10% (*p = 0.04) | 55, 44 | 10.0 ± 3.1, 11.0 ± 3.1 | ASD | 89%, 89% | Serum | Adams 2011 |
| 33% (NS) | 5, 8 | 9.23 ± 1.82, 10.22 ± 1.05 | Asperger’s | 100%, 100% | Plasma | Pasca 2009 |

Nutritional status in children with ASDs. Relative to the paucity of data connecting specific dietary factors to the relative importance of individual dietary factors, there are a plethora of studies documenting abnormalities in C1 metabolites in affected children (results summarized in Table 1). Specifcally, reduced methionine concentrations and increased folate, specifically in children with Asperger syndrome (Pasca et al. 2009). In the remaining six studies, the direction of change in folate concentrations was inconsistent; some were higher and others lower, with age, sex, and diagnosis apparently inconsequential. Within cell population, methionine concentration was reduced in blood serum reported increased folate specifically in children with Asperger syndrome (Pasca et al. 2009). Differences in the plasma composition of children with ASDs were more varied. One of seven studies examining folate concentrations in blood serum reported increased folate specifically in children with Asperger syndrome (Pasca et al. 2009). The results for folate and 5-MTHF, a derivative of folate, were more limited. One of seven studies examining folate concentrations in blood serum reported increased folate, specifically in children with Asperger syndrome (Pasca et al. 2009). Changes in the concentration of folate in folate transport across the blood-brain barrier are well known, and have been linked to folate transport abnormalities in patients with ASDs (Pasca et al. 2009). Of note, in these three studies, folate measured in the plasma of children with ASDs was not significantly different from that measured in the plasma of controls. Differences in the plasma folate levels from the same subjects were normal. The results for folate and 5-MTHF, a derivative of folate, were more limited. One of seven studies examining folate concentrations in blood serum reported increased folate, specifically in children with Asperger syndrome (Pasca et al. 2009). Changes in the concentration of folate in folate transport across the blood-brain barrier are well known, and have been linked to folate transport abnormalities in patients with ASDs (Pasca et al. 2009).
(reviewed below) and that tissue levels of folate derivatives rather than plasma folate may be a better marker for C1 metabolic dysfunction in ASDs.

The evidence for abnormalities in three other dietary metabolites—choline, glycine, and serine—is less conclusive. Choline levels were significantly elevated in one report (Adams et al. 2011b); additional studies are required to confirm this finding. Reductions in glycine and serine concentrations were described in a small cohort of autistic children (Pasca et al. 2009). However, larger studies that included children from a broad range of ASD diagnoses failed to support these findings for glycine or, conversely, reported significant increases in serine (Adams et al. 2011b). Currently, it is difficult to know whether abnormalities in glycine or serine concentrations are associated with ASDs because the ages and diagnoses of the children sampled between studies are so varied.

What effect might these abnormal substrate levels have on DNA methylation? Low levels of any of the methyl group donors (i.e., folate derivatives, methionine, and choline) are expected to reduce the capacity for DNA methylation in the affected individual (Niculescu and Zeisel 2002; Niculescu et al. 2006). When the methyl donor deficiencies are present in the pregnant mother, it has been hypothesized that deficiencies will also be present in her offspring. Indeed, in humans, low maternal folate levels are correlated with DNA hypomethylation in the fetus (Chang et al. 2011), which suggests that the nutritional status of the mother is indicative of methylation potential in her unborn child. As expected, global DNA hypomethylation is evident in the mothers of autistic children who also exhibit significantly reduced levels of folate and methionine (James et al. 2010). Although DNA methylation status was not examined in the affected offspring in this study, another cohort of autistic children exhibited significant reductions in methionine and also global DNA hypomethylation (Melnyk et al. 2011). It seems particularly salient that methionine, the C1 metabolite most closely associated with SAM concentrations and methylation potential, was reduced in both the mothers and the offspring with ASDs. In contrast, elevated concentrations of folate or choline, reported in a few studies, are predicted to increase DNA methylation (Cooney et al. 2002; Wolff et al. 1998) and may be associated with the hypermethylation of gene-specific promoters noted in children with ASDs. Currently no evidence from the human literature supports these claims.

**Insight from nutrition studies in rodents.** Studies in rodents provide additional insight into the potential mechanisms by which increased concentrations of folate or choline may alter DNA methylation patterns. Rodents fed excess dietary folic acid display phenotypic behaviors reminiscent of ASDs as well as altered methylation patterns. Juvenile rats fed three times the recommended daily allowance (RDA) of folic acid exhibited both motivation and memory deficits on a spatial water maze task. Behavior impairments are associated with DNA hypermethylation of gene-specific promoters (Sittig et al. 2011). In another study, rats fed two times the RDA of folic acid after weaning displayed global DNA hypomethylation in the colon (Sie et al. 2011). In a rodent model of axon injury, animals were supplemented with a broad range of doses of folic acid to enhance neuron regeneration. Dose-dependent changes in DNA methylation, in which both low and high doses of folic acid impaired axon regeneration and resulted in global DNA hypomethylation, were reported (Iskandar et al. 2010). Together, these three studies suggest that excessive intake of folic acid can not only account for gene-specific hypermethylation but also result in global DNA hypomethylation.

The mechanism by which excess folic acid leads to global DNA hypomethylation is still hypothetical. Excessive folic acid cannot be metabolized by the body and, therefore, leads to an increase in unmetabolized folic acid (Obeid et al. 2011). Unmetabolized folic acid binds at a higher affinity to folate transporters than naturally occurring folate, thus downregulating influx of 5-MTHF into the cell (Henderson et al. 1988). In cases where the ability to metabolize folic acid is compromised, either because of the presence of excess folic acid or genetic abnormalities (Bailey and Ayling 2009; Kalmbach et al. 2008), high concentrations of unmetabolized folic acid may lead to low cellular 5-MTHF, a reduced capacity to convert homocysteine to methionine, and diminished methylation capacity. Further studies will need to be carried out, including analysis of levels of unmetabolized folic acid in the blood of children with ASDs, to assess this possibility.

