

# Folate and methionine metabolism in autism: a systematic review<sup>1,2</sup>

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## ABSTRACT

**Background:** Autism is a complex neurodevelopmental disorder that is increasingly being recognized as a public health issue. Recent evidence has emerged that children with autism may have altered folate or methionine metabolism, which suggests the folate-methionine cycle may play a key role in the etiology of autism.

**Objective:** The objective was to conduct a systematic review to examine the evidence for the involvement of alterations in folate-methionine metabolism in the etiology of autism.

**Design:** A systematic literature review was conducted of studies reporting data for metabolites, interventions, or genes of the folate-methionine pathway in autism. Eighteen studies met the inclusion criteria, 17 of which provided data on metabolites, 5 on interventions, and 6 on genes and their related polymorphisms.

**Results:** The findings of the review were conflicting. The variance in results can be attributed to heterogeneity between subjects with autism, sampling issues, and the wide range of analytic techniques used. Most genetic studies were inadequately powered to provide more than an indication of likely genetic relations.

**Conclusions:** The review concluded that further research is required with appropriately standardized and adequately powered study designs before any definitive conclusions can be made about the role for a dysfunctional folate-methionine pathway in the etiology of autism. There is also a need to determine whether functional benefits occur when correcting apparent deficits in folate-methionine metabolism in children with autism. *Am J Clin Nutr* 2010;91:1598–620.

## INTRODUCTION

Autism spectrum disorders (ASDs) are increasingly recognized as a public health issue. ASDs are characterized by impairments in reciprocal social interaction and communication and restricted interests as well as repetitive stereotypic behaviors (1). The term *autism spectrum disorder* encompasses autistic disorder, Asperger disorder, and pervasive development disorders—not otherwise specified. Over the past 20 y, the number of diagnosed cases has significantly increased. This has been partly attributed to broadening of the diagnostic criteria and increased community awareness (2).

Recent well-designed studies using whole-genome scanning methods indicate a key role for genetic factors in the etiology of autism (3–5). These studies have shown that multiple genes contribute to the wide range of symptoms observed in autism (6). A common aberration is not consistently seen in all autism cases, which suggests that it is a cluster of disorders with each having a distinct pathophysiology. In addition, environmental factors, including heavy metal toxicity (7–9), subclinical viral infections

(10), and gastrointestinal pathology (reviewed in references 11 and 12), have also been identified as contributing to autism.

## Folate and methionine metabolism and autism

A dysfunctional folate-methionine pathway has been identified in many individuals with autism. This pathway is crucial for DNA synthesis (13), DNA methylation (14), and cellular redox balance (15). As shown in **Figure 1**, methionine, an essential amino acid, is converted to *S*-adenosyl-methionine (SAM), the body's main methyl group donor, which is converted to *S*-adenosyl-homocysteine (SAH) during methylation reactions. Thus, plasma SAM:SAH indicates methylation status. SAH is later hydrolyzed to homocysteine in a reversible reaction releasing adenosine.

Homocysteine formed from methylation reactions is metabolized by 1 of 2 pathways. The first is the *trans*-sulfuration pathway, which involves the irreversible conversion of homocysteine to cysteine through cystathionine. Cysteine is the rate-limiting amino acid for the synthesis of glutathione, which plays a key role in detoxification processes (16). Total glutathione: oxidized glutathione in plasma is an indicator for oxidative stress (17). The second pathway involves the remethylation of homocysteine to methionine, which is carried out by methionine synthase (MS) in most tissues.

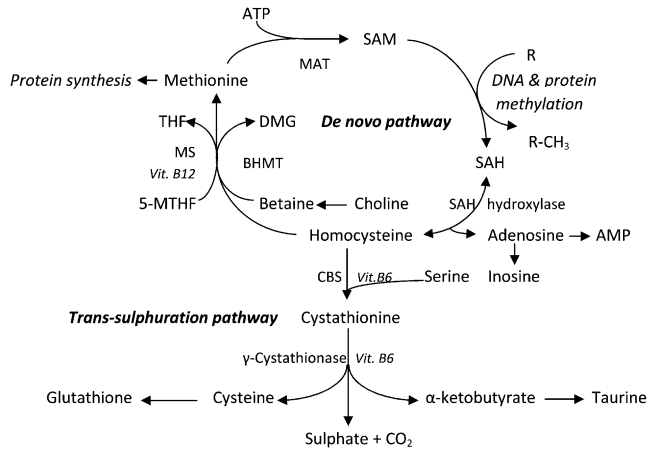
As shown in **Figure 2**, the methyl group for MS is donated by 5-methyltetrahydrofolate (5-MTHF), which is converted to tetrahydrofolate (THF). THF is methylated to become 5,10-methylene tetrahydrofolate (5,10-MTHF) either by serine hydroxyl-methyltransferase or a series of 3 reactions catalyzed by methyltetrahydrofolate dehydrogenase (MTHFD-1).

Most 5,10-MTHF is metabolized to 5-MTHF, the only form of folate used in the central nervous system (CNS) and the main form of folate in the blood, by methylene tetrahydrofolate reductase (MTHFR). The remaining 5,10-MTHF is converted to dihydrofolate (DHF) by thymidine synthase in the synthesis of thymidylate, which is required for DNA replication and may be converted back to THF by dihydrofolate reductase (DHFR).

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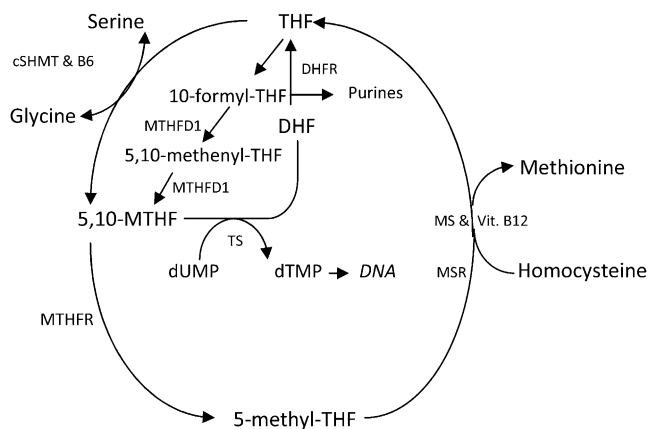
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**FIGURE 1.** Methionine cycle and *trans*-sulfuration pathway. SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine; THF, tetrahydrofolate; 5-MTHF, 5-methyl-tetrahydrofolate; R, DNA or protein; R-CH<sub>3</sub>, methylated DNA or protein; MAT, methionine adenosine transferase; MS, methionine synthase; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine B synthase; DMG, dimethylglycine; Vit. B6, vitamin B-6; Vit. B12, vitamin B-12.

