Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism1,2

S Jill James, Paul Cutler, Stepan Melnyk, Stefanie Jernigan, Laurette Janak, David W Gaylor, and James A Neubrander

ABSTRACT

Background: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced by genetic and environmental factors. Although abnormal metabolism of methionine and homocysteine has been associated with other neurologic diseases, these pathways have not been evaluated in persons with autism.

Objective: The purpose of this study was to evaluate plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism.

Design: Plasma concentrations of methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, and oxidized and reduced glutathione were measured in 20 children with autism and in 33 control children. On the basis of the abnormal metabolic profile, a targeted nutritional intervention trial with folinic acid, betaine, and methylcobalamin was initiated in a subset of the autistic children.

Results: Relative to the control children, the children with autism had significantly lower baseline plasma concentrations of methionine, SAM, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of SAH, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation (significantly lower ratio of SAM to SAH) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism. The intervention trial was effective in normalizing the metabolic imbalance in the autistic children.


KEY WORDS Autistic disorder, biomarkers, oxidative stress, methylation, methionine, S-adenosylmethionine, S-adenosylhomocysteine, adenosine, cysteine, glutathione

INTRODUCTION

Autism is a neurodevelopmental disability that is usually diagnosed before age 3 y and is characterized by deficits in social reciprocity and in language skills that are associated with repetitive behaviors and restricted interests (1). In addition to behavioral impairment, autistic persons have a high prevalence of gastrointestinal disease and dysbiosis (2), autoimmune disease (3), and mental retardation (4). Autism also affects many more males than females, occurring at a ratio of 4:1. A significant role for genetics in the etiology of the autistic disorder is supported by a high concordance of autism between monozygotic twins and increased risks among siblings of affected children and of autistic symptoms associated with several heritable genetic diseases [see: Online Mendelian Inheritance in Man (OMIM) #209850 (autism; 5)]. Autism has been reported to be a comorbid condition associated with Rett syndrome (5), fragile X (6), phenylketonuria (7), adenylosuccinate lyase deficiency (8), dihydouracil dehydrogenase deficiency (9), and 5′-nucleotidase hyperactivity (10); however, these genetic diseases account for <10% of cases of autism. Nonetheless, the association of autism with genetic deficits in specific enzymes suggests the possibility that the genetic component of primary autism could be expressed as a chronic metabolic imbalance that impairs normal neurodevelopment and immunologic function. The possibility that autism has a metabolic phenotype is less widely accepted but has been supported by several small studies (9, 11–14).

The current study was prompted by the serendipitous observation in a previous study that the metabolic profiles of dizygotic twins—one with Down syndrome and one with autism—were virtually identical with respect to methionine cycle and transsulfuration metabolites (15). Down syndrome, or trisomy 21, is a complex genetic and metabolic disease due to the presence of 3 copies of chromosome 21 and associated with an increased frequency of autism (16). In our previous study, children with Down syndrome had lower concentrations of metabolites in the methionine cycle and significantly lower glutathione concentrations than did control children (15).

The methionine cycle involves the regeneration of methionine via the vitamin B-12–dependent transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine in the methionine synthase reaction. Methionine may then be activated by methionine adenosyltransferase to form S-adenosylmethionine (SAM), the primary methyl donor for most cellular methyltransferase reactions including the methylation of DNA, RNA, proteins, phospholipids,
and neurotransmitters (Figure 1). The transfer of the methyl group from SAM to the various enzyme-specific methyl acceptors results in the formation of S-adenosylhomocysteine (SAH). The reversible hydrolysis of SAH to homocysteine and adenosine by the SAH hydrolase (SAHH) reaction completes the methionine cycle. Adenosine is further metabolized by adenosine kinase for purine synthesis or catabolized by adenosine deaminase (ADA). Homocysteine may be permanently removed from the methionine cycle by irreversible conversion to cystathionine by vitamin B-6-dependent cystathionine β-synthase (CBS). Cystathionine is converted to cysteine, which is the rate-limiting amino acid for the synthesis of the tripeptide glutathione (Glu-Cys-Gly). THF, tetrahydrofolate; 5-CH₃ THF, 5-methyltetrahydrofolate; SAHH, SAH hydrolase.

FIGURE 1. The methionine cycle involves the remethylation of homocysteine to methionine by either the folate–vitamin B-12–dependent methionine synthase (MS) reaction or the folate–vitamin B-12–independent betaine homocysteine methyltransferase (BHMT) reaction. Methionine is then activated by methionine adenosyltransferase (MAT) to S-adenosylmethionine (SAM), the major methyl donor for cellular methylenetransferase (MTase) reactions. After methyl group transfer, SAM is converted to S-adenosylhomocysteine (SAH), which is further metabolized in a reversible reaction to homocysteine and adenosine. Adenosine may be phosphorylated to adenosine nucleotides by adenosine kinase (AK) or catabolized to inosine by adenosine deaminase (ADA). Homocysteine may be permanently removed from the methionine cycle by irreversible conversion to cystathionine by vitamin B-6–dependent cystathionine β-synthase (CBS). Cystathionine is converted to cysteine, which is the rate-limiting amino acid for the synthesis of the tripeptide glutathione (Glu-Cys-Gly). THF, tetrahydrofolate; 5-CH₃ THF, 5-methyltetrahydrofolate; SAHH, SAH hydrolase.