Elevated choline concentrations in children with ASDs, on the other hand, may compensate for reduced concentrations of other substrates, including folate or methionine. During folate deficiency in rodents, C1 metabolism preferentially uses choline to regenerate methionine from homocysteine and acquires choline either through the diet or by breaking down cell membranes (Troen et al. 2008). Long-term depletion of folate, therefore, can lead to increases in choline concentrations both in blood plasma and in specific brain regions (Chew et al. 2011; Crivello et al. 2010).

**Vitamin Cofactors**

Three essential B vitamins, B2 (riboflavin), B6 (pyridoxine), and B12 (cobalamin), act as coenzymes within the folate cycle to enhance the catalysis of folate among its many derivatives (Selhub 2002). The concentrations of vitamin cofactors in ASDs are summarized in Table 1. Concentrations of vitamin B12, examined in only one cohort of mothers of autistic children, were similar to concentrations in mothers of neurotypical children, when measured 3 to 7 years after pregnancy (James et al. 2010). In children with ASDs, studies have reported normal levels of vitamin B2 (one study), B6 (one study), and B12 (five studies) (Adams et al. 2007, 2011b; Melnyk et al. 2011; Pasca et al. 2009). Suboptimal levels of vitamin B12 were reported in 7 of 12 children with ASDs (Pasca et al. 2006). In a separate study, significantly elevated concentrations of B6 were reported in a cohort of young children with ASDs (average age 4.9 ± 1.4 vs. 10.0 ± 3.1 years
from nonsignificant study) (Adams et al. 2006). The potential relevance of age to B6 concentrations suggests that, similar to that found with methionine, C1 metabolic dysfunction may be more severe earlier in ASDs. Increased levels of B6 in the blood of children with ASDs may be indicative of a failure to transport this vitamin out of the blood and into tissues or, conversely, may represent the body’s attempt to compensate for genetic mutations that result in defective C1 metabolic function (reviewed below). There is little evidence to support a causal role for vitamin B2 and B12 cofactors in the etiology of ASDs.

Altogether, these studies, which examine the concentrations of a number of dietary factors in ASDs, suggest that children exposed to insufficient concentrations of C1 metabolites in utero as well as children exposed to excessive concentrations of C1 metabolites in utero are at a significantly higher risk for developing ASDs and that abnormalities in C1 metabolic function likely persist throughout childhood in individuals with ASDs. These findings underscore a potential role for specific dietary factors, especially methionine deficiency and folate deficiency or excess in ASDs, and hint at a critical window prior to adolescence, coincident with the phase of stagnated brain growth, that is particularly sensitive to C1 metabolic dysfunction. With all C1 metabolites, however, it is difficult to determine whether the abnormalities apparent are the primary cause of ASDs or reflect secondary dysregulation of C1 metabolism.

Genetic Risk Factors

In addition to dietary factors, increased risk of ASDs has also been linked to a number of single nucleotide polymorphisms within genes involved in C1 metabolism. For the purpose of this review, these genes are broadly grouped according to their function within the C1 metabolic pathway. A summary of the studies that have reported risk for these polymorphisms in ASDs is provided in Table 2.

Genes That Regulate Processing and Transport of Folate

Two genes play critical roles in ensuring adequate folate is shuttled into the cell for use in C1 metabolism. Glutamate carboxypeptidase II (GCPII) encodes an enzyme involved in hydrolysis of dietary folate into a bioavailable form in the intestines (Devlin et al. 2000) and may also modify folate to facilitate transport in and out of cells (Yao et al. 2010). The reduced folate carrier (RFC-1) is a transport protein responsible for uptake of folate across the blood–brain barrier (function reviewed in Matherly 2001). Polymorphisms in both GCPII (GCPII 1561C>T) and RFC-1 (RFC-1 80G>A) are associated with high plasma folate concentrations (Halsted et al. 2007; Yates and Lucock 2005) that likely correspond to low tissue concentrations of folate (DeVos et al. 2008). In individuals with the RFC-1 80G>A polymorphism, low concentrations of 5-MTHF have been noted in the CSF (Yates and Lucock 2005). By reducing tissue folate levels, these polymorphisms are expected to result in reduced methylation capacity. As predicted, the RFC-1 80G allele has been associated with global DNA hypomethylation (James et al. 2010); DNA methylation status has yet to be examined in individuals with the GCPII 1561T allele.

Parental gene variants. To date, no studies have investigated GCPII 1561C>T polymorphisms in either mothers or fathers of children with ASDs. On the other hand, the RFC-1 80G allele in the mother, but not the father, has been associated with a significantly increased risk of having a child with autism (James et al. 2010).

Gene variants in children with ASDs. No association has been noted between the GCPII 1561C>T variant and risk of ASDs in the child (Adams et al. 2007; James et al. 2006), which suggests that this gene is unlikely to play a major role in the disorder. In contrast, the RFC-1 80G>A variant has been shown to confer an increased risk of developing autism in some studies (James et al. 2006, 2010) but not others (Adams et al. 2007).

Genetic Variants in the Folate Cycle

Folate is converted between a number of derivates within the folate cycle (Figure 1). Dihydrofolate reductase (DHFR) converts synthetic folic acid and dihydrofolate into THF. DHFR is therefore required to catabolize folic acid into physiologically functional forms for C1 metabolism (Kalmbach et al. 2008). THF is converted into 5,10-MTHF by serine hydroxymethyltransferase (SHMT) with the help of the coenzyme vitamin B6. This reaction is reversible and results in the simultaneous conversion of the methyl donor serine to glycine (Schirch and Peterson 1980). Finally, 5,10-MTHF is irreversibly converted into 5-MTHF by methylene tetrahydrofolate reductase (MTHFR) using vitamin B2 as a coenzyme (Anguera et al. 2003). The functional consequence of polymorphisms to DHFR (a 19–base pair deletion [19bp del]) and SHMT (SHMT 1420C>T) remain controversial. A low activity variant of DHFR limits the capacity to convert THF into 5,10-MTHF (reviewed in Askari and Krajnovic 2010), whereas the SHMT 1420T allele appears to drive C1 units destined for DNA repair and synthesis toward methylation reactions (Heil et al. 2001; Herbig et al. 2002). Two polymorphisms in MTHFR are the most commonly studied variants associated with susceptibility to ASDs. The MTHFR 677C>T polymorphism produces a protein with about 35% of normal activity, whereas the MTHFR 1298A>C polymorphism is less severe and reduces enzyme activity to around 60% of normal (Chango et al. 2000). In general, reduced enzymatic activity in individuals with the MTHFR 677TT genotype leads to an increase in the production of 5-FTHF for DNA synthesis and repair and decreased production of 5-MTHF (Friso et al. 2002). Polymorphisms in DHFR, SHMT, and MTHFR are likely to have different effects on methylation capacity, DHFR 19bp del, MTHFR 677T, and MTHFR 1298C and limit the production of methionine and decrease methylation potential, whereas SHMT 1420T should drive C1 units toward methylation reactions.
### Table 2 Summary of studies reporting autism spectrum disorder susceptibility for genetic polymorphisms in one-carbon metabolism