Significant cytogenetic alterations in both lymphocytes and/or buccal cells have been found in other neurologic conditions, including Down syndrome, Parkinson disease, Alzheimer disease, and schizophrenia (18–21). Although it is plausible that folate deficiency increases chromosomal instability (22), there is currently no direct evidence that chromosomal DNA damage is the cause of neurodegenerative disease. Other plausible mechanisms for a role of folate deficiency in neurodegenerative diseases include impaired mitochondrial function due to mitochondrial DNA deletions, reduced availability of methyl groups from folate for neurotransmitter synthesis, and reduced proliferative potential of regenerative cells in critical regions of the brain caused by diminished nucleotide synthesis (23, 24).



**FIGURE 2.** Folate cycle. THF, tetrahydrofolate; 5-methyl-THF, 5-methyl-tetrahydrofolate; 5,10-MTHF, 5,10-methylene-tetrahydrofolate; DHF, dihydrofolate; 10-formyl-THF, 10-formyl-tetrahydrofolate; 5,10-methenyl-THF, 5,10-methenyl-tetrahydrofolate; dUMP, deoxy-uracil-monophosphate; dTMP, deoxy-thymidine-monophosphate; cSHMT, cyclo-serine-hydroxy methyl transferase; MTHFR, 5,10-methylene tetrahydrofolate reductase; MS, methionine synthase; MSR, methionine synthase reductase; MTHFD1, 5,10-methylenetetrahydrofolate dehydrogenase/5,10-methenyl-tetrahydrofolate cyclohydrolase/10-formyl-tetrahydrofolate synthetase; DHFR, dihydrofolate reductase; TS, thymidine synthase; B6, vitamin B-6; Vit. B12, vitamin B-12.

**Folate transport into the CNS**

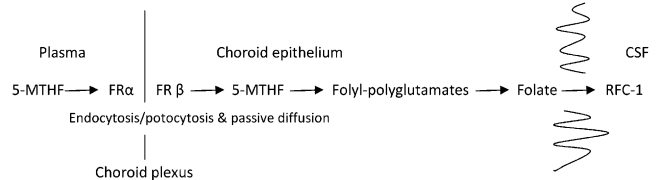
Folate transport through the choroid plexus is mainly mediated by a family of folate receptor (FR) proteins, and the reduced folate carrier 1 (RFC-1) (Figure 3) FR proteins located on the plasma side of the choroid plexus bind and transfer folate via endocytosis into the intracellular compartment where it is concentrated. The RFC-1 is located on the cerebrospinal fluid (CSF) side of the choroid plexus, where it facilitates transport of the concentrated folate into the CSF. Defective folate transport into the CNS has been linked with cerebral folate deficiency (CFD), a condition associated with developmental delays (with or without autistic features), providing plausibility for involvement of the folate-methionine pathway in the etiology of autism (reviewed in reference 25). This article systematically reviews the evidence for a role of the folate-methionine pathway in the etiology of autism, because, to our knowledge, no such article has been published to date.

**METHODS**

A systematic literature review was conducted to identify folate-methionine pathway studies in autism, including metabolite concentrations in blood, interventions directed at normalizing a dysfunctional pathway and genes, and related polymorphisms of the pathway. The search used the following electronic databases (all databases were accessed through our institution’s subscription, with the exception of The Cochrane Library): Embase, Medline, Cinahl, Scopus, Web of Science, International Pharmaceutical Abstracts, and the Cochrane database (available from <http://www.thecochranelibrary.com>). The reference lists for all obtained studies were hand-searched for additional studies.

The criteria for study inclusion were as follows: 1) studies in children with autistic disorder as described in the *Diagnostic and Statistical Manual of Mental Disorders: Revised Text* (DSM-IV-R) (1) or diagnosed by using a standard diagnostic instrument, eg, the Childhood Autism Rating Scale (CARS) (26); and 2) studies including data for receptors, carriers, metabolites, cofactors or genes of the folate-methionine pathway, and/or 3) interventions using metabolites or cofactors of the folate-methionine pathway. Only full-text English-language articles published between 1978 and October 2008 were included.

All potential studies identified were independently evaluated for inclusion by 2 primary reviewers (PM and MA). The primary reviewers were not blinded to the authors, institutions or source of publication at any time during the selection process. Disagreements about the inclusion/exclusion of studies were discussed and consensus achieved. Provision was made for a third reviewer if consensus was unattainable but did not prove necessary. A level of evidence was assigned to each study by using the Australian



**FIGURE 3.** Folate transport across the choroid plexus. 5-MTHF, 5-methyl-tetrahydrofolate; FR $\alpha$  and  $\beta$ , folate receptor  $\alpha$  and  $\beta$ ; RFC-1, reduced folate carrier-1; CSF, cerebral spinal fluid.

National Health and Medical Research Council criteria (27) (Table 1). The large number of variables and case definitions across studies prohibited statistical assessment of heterogeneity and meta-analysis.

## RESULTS

Forty-nine abstracts were identified via the electronic and hand-search strategy. Of these abstracts, 31 were ineligible for inclusion because they did not include data about the folate-methionine pathway, data about children with autism was not presented separately from other disorders, and/or because they were not written in the English language. Eighteen studies met the inclusion criteria, of which 17 provided data on metabolites or cofactors of the folate-methionine pathway, 5 provided the results of interventions, and 6 included genetic data.

A summary of studies that measured metabolites and/or cofactors of the folate-methionine pathway is shown in Table 2. Three studies presented data for multiple metabolites of the folate-methionine and *trans*-sulfuration pathways (28–30). Both studies by James et al (28, 29) showed that, with the exception of SAH and reduced glutathione, specific metabolites of the methionine and *trans*-sulfuration pathways were significantly decreased. The metabolites measured were methionine, SAM, homocysteine, cysteine, and total glutathione. The authors concluded that the resultant decrease in the SAM:SAH ratio indicates a decreased capacity for methylation in children with autism, and the total glutathione:oxidized glutathione ratio suggests that oxidative stress may play a role in the etiology of autism. In contrast, the study by Suh et al (30) showed no significant change in plasma metabolites of the folate-methionine and *trans*-sulfuration pathways; lower concentrations of SAM, cysteine, and glutathione; and significantly higher homocysteine concentrations in peripheral leukocytes when children with autism were compared with controls. The discrepancies may have been due to differences in methodology. James et al's studies

(28, 29) used HPLC/electrocoulometric detection and the other used liquid chromatography-linked tandem mass spectrometry.

Eleven studies measured plasma concentrations of amino acids associated with the folate-methionine pathway (28–38). The findings were inconsistent between studies. For example, 3 reported low methionine in plasma of children with autism (28–30), 2 others reported no association (30, 32), and another reported significantly increased concentrations (31). Three studies reported low concentrations of cysteine (28, 29, 31), whereas others reported no significant differences (30, 32) and, although James et al (28, 29) reported a decreased concentration of homocysteine, 2 later studies reported significantly increased concentrations of homocysteine (37, 38) and 2 reported no significant difference (30, 36).

Ten studies examined cofactors required for folate-methionine metabolism (31, 35–37, 39–44). Of these, 4 studies detected significantly higher serum vitamin B-6 in children with autism than in controls (31, 39, 41, 43), of which one also found elevated serum concentrations of riboflavin (39). In addition, a case study reported high vitamin B-12 in a child with autism and CFD (35); however, a later study found no significant difference in vitamin B-12 between children with autism and controls (37). None of the studies found any significant difference in serum or erythrocyte folate between children with autism and controls.