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Results</th>
<th>Subjects(^a)</th>
<th>Diagnosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genes that regulate processing and transport of folate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCPII 1561C&gt;T: child</td>
<td>Not significant</td>
<td>17, 16</td>
<td>Autism</td>
<td>Adams 2007</td>
</tr>
<tr>
<td>RFC-1 80A&gt;G: maternal</td>
<td><strong>GG ↑ OR = 1.46 (CI = 1.01 to 2.11; (p = 0.036))</strong></td>
<td>1056, 1132</td>
<td>Autism</td>
<td>James 2010</td>
</tr>
<tr>
<td>RFC-1 80A&gt;G: paternal</td>
<td>Not significant</td>
<td>1042, 1132</td>
<td>Autism</td>
<td>James 2010</td>
</tr>
<tr>
<td>RFC-1 80A&gt;G: child</td>
<td><strong>GG ↑ OR = 1.96 (CI = 1.15 to 3.3)</strong></td>
<td>360, 205</td>
<td>Autism</td>
<td>James 2006</td>
</tr>
<tr>
<td></td>
<td>AG ↑ OR = 1.40 (CI = 0.99 to 1.98; (p = 0.047))</td>
<td>1056, 1132</td>
<td>Autism</td>
<td>James 2010</td>
</tr>
<tr>
<td></td>
<td>Not significant</td>
<td>17, 16</td>
<td>Autism</td>
<td>Adams 2007</td>
</tr>
<tr>
<td><strong>Genetic variants in the folate cycle</strong></td>
<td></td>
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</tr>
<tr>
<td>DHFR 19 bp: child</td>
<td><strong>19 bp ↑ OR = 2.69 (CI = 1.00 to 7.28; (p &lt; 0.05))</strong></td>
<td>17, 16</td>
<td>Autism</td>
<td>Adams 2007</td>
</tr>
<tr>
<td>SHMT1 1420T&gt;C: child</td>
<td><strong>T ↑ OR = 0.44 (CI = 0.31 to 0.62; (p &lt; 0.0005))</strong></td>
<td>138, 138</td>
<td>Autism</td>
<td>Mohammad 2009</td>
</tr>
<tr>
<td>MTHFR 677C&gt;T: maternal</td>
<td><strong>TT ↑ OR = 4.5 (CI = 1.4 to 14.6; (p = 0.04))</strong></td>
<td>204, 185</td>
<td>Autism</td>
<td>Schmidt 2011</td>
</tr>
<tr>
<td>MTHFR 677C&gt;T: parental</td>
<td>Not significant</td>
<td>400, 384</td>
<td>ASD</td>
<td>Liu 2011</td>
</tr>
<tr>
<td>MTHFR 677C&gt;T: child</td>
<td><strong>CT + TT ↑ OR = 1.38 (CI = 0.96 to 1.98)</strong></td>
<td>360, 205</td>
<td>Autism</td>
<td>James 2006</td>
</tr>
<tr>
<td></td>
<td><strong>TT ↑ ((p = 0.09))</strong></td>
<td>15, 80</td>
<td>Autism</td>
<td>Pasca 2009</td>
</tr>
<tr>
<td></td>
<td><strong>T ↑ OR = 2.79 (CI = 1.58 to 4.93; (p &lt; 0.0001))</strong></td>
<td>138, 138</td>
<td>Autism</td>
<td>Mohammad 2009</td>
</tr>
<tr>
<td></td>
<td>Not significant</td>
<td>205, 384</td>
<td>ASD (simplex)</td>
<td>Liu 2011</td>
</tr>
<tr>
<td></td>
<td><strong>TT ↓ OR = 0.59 (CI = 0.26 to 1.35; trend)</strong></td>
<td>201, 180</td>
<td>Autism</td>
<td>Schmidt 2011</td>
</tr>
<tr>
<td>MTHFR 1298A&gt;C: parental</td>
<td>Not significant</td>
<td>401, 382</td>
<td>ASD</td>
<td>Liu 2011</td>
</tr>
<tr>
<td>MTHFR 1298A&gt;C: child</td>
<td>Not significant</td>
<td>17, 16</td>
<td>Autism</td>
<td>Adams 2007</td>
</tr>
<tr>
<td></td>
<td>Not significant</td>
<td>138, 138</td>
<td>Autism</td>
<td>Mohammad 2009</td>
</tr>
<tr>
<td></td>
<td><strong>A ↑ ((p = 0.059))</strong></td>
<td>205, 382</td>
<td>ASD (simplex)</td>
<td>Liu 2011</td>
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<tr>
<td><strong>Genes in the methionine cycle</strong></td>
<td></td>
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<tr>
<td>MTR 2756A&gt;G: child</td>
<td>Not significant</td>
<td>17, 16</td>
<td>Autism</td>
<td>Adams 2007</td>
</tr>
<tr>
<td>TCN2 776C&gt;G: maternal</td>
<td>Not significant</td>
<td>201, 187</td>
<td>Autism</td>
<td>Schmidt 2011</td>
</tr>
<tr>
<td>TCN2 776C&gt;G: child</td>
<td><strong>GG ↑ OR = 1.70 (CI = 1.02 to 2.8)</strong></td>
<td>360, 205</td>
<td>Autism</td>
<td>James 2006</td>
</tr>
<tr>
<td></td>
<td>Not significant</td>
<td>198, 177</td>
<td>Autism</td>
<td>Schmidt 2011</td>
</tr>
<tr>
<td>MTRR 66A&gt;G: maternal</td>
<td>Not significant</td>
<td>211, 194</td>
<td>Autism</td>
<td>Schmidt 2011</td>
</tr>
<tr>
<td>MTRR 66A&gt;G: child</td>
<td><strong>GG ↑ OR = 0.61 (CI = 0.36 to 1.03)</strong></td>
<td>360, 205</td>
<td>Autism</td>
<td>James 2006</td>
</tr>
<tr>
<td></td>
<td><strong>AA ↑ OR = 0.55 (CI = 0.35 to 0.86; (p &lt; 0.0005))</strong></td>
<td>138, 138</td>
<td>Autism</td>
<td>Mohammad 2009</td>
</tr>
<tr>
<td></td>
<td>Not significant</td>
<td>17, 16</td>
<td>Autism</td>
<td>Adams 2007</td>
</tr>
<tr>
<td></td>
<td>Not significant</td>
<td>198, 177</td>
<td>Autism</td>
<td>Schmidt 2011</td>
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<tr>
<td><strong>Methyltransferases</strong></td>
<td></td>
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</tr>
<tr>
<td>COMT 472G&gt;A: maternal</td>
<td>Not significant</td>
<td>198, 187</td>
<td>Autism</td>
<td>Schmidt 2011</td>
</tr>
<tr>
<td>COMT 472G&gt;A: child</td>
<td><strong>GG ↑ OR = 1.74 (CI = 1.02, 2.9)</strong></td>
<td>360, 205</td>
<td>Autism</td>
<td>James 2006</td>
</tr>
<tr>
<td></td>
<td><strong>AA ↑ OR = 7.2 (CI = 2.3, 22.4; (p = 0.05))</strong></td>
<td>202, 180</td>
<td>Autism</td>
<td>Schmidt 2011</td>
</tr>
</tbody>
</table>