Five studies reported significantly low CSF folate concentrations together with normal serum folate concentrations in children with autism (35, 38, 40, 42, 44). High titers of FR1 antibodies were found in 19 of 23 children with autism and at least one symptom of CFD (44).

The findings of the 5 studies that reported the outcome of interventions (28, 35, 38, 42, 44) are presented in Table 3. A pilot study conducted in a small group of children with autism showed that supplementation with folic acid and betaine for 3 mo significantly normalized the methionine pathway metabolite profile in plasma, particularly the SAM:SAH ratio (28). The addition of vitamin B-12 to this regimen for an additional 1 mo in a subset of participants acted mainly on the *trans*-sulfuration pathway, improving the total glutathione:oxidized glutathione ratio, although it also led to further normalization of methionine metabolites. Quantitative psychometric measures were not, however, included in the study.

The remaining studies reported the effect of treatment with folic acid on low CSF concentrations of 5-MTHF in children with autism and at least one symptom of CFD (35, 37, 42, 44). The most autism-specific of these studies showed that treatment with folic acid resulted in improved autistic, motor, and other neurologic symptoms in young children (<3.5 y) and improvements in motor and neurologic symptoms in older children, although there was no change in autistic symptoms in the older age group (44).

Six studies examined genes of the folate-methionine pathway or folate transport system in children with autism, which are summarized in Table 4. The results from these studies were inconsistent. For example, an early study found that the *T* allele of the *MTHFR* 677C→T polymorphism was of significantly higher frequency in autistic patients than in controls ( $P < 0.0001$ ) (45). The homozygote *MTHFR* 1298A→C genotype ( $P = 0.0005$ ) and compound *MTHFR* 677C→T/1298A→C genotype ( $P = 0.01$ ) were also significantly associated with the condition. A subsequent larger study, however, failed to confirm

**TABLE 1**

Australian National Health and Medical Research Council designated levels of evidence<sup>1</sup>

Level of evidence	Description
I	Evidence obtained from a systematic review of all relevant randomized controlled trials.
II	Evidence obtained from at least one properly designed randomized controlled trial.
III-1	Evidence obtained from well-designed pseudo-randomized controlled trials (alternate allocation or some other method).
III-2	Evidence obtained from comparative studies with concurrent controls and allocation not randomized (cohort studies), case control studies, or interrupted time series with control group.
III-3	Evidence obtained from comparative studies with historical control, ≥2 single-arm studies, or interrupted time series without a parallel control group.
IV	Evidence obtained from a case series, either posttest or pretest and posttest.

<sup>1</sup> Data from reference 27.