\(^a\)Numbers given as cases, controls.

A diagnosis of autism spectrum disorder (ASD) refers to studies that combined individuals with typical autism, pervasive developmental disorder not otherwise specified, and Asperger syndrome in the analysis. Values in bold are either statistically significant or are reported as trends. bp, base pair; CI, 95% confidence interval; COMT, catechol-O-methyltransferase; DHFR, dihydrofolate reductase; GCPII, glutamate carboxypeptidase II; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; OR, odds ratio; RFC-1, reduced folate carrier; SHMT, serine hydroxymethyltransferase; TCN2, transcobalamin II.

A diagnosis of autism spectrum disorder (ASD) refers to studies that combined individuals with typical autism, pervasive developmental disorder not otherwise specified, and Asperger syndrome in the analysis. Values in bold are either statistically significant or are reported as trends. bp, base pair; CI, 95% confidence interval; COMT, catechol-O-methyltransferase; DHFR, dihydrofolate reductase; GCPII, glutamate carboxypeptidase II; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; OR, odds ratio; RFC-1, reduced folate carrier; SHMT, serine hydroxymethyltransferase; TCN2, transcobalamin II.

\(^b\)Mother took prenatal vitamins while child was in utero.
Parental gene variants. To date, the risk of having a child with ASDs has been evaluated only in parents with polymorphisms in MTHFR. In mothers and fathers combined, neither the MTHFR 677T nor the MTHFR 1298C alleles significantly increased susceptibility to ASDs in the offspring (Liu et al. 2011). In a separate study, the offspring’s risk of autism was significantly higher in mothers with the MTHFR 677TT genotype, but only when the mothers reported no prenatal vitamin intake prior to or around the time of conception (Schmidt et al. 2011).

Gene variants in children with ASDs. Polymorphisms within all three enzymes, DHFR, SHMT1, and MTHFR, have been shown to modify risk for autism in the child. DHFR 19bp del has been shown to increase susceptibility to autism (Adams et al. 2007). Consistent with this finding, inhibition of DHFR activity in young children with methotrexate, an anticancer drug, has been shown to significantly increase the prevalence of autistic-like symptoms (Kamen and Chukoskie 2011). The SHMT 1420T allele has been shown to confer protection against autism (Mohammad et al. 2009). Multiple studies have shown that at least one copy of the low-activity MTHFR 677T allele significantly increased the risk for autism (James et al. 2006; Liu et al. 2011; Mohammad et al. 2009; Pasca et al. 2009). Goin-Kochel and colleagues (2009) went one step further and demonstrated significant genotype–phenotype correlations for four autism-associated behaviors (MTHFR 677CC < MTHFR 677CT < MTHFR 677TT in increasing scores of severity). Two additional studies reported no association between the MTHFR 677T allele and risk for autism. In the first, the lack of association was likely due to small numbers (n = 17 autistic subjects, n = 16 control subjects) (Adams et al. 2007). Interestingly, the second study reported a trend toward an inverse association with the MTHFR 677T allele conferring protection against autism (Schmidt et al. 2011). Finally, although the MTHFR 1298A>C variant does not by itself confer risk, it does amplify susceptibility to ASD in combination with MTHFR 677C>T (Adams et al. 2007; James et al. 2006; Mohammad et al. 2009). A number of other significant gene–gene interactions have also been noted. Combinations of RFC-1 80A>G and MTHFR 677C>T (Adams et al. 2007; James et al. 2006) and DHFR and MTHFR 677C>T (Adams et al. 2007) further increase the risk of developing autism in the child. Presumably, the increase in risk is compounded when multiple polymorphisms further reduce the availability of folate derivatives for important C, metabolic reactions, including those associated with DNA methylation.

Genes in the Methionine Cycle

At the intersection between the folate and methionine cycles, one critical determinant of methylation capacity is the ability to remethylate homocysteine into methionine through the conversion of 5-MTHF to THF. Three proteins play important roles in this reaction in the brain. Methionine synthase is directly responsible for the transfer of a methyl group from 5-MTHF to homocysteine (Chen et al. 1997). Transcobalamin II (TCN2) is a transport protein required for cellular uptake of vitamin B12 (Seetharam 1999), the essential cofactor for methionine synthase (MTR). Following conversion of homocysteine to methionine, MTR becomes inactive due to oxidation of vitamin B12. Methionine synthase reductase (MTRR) is then responsible for regenerating MTR to its active form (Wolthers and Scrutton 2009). Polymorphisms in MTR (MTR 2756A>G), TCN2 (TCN2 776C>G), and MTRR (MTRR 66A>G) are all expected to limit the conversion of methionine from homocysteine, thereby decreasing methylation capacity (Afman et al. 2001; Terruzzi et al. 2011).

Maternal genetic variants. Maternal polymorphisms in neither TCN2 nor MTRR have been shown to increase susceptibility for autism in the offspring (Schmidt et al. 2011).

Genetic variants in children with ASDs. No association between MTR 2756A>G and risk of autism has been found (Adams et al. 2007; Mohammad et al. 2009). Potential associations between the TCN2 776G and MTRR 66G alleles and autism are inconsistent across studies. TCN2 776C>G has been correlated with increased risk of autism (James et al. 2006), but a more recent study failed to confirm this association (Schmidt et al. 2011). The MTRR 66A allele, in comparison with MTRR 66G, has been shown to confer protection against autism (Mohammad et al. 2009), likely because MTRR 66A is associated with normal MTRR activity levels (Wilson et al. 1999). In contrast, the MTRR 66G allele was shown to confer protection in another study (James et al. 2006), whereas two additional studies found no association between MTRR 66A>G and risk for ASDs (Adams et al. 2007; Schmidt et al. 2011). Additional studies appear to be required to parse the potential roles for TCN2 and MTRR in ASDs.

Methyltransferases and Beyond

Although no genetic abnormalities or changes in gene expression have been reported in DNMTs in children with ASDs (Voineagu et al. 2011), mutations have been noted in other methyltransferases. The most frequently studied methyltransferase associated with ASD is catechol-O-methyltransferase (COMT). COMT methylates the neurotransmitter dopamine, which is involved in cognitive and reward pathways in the brain (Phillips et al. 2008), and inactivates it (Axelrod 1971). Individuals with the wildtype COMT 472A allele have less enzymatically active COMT and consequently higher dopamine levels than those with the COMT 472G allele (Mannisto and Kaakkola 1999). In generally, the COMT 472GG genotype is associated with poor performance on cognitive tasks and antisocial behavior (Nedic et al. 2011; Thapar et al. 2005). COMT 472A>G polymorphisms in the mother have not been shown to affect autism risk in the offspring (Schmidt et al. 2011), whereas studies of the effects of the COMT genotypes in the children have given conflicting
results. Susceptibility to autism was increased in individuals with the COMT 472GG genotype in one study (James et al. 2006) and with the COMT 472AA genotype in another (Schmidt et al. 2011). Increased susceptibility to ASDs has also been noted in children with polymorphisms in the genes encoding acetylsalicycدين-O-methyltransferase (ASMT) and guanidinoacetate methyltransferase (GAMT), which are necessary for melatonin (Pagan et al. 2011) and creatine synthesis, respectively (Almeida et al. 2007).

Mutations in MeCP2 are responsible for approximately 80% of cases of Rett syndrome, an ASD (Bienvenu and Chelly 2006). MeCP2 binds to specific patterns of methylated DNA to alter expression of target genes. Although MeCP2 is canonically a transcriptional repressor (Nan et al. 1997), more recent studies have suggested that MeCP2 can both repress and activate transcription of downstream genes through chromatin remodeling mechanisms (Chahrour et al. 2008; Guy et al. 2010). These examples, which demonstrate susceptibility to ASD in individuals with genetic mutations in methyltransferases as well as proteins responsible for “readout” of methylation patterns, further support the important role of the establishment and maintenance of methylation patterns in ASD.

Diet–Gene Interactions

Maternal diet–gene interactions. Diet–gene interactions have been examined only very recently in the mothers of children with ASDs. The ability of both genes and nutritional factors to independently influence C1 metabolism and methylation patterns makes it likely that they could do so in combination and perhaps interact in unpredictable ways. One study showed that lack of prenatal vitamin intake in mothers with the MTHFR 677C>T polymorphism significantly increased the risk of having a child with autism (Schmidt et al. 2011). However, maternal prenatal vitamin intake or lack thereof did not modify the offspring’s risk of developing autism in the presence of COMT, MTRR, or TCN2 polymorphisms (Schmidt et al. 2011). In another study, pregnant mothers with the MTHFR 677C>T genotype and vitamin B6 deficiency (coenzyme for SHMT) displayed a higher risk for DNA hypomethylation (La Merrill et al. 2011), which suggests a potential mechanism through which low-activity genetic variants of MTHFR and a specific nutrition deficiency may alter DNA methylation and increase susceptibility to ASDs.

Diet–gene interactions in children with ASDs. In affected children, a child carrying the COMT 472 allele was more likely to develop autism if the child’s mother did not take prenatal vitamins during pregnancy; however, maternal vitamin intake did not modify risk associated with MTHFR, MTRR, or TCN2 polymorphisms (Schmidt et al. 2011). The allele frequencies for many of these polymorphisms in the general population are quite high (approximately 35% for MTHFR 677T [Schmidt et al. 2011]) and may be expected to increase the prevalence of ASDs in developing countries, such as China or Iran, where inadequate nutrition is a concern. Although recent studies suggest that rates of ASD in China and Iran (11.0 and 6.26 per 10,000 respectively for 5 year olds) may not be as high as in the United States (40 per 10,000), there are many reasons to think that these rates are not yet accurate reflections of the true prevalence of ASDs in these countries (Samadi and McConkey 2011). In contrast, there is some evidence to suggest that recent folic acid fortification of food may have affected the risk associated with at least one polymorphism. The MTHFR 677T allele has been associated with increased risk for ASDs or has shown changes in allele frequencies in a similar direction in all but one study (see “Genetic Variants in the Folate Cycle”). Accounting for the country of study origin (Australia, Canada, India, Romania, or the United States) and the potential range of birth years for the cohorts, children were almost certainly not exposed to fortified foods in four studies; fortification is probable in a fifth; and in the last, children (born between 2003 and 2009 in California) were certainly exposed to fortified foods from conception. In the later study, the MTHFR 677TT genotype was protective and implicated excessive folic acid intake, in combination with normal C1 metabolic function, in increased risk for ASDs. The ability of the diet to modify the risk associated with genetic polymorphisms, such as MTHFR, suggests that it may be critical to account for levels of food fortification in future studies of genetic risk factors, especially factors that affect the folate cycle. The currently available studies only begin to scratch the surface of potential diet–gene interactions in affecting ASD susceptibility and provide little insight into the specific nutrients that may be most important.