**TABLE 2**  
Studies of metabolites or cofactors of the folate-methionine pathway in children with autism<sup>1</sup>

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Sankar, 1979 (39) (USA) Case control III-3	Cases = 19. Controls = 78 schizophrenic, 6 behavioral disturbance, 12 psychosis, 5 metabolic deficiency.	Male, age = 5–16 y, admitted to Child Psychiatric Research Unit, Creedmore State Hospital, USA. Inclusion: No supplementation for 3 wk before the study.	Onset from infancy with severe emotional isolation; failure to relate to objects and persons; failure to develop speech and communication. If speech present, it is a noncommunicative type. Stereotypy of motor behavior.	Serum folic acid, riboflavin, vitamin B-6, and vitamin C concentrations.	Results presented as mean serum concentrations for each vitamin $\pm$ SD compared with concentrations of published normal range. No measures of statistical significance. Serum riboflavin ( $P =$ 0.0029) and vitamin B-6 ( $P < 0.0001$ ) were significantly higher in cases than in controls.	Study was undertaken before a standardized definition of autism was available. Standard photometric and microbiological assays used; however, the method used to determine vitamin B-6 differed for cases and controls. Washout time for folate is 4 mo; therefore, the inclusion criteria do not preclude interference from prior folate ingestion. Conducted before recommendations for folate supplementation. Previous dietary intake and medications were not considered. Study unblinded.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Khaleeluddin and Philpott, 1980 (31) (USA) Case control III-3	Cases = 9 (plus 32 schizophrenics and 114 with chronic degenerative diseases).	Consecutive patients at the same clinic. Details of age, sex, etc not provided.	None provided.	Plasma and urinary amino acids. Serum and urinary vitamin concentrations.	Results presented as the percentage of cases with plasma or urinary vitamin and amino acid concentrations higher or lower than normative values (not provided). Methionine: 20% had high plasma concentrations and 33% low urinary concentrations. Cysteine: 83% had low plasma concentrations and 66% had low urine concentrations. Cystathione: 20% had high plasma concentrations, 33% had low urinary concentrations, and 33% had high urinary concentrations. Serum folic acid: 11% high, 11% low. Serum vitamin B-5: 11% high; serum vitamin B-6: 43% high.	Very small sample size (6/9 plasma 3/9 urinary amino acids). Standard photometric and microbiological tests. Previous dietary intake, supplementation and medications were not considered. Conducted before recommendations for folate supplementation. Study unblinded.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Lowe et al, 1981 (40) (USA) Case control III-2	Cases = 43. Controls = 59 children/ adolescents with other psychiatric or developmental disorders. 19 family and community members.	Cases aged 12.4 y (range: 4–22 y); controls: other psychiatric/ developmental disorders; age = 11.4 y (range: 2–32 y). Family and community members aged 26 y (range: 12–46 y).	DSM-III.	Serum and erythrocyte folate, serum vitamin B-12 concentrations. CSF folate and monoamine concentrations in a subgroup comprising 6 cases and 10 with nonautistic psychiatric or developmental disorders.	Results were presented as mean serum and erythrocyte concentrations for vitamins and CSF monoamines $\pm$ SDs and range. The correlation between serum folate and vitamin B-12 is shown by using Pearson's coefficient. Serum and erythrocyte folate and serum vitamin B-12 were normal compared with family and community controls. There was no correlation between them. There was no significant difference in CSF folate between children with autism and those with other psychiatric/developmental disorders.	Eight cases were taking folic acid supplements. These children had higher serum and erythrocyte folate concentrations. Serum and CSF folate concentrations were low compared with current reference values [AMH2008, Ramaekers et al (44)]. No information about previous dietary intake or medication. Conducted before recommendations for folate supplementation. The inclusion of family members and adults as controls was not appropriate. Study unblinded.
Visconti et al 1994 (32) (Italy) Case control III-2	Cases = 37. Controls = 19.	Controls: mean age = 7 y (range: 3–13 y). No neurologic/metabolic/psychiatric disorders.	DSM-III-R.	Serum and urinary amino acid concentrations.	Results presented as mean ( $\pm$ SD) serum and urinary concentrations. There were no statistically significant differences in plasma or urinary methionine or plasma cysteine between cases and controls or between cases with and without neurologic symptoms. Urinary cysteine concentrations were lower in children with autism and neurological abnormalities ( $P < 0.05$ ) than in controls.	Age and sex are identified as possible confounders. No information about previous dietary intake, medications, or supplementation. Study unblinded.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
D'Eufemia et al, 1995 (33) (Italy) Case control III-2	Cases = 40. Controls = 46.	Cases aged 12 y 4 mo (range: 7–17 y; 27 male, 13 female). Mean IQ = 68.1 (62–78) (Stanford and Binet scale), no epilepsy. Controls aged 11 y 2 mo (range: 5–15 y; 27 males, 19 females). Normal IQ, no personal or family history of psychiatric or neurologic disorders, healthy. All participants of normal height and weight range and on an unrestricted diet. No medications in the month before the study.	DSM-III-R.	Plasma amino acid concentrations. Tryptophan:LNAA ratio.	Results for plasma amino acid concentrations were presented as means $\pm$ SDs. Comparison of means was carried out by using the Mann-Whitney <i>U</i> test for nonparametric data. Spearman's test was used to calculate the correlation coefficients between the tryptophan:LNAA ratio and age, height, weight, and IQ. There was no significant difference in methionine or cysteine concentrations between cases and controls. The tryptophan:LNAA ratio was significantly lower in cases than in controls ( $P < 0.01$ ). No correlation between tryptophan:LNAA and age, height, weight, or IQ.	Small study. Cases sourced from Italian Association of Parents of Autistic Children. LNAA did not include methionine. Study unblinded.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Arnold et al, 2003 (34) (USA) Case control III-2	Cases = 36. Controls = 24.	Age <5 y. Cases: 10 gluten/casein-free diet and 26 unrestricted diet. Controls: age- and sex-matched children with other developmental delays.	DSM-IV-R autistic disorder, PDD-NOS CARS, Pervasive Developmental Disorders Screening Test.	Plasma amino acid concentrations. Tryptophan: LNAA ratio.	Results presented as mean ( $\pm$ SD) plasma amino acid concentrations and <i>P</i> values where significant. Bonferroni correction and Tukey's honestly significant difference test applied. Plasma methionine was significantly lower in children with autism with an unrestricted diet than in controls ( $P < 0.02$ ) but not when Bonferroni correction applied as the statistical significance was lowered to $P < 0.0028$ . No significant difference was found between any of the groups for tryptophan: LNAA.	Small study comprising a retrospective review of medical records. Source of controls not optimal. LNAA did not include methionine. No information provided about previous supplementation or medications. Study unblinded.
Adams et al, 2004 (41) (USA) Cohort III-3	Cases = 24 recruited for randomized controlled trial.	Cases aged $4.9 \pm 1.4$ y (range: 3–8 y; 22 males, 2 females). No prior supplementation.	Pooled ASDs diagnosed by psychiatrist or developmental pediatrician.	Serum vitamin B-6.	Results are presented as mean and median serum vitamin B-6 concentrations $\pm$ SDs with <i>P</i> values where significant. Vitamin B-6 concentrations were significantly higher in cases than in controls ( $P = 0.0000001$ ).	Small study size. Case definition not standardized. Standard micro-biological assay for vitamin B-6. Interpretation depends on reference values from the testing laboratory. Samples not measured simultaneously. Study unblinded.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
James et al, 2004 (28) (USA) Case control III-2	Cases = 20. Controls = 33.	Idiopathic (14 male, 6 female). 16/20: 400 $\mu$ g folic acid and 3 $\mu$ g vitamin B-12/d. Controls aged 7.4 $\pm$ 1.3 y; age- and sex-matched. Exclusions (both groups): medications known to affect methionine metabolism. Diagnosis of malnutrition, active infection, or genetic disease. Exclusions (controls): chronic disease.	DSM-IV.	Metabolites of the methionine/trans-sulfuration pathways in plasma.	Results presented as mean ( $\pm$ SD) metabolite concentrations and range. <i>P</i> values were provided where there were statistically significant differences. All metabolites were significantly lower in cases than in controls except for SAH, adenosine, and GSSH, which were higher. The SAM:SAH ratio decreased by 46%, and the tGSH:GSSH ratio decreased by 66%.	The source of the controls was not provided. No information about dietary intake. No details provided about OTC supplementation taken by controls. Study unblinded.
Moretti et al, 2005 (35) (USA) Case report IV	Cases = 1.	Females with CSF folate deficiency and autism followed from second day after birth to $\geq$ 6 y. Neonatal EEG showed multifocal seizure discharges; mild spasticity at 2-3 mo; developmental delay at 9 mo and regression at 3.5 y resulting in an inability to walk or feed orally.	ADOS, ADI-R, Bailey Scale of Infant Development, VABS.	Folate and vitamin B-12 in peripheral tissues. Vitamin B-12 in serum. MTHFR activity. CSF folate, SAM, SAH, and homocysteine.	Results presented as point values compared with normal range. MTHFR activity slightly lower than control reference. Serum vitamin B-12 concentrations high but normal in peripheral tissue. CSF folate and SAM low; homocysteine and SAH concentrations high.	Source of reference range not provided.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Ramaekers et al, 2005 (42) (Germany) Case control III-3	Cases = 5/28 cases of autism in children with idiopathic CFD. Controls = 28 age-matched. 41 unrelated CNS conditions; 5 mothers.	One child with autism and normal IQ diagnosed at 3 y. Four children with autism and mental retardation diagnosed at 2, 3, 5, and 12 y. The youngest also had intractable epilepsy.	ADOS.	Serum folate and CSF 5-MTHF concentrations.	Serum and CSF folate concentrations were presented as means and ranges. Blocking antibodies were presented with chi-square and <i>P</i> values. The mean titer and affinity constants were presented with ranges. Serum folate concentrations were normal. CSF folate concentrations in cases were significantly low before treatment and normalized after treatment.	Source of the age-matched controls was not provided. No correction for multiple comparisons.
James et al, 2006 (29) (USA) Case control III-2	Cases = 80. Controls = 73. Subjects from the 2004 study were not included.	Cases aged 7.3 ± 3.2 y (71 males, 9 females). Controls aged 10.8 ± 4.1 y. Age- and sex-matched controls from similar studies. Exclusions (both groups): medications and supplements known to affect methionine metabolism, known genetic disease, and childhood disintegrative disorders. Exclusions (control): chronic disease, autism, or other neurologic disorder.	DSM-IV, ADOS, CARS.	Plasma metabolites of the methionine and <i>trans</i> -sulfuration pathways.	Results were presented as means, SDs, and ranges for each metabolite with <i>P</i> values for statistically significant differences. All metabolites were significantly lower in cases than in controls except for SAH, adenosine, and GSSH, which were higher. The SAM:SAH ratio decreased by 27%, and the IGSH-GSSH ratio decreased by 48%. The proportion of cases with more clinically severe metabolic alterations was determined, and the findings are presented as percentages.	Relatively small study. No correction for multiple comparisons. The authors do not define clinical severity. Unblinded.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Pasca et al, 2006 (37) (Romania) Case control III-2	Cases = 12. Controls = 9.	Cases aged 8.3 ± 2.76 y (75% male, 25% female). Controls aged 8.3 ± 1.82 y (67% male, 33% female). Exclusions (both groups): medication/supplementation known to interfere with methionine metabolism in the previous 6 mo.	DSM-IV.	Plasma homocysteine and vitamin B-12 concentrations. Paraoxonase I activity in plasma and glutathione peroxidase activity in erythrocytes.	Homocysteine significantly higher in cases than in controls ( $F = 6.78, P = 0.01$ ). Statistically significant reduction of PON1 arylesterase activity in cases compared with controls ( $F = 10.37, P = 0.005$ ). No significant difference in paraoxonase I or glutathione peroxidase activity. Negative correlation between glutathione peroxidase activity and homocysteine ( $r = -0.769, P = 0.023$ ) when age and sex used as control variables.	Very small sample size. Source of cases and controls not provided. No information about treatment of samples before analysis. Homocysteine concentrations will increase if the samples are not placed immediately on ice. Scheffe correction for post hoc pairwise comparisons applied. Study unblinded.
Adams et al, 2006 (43) (USA) Case control III-2	Cases = 11 + 24 from previous study. Controls = 11.	Cases aged 7.2 ± 1.4 y (8 males, 3 females). Previous study age = 4.9 ± 1.4 y (22 males, 2 females). Controls aged 7.8 ± 1.2 y (10 males, 1 female). Exclusions: supplementation with vitamin B-6 in previous 2 mo. Inclusion (cases): no family history of ASDs, good mental and physical health.	Diagnosis of autism, PDD-NOS, or Asperger syndrome from a psychiatrist or developmental pediatrician.	Serum vitamin B-6 concentrations.	Results presented as mean and median serum vitamin B-6 concentrations ± SDs with $P$ values where significant. Vitamin B-6 concentrations were significantly higher in cases than in controls ( $P = 0.001$ ).	Small sample size. Case definition not standardized. Standard microbiological assay for phosphorylated and unphosphorylated forms of vitamin B-6. Results not stratified by type of ASD. Samples blinded for measurement.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Adams et al, 2007 (36) (Australia) Case control III-2	Cases = 17. Controls = 16.	Cases aged 2–16 y. Exclusions: PDD-NOS and Asperger syndrome.	DSM-IV, ADOS, Adaptive Behavioral Assessment Questionnaire.	Folate metabolites.	Results for metabolites presented as mean concentrations $\pm$ SDs and <i>P</i> values. No significant difference was detected for homocysteine, serum, or erythrocyte folate or vitamin B-12.	Small sample numbers. Source of cases and controls not provided. No information about dietary intake, supplementation, or medications. Homocysteine concentrations will increase if the samples are not placed immediately on ice. Study unblinded.
Ramaekers et al, 2007 (44) (Germany) Case series IV	Cases = 25.	Cases aged 6.88 y (2.8–12.3 y; 18 males, 7 females). Autism plus one or more features of CFD. Controls aged 6.76 y (3.3–11.4 y; 14 males, 11 females). Exclusions: infections during pregnancy, birth/neonatal injuries, hearing deficits, inborn errors of metabolism, known genetic abnormalities, and defects of intracellular signaling.	DSM-IV, ADOS, ADI, VABS.	Serum folate concentrations. CSF 5-MTHF concentrations. Blocking folate receptor autoantibodies in serum.	Results of the serum and CSF folate concentrations were presented as means with a test statistic and <i>P</i> value. No significant difference was found in serum folate ( <i>t</i> = 0.76) between cases and controls. CSF 5-MTHF was significantly lower in cases before treatment ( <i>t</i> = 7.77, <i>P</i> < 0.0001).	It is not clear whether the cases presented in Ramaekers et al (42) are included. No information about dietary or supplemental folate intake. Study unblinded.
Moretti et al, 2008 (38) (USA) Case series IV	Cases = 7. Patient 1 = previous case report.	Cases aged 8, 9, 8, 10, 7, 2, and 15 y. CFD plus autism (includes Moretti et al; 28); 5 children had severe and 2 had profound developmental delays.	ADOS, ADI-R, Bailey, VABS.	CSF 5-MTHF concentrations.	Results for CSF 5-MTHF concentrations were presented as point values compared with normal range. CSF folate was significantly low for all cases at diagnosis.	Small sample size. No information about supplements or medication. Cases older than Ramaekers et al (42, 44). Pre- and postintervention behavioral scores not presented. Unblinded.