Diet–gene interactions in mice. In general, transgenic knockout mice recapitulate the C1 metabolic abnormalities reported in peripheral tissues in humans (Davis et al. 2005; Ghandour et al. 2004), which suggests that they are valuable models for further understanding the mechanisms through which genes and nutrients interact to affect DNA methylation status in the brain. Shmt1 knockout mice provide insight into the mechanism through which the Shmt 1/2 knockout mice may reduce the likelihood of autism in humans. In Shmt1 mice, the SAM/SAH ratio is significantly higher than in wild-type mice, which suggests that the lack of Shmt1 drives C1 metabolism toward the production of SAM and methylation reactions and away from DNA synthesis and repair (MacFarlane et al. 2008). Increases in the SAM/SAH ratio remain within normal ranges in these mice, even after severe folate deficiency (MacFarlane et al. 2008). These data support another potential mechanism by which this gene could provide protection in the presence of a nutrition deficiency. In contrast with the protective effect of Shmt variants, Mthfr+/− and Mthfr−/− mice exhibit a dose-dependent reduction in the SAM/SAH ratio associated with a decrease in global DNA methylation (Chen et al. 2001). Combined deficiencies in Mthfr and folic acid in the dam result in small, developmentally delayed offspring with a variety of anatomic defects (Pickett et al. 2009). The deficits in the offspring of Mthfr folate-deficient mice mostly mimic reported defects in the offspring of genetically normal dams that are fed a diet...
containing 20 times the RDA for folic acid (Pickell et al. 2011). When the Mthfr<sup>+/−</sup> dams are fed a diet with excess folic acid, all abnormalities are rescued (Pickell et al. 2011). These experiments suggest that both reduced function of Mthfr and excess folic acid intake, although opposite in function, as demonstrated by rescue experiments, similarly reduce methylation potential. Together these studies highlight the importance of measuring nutritional status in combination with genetic analyses to determine risk factors for ASDs.

Can changes in diets, genes, and the interaction between them account for the abnormal DNA methylation patterns observed in children with ASDs (e.g., global DNA hypomethylation and gene-specific DNA hypermethylation)? With the exception of SHMT, which increases DNA methylation and confers protection against ASDs, the nutritional and genetic risk factors reviewed here are hypothesized to reduce methylation capacity (SAM/SAH) and increase the risk of ASDs. A reduction in the availability of methyl groups, although counterintuitive, may explain both global DNA hypomethylation and gene-specific hypermethylation. In a normal individual, the majority of methylated CpGs are located within repetitive genomic regions, whereas CpG islands, located near 70% of gene promoters, are generally unmethylated (Jones and Liang 2009). A reduction in methylation capacity would most significantly affect methylation within regions that are normally highly methylated—in other words, within repetitive genomic regions. Thus, reduced nutrition intake and abnormal C<sub>1</sub> metabolic enzyme function are expected to lead to global DNA hypomethylation. Conversely, gene-associated CpG islands are normally unmethylated (Saxonov et al. 2006). One speculative mechanism by which CpG islands normally remain hypomethylated may also explain how a reduced methylation capacity can result in gene-specific DNA hypermethylation. CpG islands are highly associated with trimethylation of the H3 histone tail at lysine 4 (H3K4me3) (Guenther et al. 2007). This epigenetic histone mark interferes with DNMT3L function and prevents de novo DNA methylation. In instances when H3K4 is unmethylated, possibly because of reduced availability of methyl groups, DNA hypermethylation of gene-specific promoters may occur (Ooi et al. 2007). Consistent with this hypothesis, a comparison of H3K4 trimethylation levels in prefrontal cortical neurons of autism cases and controls revealed a number of disease-associated changes in the CpG islands of genes that have previously been implicated in ASDs (Shulha et al. 2011).

**Therapeutic Implications**

There appear to be close and consistent links between abnormalities in C<sub>1</sub> metabolism and ASDs. Even more important, the data reviewed here suggest strongly that cases of ASD associated with metabolic imbalances in the C<sub>1</sub> pathway are likely preventable. An important question remains: When do therapeutic interventions need to occur to prevent ASDs? Critical periods for epigenetic programming begin at conception (Morgan et al. 2005). There is a decreased risk of ASDs in offspring whose mothers took prenatal vitamins prior to conception (Schmidt et al. 2011), when a woman may not realize she is pregnant. Therefore, a preconception checkup to assess nutritional status and identify C<sub>1</sub> metabolic polymorphisms, which may affect nutritional requirements, is an important step in early prevention. As we continue to understand better the intersection between diet and genetics in C<sub>1</sub> metabolic function, clinicians will be in a better position to ensure appropriate availability of methyl groups for early epigenetic programming of the fetus and for proper organization of neuron networks in the infant. By the time a toddler is diagnosed with ASDs, we hypothesize that symptoms will be more difficult to treat. Autistic-like behaviors likely become apparent as important cortical networks that support social and cognitive processes are already wiring (Berger-Sweeney 2011; Berger-Sweeney and Hohmann 1997; Lagercrantz and Ringstedt 2001).

Although epigenetic programming is liable to be most important during the prenatal and early postnatal periods, DNA methylation patterns can also be manipulated during early postnatal development and even into adulthood (Praga et al. 2005; Szyf 2009). A number of therapies, including supplementation with vitamins and amino acids, can alter epigenetic regulation of gene expression later in life by affecting C<sub>1</sub> metabolism (Davison et al. 2009; Nag et al. 2009; Troen et al. 2008). Given the number of abnormalities in C<sub>1</sub> metabolites in ASDs demonstrated across multiple studies (Adams et al. 2011b; Main et al. 2010), surprisingly few studies focus on correcting metabolic abnormalities and assessing improvements in autism-associated behaviors. Folic acid, a synthetic 5-FTHF, or folic acid was administered in five studies where CSF levels of 5-MTHF were significantly lower than normal (reviewed in Main et al. 2010). Following treatment, 5-MTHF levels were normalized, and significant improvements in epileptic seizures and motor dysfunction were reported. Two of the youngest patients (both approximately 3 years old) completely recovered; whereas some, but not all, of the core behavior features of autism were ameliorated in the remaining patients (Ramaekers et al. 2007). Vitamin B12 supplementation with or without folic acid has also been assessed. Overall improvements in behavior and measures of redox homeostasis, but not methylation potential, were noted in a subset of children (Bertoglio et al. 2010; James et al. 2009). Vitamin B6 supplementation with magnesium also improved a range of clinical symptoms in a subgroup of ASD children diagnosed with pervasive development disorder (Mousain-Bosc et al. 2006; Nye and Brice 2005; Xia 2011). Most recently, multivitamins, containing a number of essential vitamins and minerals, have been shown to increase markers of methylation capacity, such as SAM, and improve symptoms of autism, including hyperactivity, throwing tantrums, and receptive language in individuals with ASDs (Adams et al. 2011a). Although results were not significant, the multivitamin appears to provide the most benefit to affected individuals aged less than 20 years.
Although the number of participants in each of the aforementioned studies was small (ranging from 1 to 50 children), collectively the studies suggest a number of important principles for treating ASDs. First, vitamin supplementation to correct metabolic imbalances improves some aspects of ASD symptomatology. However, supplementation appears to be least effective at reducing social and cognitive deficits; those cortical networks are presumably set during critical periods in early postnatal life (Berger-Sweeney 2011) before supplementation is given. Second, blanket vitamin supplementation of all affected children is unlikely to be effective. Although unlikely to be harmful within moderation, supplementation may be most helpful to those children with diagnosed nutritional deficiencies or with genetic mutations known to limit the transport or use of vitamins and amino acids required for C1 metabolism. There is increasing evidence that excessive supplementation may also adversely affect epigenetic gene regulation and negatively affect behavior (Iskandar et al. 2010; Sittig et al. 2011), which suggests that supplementation should be monitored closely. Third, early nutraceutical interventions are more likely to be effective in ameliorating symptomatology in ASDs than are later interventions. Early abnormalities in the brains of individuals with ASDs probably stem from altered proliferation and long-range neuron connectivity (Schumann and Nordahl 2011) that may be irreversible later in life. Thus, the ability to rescue fully autistic-like symptoms presumably will require the ability to restore the underlying anatomic alterations present during development of neuron networks, not just later changes in synaptic function (Berger-Sweeney 2011).

Studies demonstrating the importance of early-life experience on epigenetic programming provide another intriguing possibility for treatment in ASDs. In rodents, the quality of maternal care early in life can significantly impact the behavior phenotype and epigenome of offspring in adulthood (reviewed in Roth and Sweatt 2011). Specifically, poor maternal care, such as long periods of separation, early in life results in a vast array of persistent changes in DNA methylation and histone acetylation patterns (McGowan et al. 2011) that can be reversed by treatment with L-methionine or a pharmacologic inhibitor of chromatin remodeling, trichostatin A, in the adult (Weaver et al. 2005, 2006). Based on these principles, it is not surprising that the first trial of early intensive behavior intervention showed significant improvements in the behavior outcome of children diagnosed with ASDs (Dawson et al. 2010).

Certainly, we are not to the point of understanding the complex interplay between hypomethylation and hypermethylation of genes in ASDs. However, there is hope that one day we may understand how best to use the plethora of chromatin remodeling drugs already available, including trichostatin A (Szyf 2009), to more effectively modulate DNA and histone methylation patterns than can currently be achieved with nutritional supplementation. There is significant potential for the use of these drugs to reduce impairments in ASDs, although very early interventions will likely be necessary to rescue symptoms fully (Berger-Sweeney 2011).

Concluding Thoughts

We have provided evidence to suggest that epigenetic programming plays a critical role in ASDs and have reviewed etiologic studies that support a role for both nutrition and genetic components of the C1 metabolic pathway on increasing susceptibility to ASDs. Consistent with the heterogeneous etiology of ASDs, no single dietary or genetic factor within the C1 metabolic pathway was found to reliably contribute to susceptibility across studies; instead a number of different metabolic and genetic abnormalities were apparent. Our understanding of how C1 metabolic dysfunction may affect susceptibility to ASDs is still limited. It is clear that a number of additional studies are required to parse the importance of these various risk factors and the critical periods during which these factors are most essential for normal brain development.
Based on the potential importance of a critical period around conception (Schmidt et al. 2011), a greater focus on the parents of children with ASDs is warranted. In particular, diet and gene interactions need to be more thoroughly explored. In the few examples where maternal diet was considered, changes in diet significantly impacted how C1-associated genetic polymorphisms, from either the mother or the offspring, altered development, DNA methylation status, and risk for developing ASDs in the offspring (Pickell et al. 2009, 2011; Schmidt et al. 2011). In addition, the influence of paternal diet and genes on ASD susceptibility is virtually unexplored. This is surprising given that advanced paternal age, which significantly elevates risk for ASDs in the offspring (Shelton et al. 2010), is hypothesized to be the result of increased genomic and epigenomic abnormalities in germ cells (reviewed in Foldi et al. 2011). Additional studies examining nutritional status in affected children are also required to determine the significance of a potential correlation between lower methionine concentrations and increased clinical severity, as well as the potential presence of a period of greater C1, metabolic dysfunction in younger children with ASDs (both methionine and B6 levels may be significantly altered in children with ASDs who are, on average, younger than 8 years, but not older.) Of note, it is also around this same age (8 years) that researchers propose that there is a shift from stagnant brain growth to premature degeneration in individuals with ASDs (Courchesne et al. 2011).