(Continued)

TABLE 2 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Suh et al, 2008 (30) (USA) Case control III-2	Cases = 31. Controls = 11.	Cases aged $4.17 \pm 1.3$ y (26 male, 4 female); 70% GI problems; 38.5% pica; 40.7% delayed motor development; 64.3% delayed speech development. Controls aged $6.9 \pm 1.6$ y (9 male, 2 female). Exclusions (both): nutritional or antioxidant supplementation in the previous 6 mo. Exclusions (cases): chronic disease; acute illness in prior 2 wk; psychiatric, antiinflammatory, or chelation therapy. Exclusions (controls): family or personal history of autism, medications.	ASDs defined by DSM-IV plus ADI-R.	Methionine cycle and <i>trans</i> -sulfuration pathway metabolites in leukocytes and plasma.	Leukocyte metabolite concentrations presented as a Forest plot for each metabolite that included the mean score and, where significant, the <i>P</i> value. Plasma metabolite concentrations were presented as means ( $\pm$ SDs) for individual metabolites and <i>P</i> values where significant. Leukocyte SAM concentrations were significantly lower in cases than in controls ( <i>P</i> = 0.03), reducing the SAM:SAH ratio by 50%. Leukocyte homocysteine was significantly higher in cases than in controls ( <i>P</i> = 0.03) and cysteine + cysteine and GSH significantly lower ( <i>P</i> = 0.004 and <i>P</i> = 0.02, respectively). There was no significant difference in plasma metabolites between cases and controls except for cysteinylglycine, which was higher in cases ( <i>P</i> = 0.0008).	Small sample size. Source of cases and controls not provided. Case definition does not distinguish between different types of ASDs. Details of treatment of blood samples immediately after venipuncture not provided. Homocysteine concentrations are likely to increase if the samples are not immediately placed on ice. Forest plot hard to interpret. Some significant values were presented in the text. Study unblinded.

<sup>1</sup> tGSH, total glutathione; GSSH, oxidized glutathione; CFD, cerebral folate deficiency; GI, gastrointestinal; CSF, cerebrospinal fluid; SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine; DSM-III, DSM-III-R, DSM-IV, and DSM-IV-R, Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, 3rd edition, 3rd edition, and 4th edition revised, 4th edition, and 4th edition revised, respectively; CARS, Childhood Autism Rating Scale; 5-MTHF, 5-methyltetrahydrofolate; MTHFR, methylene tetrahydrofolate reductase; OTC, over the counter; CNS, central nervous system; IQ, intelligence quotient; ASD, autism spectrum disorder; LNAA, large neutral amino acids; EEG, electroencephalograph; PDD-NOS, pervasive developmental disorder—not otherwise specified; VABS, Vineland Adaptive Behaviour Scale; ADOS, Autism Diagnostic Observation Schedule; ADI-R, Autism Diagnostic Interview—revised; AMH, Australian Medicines Handbook.