In addition, we must question whether the biochemical measurements of nutrition and methylation status made in peripheral tissues accurately reflect brain concentrations. Our understanding of how nutrition and genetic factors influence DNA methylation patterns and risk for ASDs is based primarily on biochemical measurements made in the periphery (e.g., blood and buccal cells in human studies as well as liver, kidneys, and so on in mice). From a review of the vast literature on diet- and gene-based modulation of DNA methylation patterns, it is clear that body tissues in both humans and rodents are differentially susceptible to genetic and environmental factors (Chen et al. 2001; Ghandour et al. 2004; Sohn et al. 2009). Even subregions of the same tissue within the brain show different methylation patterns in response to genetic or environmental factors (Mehedint et al. 2010). Very few studies examine the effects of genetic and environmental factors that affect C1 metabolism on brain DNA methylation in rodents, let alone in humans. To our knowledge, no studies have determined the maternal or paternal effects of modifying C1 metabolic parameters on behavior outcomes or DNA methylation in the brains of postweaning offspring. Because dynamic DNA methylation status in the brain is likely most relevant to symptomatology in ASDs, additional studies are needed to confirm that peripheral measurements are reliable indicators of central nervous system dysfunction or to assess directly methylation status in the brain.

Can recent changes in environmental influences (specifically dietary), frequency of genes related to C1 metabolism, or the combination explain the recent explosion in the incidence of ASDs? Many researchers hypothesize that recent changes in nutrition intake and, perhaps secondarily, changes in the frequency of genetic polymorphisms associated with C1 metabolism may account for the recent increases in ASD prevalence (Rogers 2008; Smith et al. 2008). Specifically, an increase in diagnosis of ASDs occurred concurrently with mandated fortification of food with folic acid in the United States (Sugden 2006). At the same time, pregnant women are advised to take prenatal vitamins with increasingly high levels of folic acid (Beard et al. 2011). Are pregnant women getting too much folic acid? Studies in rodents suggest that high levels of maternal folic acid are as detrimental to early programming
of the epigenome as folate deficiency (Pickell et al. 2011). One might question whether the levels of folic acid necessary to induce changes in DNA methylation in rodents are comparable with the levels of supplementation in modern diets during pregnancy and early life in human offspring? In rodents, supplementation of folic acid two or more times that of the RDA has significant negative health consequences (Pickell et al. 2011; Sie et al. 2011; Sittig et al. 2011). In a study in North Carolina, 1 in 10 pregnant women reported taking more than 1 mg of folic acid in vitamin supplements (Hoyo et al. 2011), which does not include additional folate and folic acid from natural and fortified food sources. Development milestones are being monitored in these offspring. In children, early reports of the effects of food fortification estimate that 43% of children aged less than or equal to 5 years are consuming two times the RDA of folic acid, and 10% of those consume more than three times the RDA of folic acid (Pfeiffer et al. 2005). These numbers suggest that upwards of 10% of fetuses and an even higher percentage of young children may be exposed to levels of folic acid shown to have adverse health effects in rodents. Certainly, excess folic acid is not always detrimental and may protect embryos with polymorphisms in C1-associated genes from spontaneous abortion (Govindiaiah et al. 2009) and thus alter allelic frequencies in the population. Indeed, an increase in the frequency of the MTHFR 677T allele over a period of 20 years has been suggested (Agodi et al. 2011; Munoz-Moran et al. 1998). Additionally, early indications suggest that fortification may have shifted the MTHFR 677T allele from a susceptibility to a protective factor for ASDs (Schmidt et al. 2011).

Where do we go from here? Clearly, C1 metabolism is an extremely complex pathway with a large number of players, and knowledge of the individual and combined effects of diet and genes is still limited. Animal models will be crucial to better understanding the interplay between specific nutrients and genes on C1 metabolic function and epigenetic patterning and the importance of critical periods for autism-related behavior outcomes. Already, a number of transgenic mice exist with mutations in ASD susceptibility genes, including *GCH1* (Han et al. 2009), *RFC-1* (Gelineau-van Waes et al. 2008), *SHMT1* (MacFarlane et al. 2008), *MTHFR* (Chen et al. 2001), *MTR* (Lawrence et al. 2007), and *MTRR* (Elmore et al. 2007). Studies to date in these knockouts focus primarily on how gene–nutrient interactions regulate cell proliferation in cancer. However, further research in mouse models, already in existence, could be used to confirm the importance of the C1 metabolic pathway in the development of autism-like symptoms. A large number of assays have been developed to assess behaviors reminiscent of the clinical symptoms of ASDs (Roulet and Crawley 2011; Silverman et al. 2010). Additional research in these mice can extend our understanding of complex gene–nutrient interactions and their effects on behavior, as well as provide valuable models for testing nutraceutical and pharmacologic interventions for ASD.

The final piece of this complex puzzle will involve translating changes in the epigenome to the genes and molecular pathways responsible for altering brain organization and function in ASDs. Although DNA methylation appears to be affected in ASDs, the identity and significance of the affected genes remains, for the most part, a mystery. Gene expression array studies have identified a number of candidate genes in ASDs (Voineagu et al. 2011). One interesting prospect is SHANK3 (SH3 and multiple ankyrin repeat domain 3), a scaffolding protein at the glutamatergic synapse, which increases susceptibility to ASDs (reviewed in Herbert 2011). Expression of SHANK3 protein is tissue-specifically regulated by methylation of DNA that can be altered by methionine supplementation (Beri et al. 2007), a C1-metabolite that is consistently affected in children with ASDs (reviewed in “Nutrition Status in Children with ASDs”)

Groundwork that suggests that C1 metabolism plays a critical role in ASD has been laid by a number of laboratories. Given the central position of C1 metabolism in regulating the epigenome and its ability to be modulated by a large number of genetic and environmental factors, it is in an ideal candidate pathway in development disorders of heterogeneous etiology such as ASDs. Thus, by understanding gene–diet risk factors and the critical time windows during which C1 metabolic function affects neuron development, there is the significant potential to prevent, not just to treat, ASDs.

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