**TABLE 3**  
Studies reporting interventions of the folate-methionine pathway in children with autism'

Reference and country	No. of subjects	Patient characteristics	Intervention	Outcome measure	Results	Comments
James et al, 2004 (28) (USA)	Cases = 8	Most severely abnormal metabolic profile from study of 20 children (see Table 1).	Intervention 1: 800 µg folic acid and 1000 µg betaine twice daily for 3 mo. Intervention 2: additional month on same regimen plus an injection of 75 µg vitamin B-12/kg twice weekly.	Metabolites of the methionine/trans-sulfuration pathways in plasma.	The first intervention normalized plasma methionine, SAM, SAH, adenosine, and homocysteine concentrations. In addition, tGSH, GSSH, and tGSH:GSSH were significantly improved but not normalized. The addition of vitamin B-12 normalized cysteine, tGSH, and GSSH concentrations as well as the tGSH:GSSH ratio. It also further increased the SAM:SAH ratio ( $P = 0.04$ ) and decreased the concentration of adenosine ( $P = 0.002$ ).	Very small sample size for the intervention. Criteria for inclusion in the intervention group were not provided. Bonferroni correction was only applied to compare individual metabolites after each intervention. The results were not linked to objective changes in behavior. Study was unblinded.
Moretti et al, 2005 (35) (USA)	Cases = 1	See Table 1.	0.5 mg folic acid/kg daily for 2 wk then 1.0 mg/kg daily for 3 mo for 1 y beginning at age 6 y.	CSF metabolites including those of the methionine cycle before and after intervention. EEG before and after intervention. Behavioral changes.	Treatment with folic acid normalized CSF metabolites and improved motor control. Epileptiform discharges ceased. No change to the symptoms associated with autism.	Source of normal range not provided. Dose of intervention based on previously published reports.

(Continued)

TABLE 3 (Continued)

Reference and country	No. of subjects	Patient characteristics	Intervention	Outcome measure	Results	Comments
Ramaekers et al, 2005 (42) (Germany)	Cases = 5/28 cases of autism in children with idiopathic CFD.	See Table 1.	The child with a normal IQ who was treated with a multivitamin with 400 µg folic acid daily. The rest of the children were treated with 0.5–1.0 mg folic or folic acid/kg daily on an ongoing basis.	CSF 5-MTHF concentrations before and 6 mo after the intervention. Blocking folate receptor antibodies in serum.	Serum folate concentrations were normal. CSF folate concentrations in cases were significantly low before treatment and normalized after treatment. The child with a normal IQ had no blocking FR antibodies. The other cases had very high titers of blocking FR antibodies. The child with a normal IQ recovered completely. Treatment improved communication skills and neurologic abnormalities in the 2 younger children with mental retardation, whereas the older children remained autistic. Epileptic seizures were fully controlled with folic acid.	No correction for multiple comparisons. The results were not linked to objective changes in behavior.

(Continued)

TABLE 3 (Continued)

Reference and country	No. of subjects	Patient characteristics	Intervention	Outcome measure	Results	Comments
Ramaekers et al, 2007 (44) (Germany)	Cases = 25	See Table 1.	1–3 mg folic acid/kg daily (starting dose: 1 mg/kg daily). If CSF 5-MTHF still low after 3–6 mo, dose increased to 2–3 mg/kg daily.	CSF 5-MTHF concentrations before and after intervention. Blocking folate receptor auto-antibodies in serum.	CSF 5-MTHF significantly lower in cases before treatment ( $t = 7.77$ , $P < 0.0001$ ) and normalized after treatment. Nineteen of 25 cases had blocking FR auto-antibodies in serum. The titer was significantly high in these subjects ( $P < 0.0001$ ); 17/19 of these cases also had neurologic deficits. Spontaneous normalization of auto-antibodies and CFD in 1 patient, 1 lost to follow-up, no results available for 3 patients. Two patients (diagnosed at 2 and 3.2 y) had complete recovery of neurologic symptoms and autism. Thirteen patients (diagnosed at 3–7 y) had improved neurology and partial recovery of autism. Three older patients (diagnosed at 4.9, 8, and 11.9 y) showed partial recovery of neurology and no change in autism.	It is not clear whether the cases presented by Ramaekers et al (42) are included. No information about dietary or supplemental folate intake. Unblinded.

(Continued)

TABLE 3 (Continued)

Reference and country	No. of subjects	Patient characteristics	Intervention	Outcome measure	Results	Comments
Moretti et al, 2008 (38) (USA)	Cases = 7	See Table 1.	0.5 mg folic acid/kg daily for 2 wk then 1.0 mg/kg daily for 3 mo.	CSF 5-MTHF concentrations before and after intervention.	Results for CSF 5-MTHF concentrations were presented as point values compared with normal range. CSF folate was significantly low for all cases at diagnosis and normalized after treatment with folic acid. Treatment led to improvements in cognition, language, and motor domains with no change in autistic symptoms. Improved seizure control in 2/6.	Small sample size. No information about supplements or medication. Cases older than those in the study by Ramaekers et al (42, 44). Pre- and postintervention behavioral scores not presented. Unblinded.

<sup>†</sup> tGSH, total glutathione; GSSH, oxidized glutathione; CFD, cerebral folate deficiency; CSF, cerebrospinal fluid; SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine; 5-MTHF, 5-methyl-tetrahydrofolate; IQ, intelligence quotient; EEG, electroencephalogram; FR, folate receptor.

these associations (29), although a later case report of a child severely affected with autism plus CFD showed that the child was homozygous for the *MTHFR* 677C→T allele and heterozygous for *MTHFR* 1298A→C (35).

In addition, a borderline association with autism was detected for the 19 base pair (bp) deletion of the dihydrofolate reductase (*DHFR*) gene [odds ratio (OR): 2.69; 95% CI: 1.00, 7.28;  $P < 0.05$ ] which is involved in folate metabolism (36). Significant associations were also found in this study for this polymorphism in combination with *MTHFR* 677C→T (OR: 2.09; 95% CI: 1.04, 4.18;  $P < 0.04$ ), *MTHFR* 677C→T and *MTHFR* 1298A→C (OR: 1.64; 95% CI: 1.0, 2.69;  $P < 0.05$ ), and *MTHFR* 677C→T and *RFC-1* 80G→A (OR: 1.8; 95% CI: 1.02, 3.18;  $P = 0.04$ ) (25). These findings have not been confirmed to date.

The findings for genes involved in folate transport were also inconsistent. Although the largest study to date found a significant association between *RFC-1* 80G→A and autism (OR: 2.13; 95% CI: 1.4, 3.4) (29), a subsequent study failed to replicate the findings (37). On the other hand, an association was found between the 19-bp deletion of *DHFR* and *RFC-1* with autism (36). Other studies have not found any mutations in genes involved in folate transport (35, 38, 44).

## DISCUSSION

Although the findings of this review indicate inconsistencies between studies, they suggest that the folate-methionine pathway may play a role in the etiology of autism; however, further study is necessary before any definitive conclusions can be made.

## Methionine cycle

The largest studies to date that have measured concentrations of the metabolites of the methionine cycle in plasma found that methionine, SAM, and homocysteine were significantly lower and SAH was significantly higher in children with autism than in controls (28, 29). Although a later study showed no significant differences between children with autism and controls, the number of participants was much lower, which suggests that the findings may be less reliable (30). The same study identified differences in methionine cycle metabolites from peripheral lymphocytes; however, the presentation of the findings together with the low sample numbers have made interpretation of the data difficult.

Inconsistencies were found between studies in plasma concentrations of all amino acids associated with the methionine-transulfuration pathways. One reason for this may be methodologic differences between studies. For example, homocysteine is released from blood cells into plasma at ≈10% h at room temperature (46). Samples, therefore, should be immediately put on ice and the plasma separated out or homocysteine concentrations will be artificially high. The 3 studies that showed higher concentrations of homocysteine in children with autism than in controls do not provide details of how the samples were handled immediately after they were taken (30, 36, 37). Both publications by James et al (28, 29), however, indicate that the samples were immediately placed on ice, which lends credence to their findings.

**TABLE 4**  
Studies of genes of the folate-methionine pathway in children with autism<sup>1</sup>

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Boris et al, 2004 (45) (USA) Case control III-3	Cases = 168.	142 male, 26 female. 73.6% autistic disorder, 28.2% PDD-NOS. 149 regressive, 19 idiopathic. All white.	DSM-IV autistic disorder or PDD-NOS.	<i>MTHFR</i> polymorphisms 677C→T and 1298A→C	Results presented as the number and proportion (%) of children with each genotype and <i>P</i> values where statistically significant. 677C→T polymorphisms were significantly associated with cases ( <i>P</i> < 0.0001). 1298AA (wild type) was more prevalent in cases than in controls ( <i>P</i> = 0.0005) and 1298 A→C was more prevalent in controls than in cases ( <i>P</i> = 0.04). Compound heterozygote 677C→T/1298A→C was significantly more prevalent in cases than in controls ( <i>P</i> = 0.01). Results for <i>MTHFR</i> polymorphism presented as photograph of electrophoresis. The patient was homozygous for <i>MTHFR</i> 677C→T and heterozygous for <i>MTHFR</i> 1298A→C. <i>MTHFR</i> activity was low. No mutations of <i>RFC1</i> or <i>FBP1</i> were detected.	Genotyping performed in 3 different laboratories. Population concentration data from 2 different studies used for controls. Hard to generalize because of a high degree of variability between ethnic groups. The findings are not stratified by type of ASDs. No correction for multiple comparisons.
Moretti et al, 2005 (35) (USA) Case report IV	Cases = 1.	See Table 1.	ADOS, ADI-R.	<i>MTHFR</i> polymorphisms 677C→T and 1298A→C. Mutations in folate receptors.	Results for <i>MTHFR</i> polymorphism presented as photograph of electrophoresis. The patient was homozygous for <i>MTHFR</i> 677C→T and heterozygous for <i>MTHFR</i> 1298A→C. <i>MTHFR</i> activity was low. No mutations of <i>RFC1</i> or <i>FBP1</i> were detected.	

(Continued)

TABLE 4 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
James et al, 2006 (29) (USA) Case control III-2	Cases = 360. Controls = 205.	See Table 1; 97% cases and 100% controls white.	DSM-IV-R, ADOS, CARS.	Genes of the methionine and <i>trans</i> -sulfuration pathways. <i>RFC-1</i> , <i>MTHFR</i> , <i>MTRR</i> , <i>TCN2</i> , <i>COMT</i> , <i>GSTM-1</i> null, <i>GCPII</i> .	Results presented as number of subjects, ORs, 95% CIs, and <i>P</i> values for each allele or gene combination. <i>RFC-1 80A</i> → <i>G</i> was significantly associated with autism (OR: 2.13; 95% CI: 1.4, 3.4). Significant gene-gene interactions found for: <i>TCN2 777C</i> → <i>T</i> ; <i>COMT472G</i> → <i>A</i> (OR: 7.0; 95% CI: 2.32, 21.2); <i>RFC-1 80A</i> → <i>G</i> ; <i>MTRR 677C</i> → <i>T</i> ; <i>RFC-1 80A</i> → <i>G</i> ; <i>GSTM-1</i> null.	Relatively small study. No correction for multiple comparisons. Unblinded.

(Continued)

TABLE 4 (Continued)

Reference and study type (country)	No. of subjects	Patient characteristics	Case definition	Outcome measures	Results	Comments
Adams et al, 2007 (36) (Australia) Case control III-2	Cases = 17. Controls = 16.	See Table 1.	DSM-IV, ADOS, Adaptive Behavioral Assessment Questionnaire.	Genes of the folate/methionine pathway. 19-bp del-DHFR, MTHFR, MS, MSR, GCPII, RFC-1.	Results presented as the number with the wild-type and mutant form plus the ORs, 95% CIs, and <i>P</i> values where the difference was statistically significant. A borderline association was detected for the 19-bp del-DHFR gene (OR: 2.69; 95% CI: 1.00, 7.28; <i>P</i> < 0.05). No significant associations were detected for RFC 80 A→G, MTHFR 677C→T, 1298A→C, MSR 756A→G, MSR 66A→G, or GCPII 1561C→T. Borderline associations were detected for 19-bp-del-DHFR and MTHFR 677 (OR: 2.09; 95% CI: 1.04, 4.18; <i>P</i> < 0.04). MTHFR 677/MTHFR 1298 (OR: 1.64; 95% CI: 1.0, 2.69; <i>P</i> < 0.05); and MTHF 677/RFC 80 (OR: 1.8; 95% CI: 1.02, 3.18; <i>P</i> = 0.04).	Small sample numbers. Source of cases and controls not provided. Unblinded.
Ramaekers et al, 2007 (44) (Germany) Case control III-3	Cases = 25.	See Table 1.	DSM-IV, ADOS, ADI, VABS.	Mutations of folate receptor genes.	Coding exons of <i>FRI</i> and <i>FR2</i> showed normal sequences.	Small sample size. Study unblinded.
Moretti et al, 2008 (38) (USA) Case series IV	Cases = 7.	See Table 1.	ADOS, ADI-R, Bailey, VABS.	Mutations of genes involved in folate metabolism.	There were no mutations of folate receptor genes ( <i>RFC-1</i> , <i>FRI</i> , <i>FR2</i> , <i>PCFT</i> ), <i>MTHFR</i> , <i>DHFR</i> , or forminotransferase cyclodeaminase.	Small sample size. Study unblinded.

<sup>1</sup> DSM-IV and DSM-IV-R, Diagnostic and Statistical Manual of Mental Disorders, 4th edition, and Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised; CARS, Childhood Autism Rating Scale; OR, odds ratio; PDD-NOS, pervasive developmental disorders—not otherwise specified; ADOS, Autism Diagnostic Observation Schedule; ADI-R, Autism Diagnostic Interview—revised; VABS, Vineland Adaptive Behaviour Scale. MTHFR, methylene tetrahydrofolate; ASDs, autism spectrum disorders; bp, base pair.

In addition, James et al (29) and Adams et al (36) reported borderline associations between some genes of the methionine pathway and autism. The power for both studies, however, was insufficient to provide more than an indication of possible gene associations and neither corrected probability values for chance effects due to multiple comparisons.

### Folate cycle

Folate metabolism is complex. 5-MTHF and vitamin B-12 are required for the conversion of homocysteine into methionine by MS. Low MS activity could lead to an accumulation of 5-MTHF, and intracellular folate retention may be impaired. No significant association between polymorphisms of MS and autism, however, has been shown (36). Furthermore, various studies have reported vitamin B-12 and erythrocyte folate concentrations are normal in children with autism (31, 36, 39, 40). Unfortunately, these studies did not identify or control for potential confounders, such as age, neurologic symptoms, or supplementation with cofactors for the folate-methionine pathway. None of the studies reported on plasma concentrations of methylmalonic acid, which is the functional indicator of vitamin B-12 status.

Many biomedical interventions for treating autism have been touted, although most lack an evidence base (47). Whereas James et al (28) showed that supplementation with folinic acid and betaine normalized the plasma concentrations of metabolites in the methionine pathway, and the addition of vitamin B-12 further improved these concentrations, significant autism behavioral outcomes were not measured or observed. On the other hand, supplementation with folinic acid led to improved CFS folate status and remarkable cognitive, motor, and neurologic changes in 15 of 18 children with low-functioning autism and at least one symptom of CFD (44). This was particularly apparent in younger children, which suggests that damage caused by metabolic dysfunction over time has a degree of irreversibility. The ability to replicate this result by showing an association of neurologic benefits with changes in CSF folate concentration is limited, however, because obtaining CSF folate is a highly invasive procedure.

Vitamin B-6 is required for the conversion of THF to 5,10-MTHF and homocysteine to cysteine via cystathionine. The significant increases in serum vitamin B-6 observed in children with autism (31, 40, 41, 43) could reflect diminished cellular uptake or inefficacy of cells to retain or store vitamin B-6. Impaired bioavailability of vitamin B-6 may affect the nervous system because it is required for the synthesis of neurotransmitters, including serotonin, dopamine, and taurine (48).

Folate metabolism can also be impaired by 2 polymorphisms of MTHFR, *MTHFR 677C* → *T*, and *MTHFR 1298A* → *C*, which lower enzyme activity, reduce DNA methylation, and possibly increase chromosomal instability (49–51). MTHFR is a pivotal enzyme that catalyses the reduction of 5,10-MTHF into 5-MTHF, which is the major circulating form of folate, and acts as a methyl donor in the remethylation of homocysteine to methionine. Whereas Boris et al (45) reported a significant association for the homozygote *MTHFR 677C* → *T* and the compound heterozygote *MTHFR 677C* → *T*/*1298A* → *C* and autism, other studies did not confirm the association (29, 36), which may be affected by folate and riboflavin status. The role

of this enzyme in autism, therefore, remains unclear; however, again, the studies lacked the power needed to more than indicate a potential association and did not correct for multiple comparisons. Furthermore, Boris et al (45) used genotype data from 2 different sources outside of the study population as controls, which means that cases and controls were not truly matched.

No studies were found that examined the association between MTHFD, an enzyme that catalyses 3 sequential reactions in the interconversion of one-carbon derivatives of tetrahydrofolate, and autism. A borderline association was, however, reported between a 19-bp deletion of *DHFR* and autism (36). *DHFR* maintains the reduced form of folate required for de novo synthesis of methionine and thymine; however, as noted above, this study lacked power and did not correct for multiple comparisons, which made the association tenuous.

James et al (29) reported a significant association between *RFC-1 80G* → *A* (OR: 2.13; 95% CI: 1.3, 3.4), and Adams et al (36) reported a borderline association between *RFC-1 80G* → *A* and 19-bp del-*DHFR* (OR: 1.8; 95% CI: 1.02, 3.18) and autism. Their findings suggest that folate transport may be involved in the development of autism; however, as discussed above, both studies lacked power and did not correct for multiple comparisons.

High titers of auto-antibodies to FR1 were reported in children with low-functioning autism and at least one symptom of CFD (44), although mutations in FR1 or FR2 were not found and mothers did not have the antibody, which led the authors to speculate that it may have been formed from milk protein (38, 44). The plausibility of an association of FR1 auto-antibodies with neural deficits is supported by the observations of antibodies against placental FR proteins being associated with neural tube defects (52).

### Conclusions

A better understanding of the metabolic basis of autism has the potential to guide the development of a laboratory-based “test” to diagnose autism, predict the outcome of disease, and assign the most appropriate intervention. Although the findings of this review do not conclusively implicate a dysfunctional folate-methionine pathway in the etiology of autism, the topic clearly deserves scrutiny. Any review of evidence will be confounded by the heterogeneity of autism, sampling issues, and the wide range of analytic techniques used. Given the increase in community awareness of autism in recent years and the consequent increased focus on autism research, the findings of the more recently published studies are likely to be more reliable, although they are still inconsistent. These findings suggest that changes in the concentrations of metabolites of the methionine cycle may be driven by abnormalities in folate transport and/or metabolism. Almost all of the genetic association studies that have examined the genes of this metabolic pathway were under powered. As autism is a complex genetic disease, the relative risk conferred by each disease-associated allele is likely to be small; therefore, large patient and control groups are required for statistical significance. Furthermore, many of the studies examined multiple polymorphisms and their interactions without correcting for multiple comparisons may have been better analyzed by using logistic regression analysis.

Whereas supplementation can normalize the concentrations of the folate-methionine metabolites, whether or not normalization



affects objective behavioral measures needs to be determined to assess the clinical relevance. Supplementation has been shown to be most effective in improving autistic behavior, motor, and neurologic symptoms in younger children (aged <3 y) with low-functioning autism and CFD (44); however, it remains to be seen whether this holds for children with autistic disorder without CFD. Large-scale studies that link normalization of metabolite concentrations with genetic polymorphisms and objective behavioral measures are needed to address these issues. In addition, a large-scale retrospective survey should be conducted in mothers of children with and without autism to ascertain the association level of folate supplementation before and during pregnancy with the risk of having a child with autism and whether susceptibility genes in the folate-methionine pathway modify such a risk if present. Overall, this review concluded that evidence suggests a role for the folate-methionine pathway in autism and suggests some future directions for research.

The authors' responsibilities were as follows—PAEM: planned, researched, and drafted the manuscript; and CEO, MF, PT, and MTA: reviewed the manuscript. There were no potential conflicts of interest.

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