None of the autistic children were taking prescribed medicines, such as valproic acid or anticonvulsants, that might have affected methionine metabolism. A quantifiable diet questionnaire was not administered as part of this study; thus, specific dietary differences within and between groups cannot be determined. The control subjects in the metabolic study were healthy white US children with no history of chronic disease or autism who had participated in a similar baseline study of children with Down syndrome (15). Control children took over-the-counter vitamin supplements and were not taking medications known to interfere with methionine metabolism. Exclusion criteria for both groups included a diagnosis of malnutrition, the presence of active infection, or known genetic disease.

The protocol and informed consent for this study were reviewed and approved by the Institutional Review Board at the University of Arkansas for Medical Sciences. The details of the study were explained to the parents of the participating children, and written informed consent was obtained from the parents.

Study design

The metabolic study consisted of 3 parts. In the first component, baseline concentrations of plasma metabolites in the methionine cycle and transsulfuration pathway were measured in 20 autistic children and compared with plasma concentrations in 33 control children to establish whether the metabolic profile of the autistic children differed significantly from that of the control children. In the second component, based on the observed abnormalities in plasma metabolites, a subset of 8 autistic children were given oral supplements of 800 μg folinic acid and 1000 mg betaine (anhydrous trimethylglycine) twice a day in an attempt to improve the metabolic profile; this is referred to as intervention 1. After 3 mo on this regimen, blood samples were again taken and the metabolite concentrations were compared with baseline concentrations of each metabolite. In the third component, the same subset of 8 children were given an injectible form of methylcobalamin (75 μg/kg) twice a week in addition to the oral folinic acid and betaine for an additional month; this is referred to as intervention 2. Each child served as his or her own control for the intervention study.

Nutritional supplements

USP-grade folinic acid was obtained from Douglas Laboratories (Pittsburgh) or Thorne Research, Inc (Dover, ID) and was given twice a day as 800 μg oral powder in juice. Betaine (trimethylglycine, USP grade) was purchased from Life Extension Foundation (Fort Lauderdale, FL) and given twice a day as 1000 μg oral powder in juice. USP methylcobalamin was obtained from Hopewell Pharmaceuticals (Hopewell, NJ) or Unique Pharmaceuticals (Temple, TX) as an injectible liquid and given subcutaneously at a dose of 75 μg/kg twice a week.

Sample treatment and HPLC method

Fasting blood samples were collected into EDTA-containing evacuated tubes (B-D Biosciences, Dallas) and immediately chilled on ice before being centrifuged at 4000 × g for 10 min at 4 °C. Plasma aliquots were transferred into cryostat tubes and stored at −80 °C until extraction and HPLC quantification. For determination of methionine, total homocysteine, cysteine, and
METABOLIC ABNORMALITIES IN AUTISTIC CHILDREN 1613

TABLE 1
Comparison of methionine cycle and transsulfuration metabolites between autistic children and control children

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control children (n = 33)</th>
<th>Autistic children (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine (µmol/L)</td>
<td>31.5 ± 5.7 (23–48)</td>
<td>19.3 ± 0.7 (15–25)²</td>
</tr>
<tr>
<td>SAM (µmol/L)</td>
<td>96.9 ± 12 (77–127)</td>
<td>75.8 ± 16.2 (68–100)³</td>
</tr>
<tr>
<td>SAH (µmol/L)</td>
<td>19.4 ± 3.4 (16–27)</td>
<td>28.9 ± 7.2 (14–41)²</td>
</tr>
<tr>
<td>SAM:SAH</td>
<td>5.2 ± 1.3 (4–8)</td>
<td>2.9 ± 0.8 (2–4)²</td>
</tr>
<tr>
<td>Adenosine (µmol/L)</td>
<td>0.27 ± 0.1 (0.1–0.4)</td>
<td>0.39 ± 0.2 (0.17–0.83)⁴</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>6.4 ± 1.3 (4.3–9.0)</td>
<td>5.8 ± 1.0 (4.0–5.8)⁵</td>
</tr>
<tr>
<td>Cystathionine (µmol/L)</td>
<td>0.17 ± 0.05 (0.1–0.27)</td>
<td>0.14 ± 0.06 (0.04–0.2)⁴</td>
</tr>
<tr>
<td>Cysteine (µmol/L)</td>
<td>202 ± 17 (172–252)</td>
<td>163 ± 15 (133–189)⁷</td>
</tr>
<tr>
<td>tGSH (µmol/L)</td>
<td>7.6 ± 1.4 (3.8–9.2)</td>
<td>4.1 ± 0.5 (3.3–5.2)⁷</td>
</tr>
<tr>
<td>Oxidized glutathione (µmol/L)</td>
<td>0.32 ± 0.1 (0.11–0.43)</td>
<td>0.55 ± 0.2 (0.29–0.97)²</td>
</tr>
<tr>
<td>tGSH:GSSG</td>
<td>25.5 ± 8.9 (13–49)</td>
<td>8.6 ± 3.5 (4–11)²</td>
</tr>
</tbody>
</table>

¹ All values are x ± SD; range in parentheses. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; tGSH, total glutathione; GSSG, oxidized glutathione.
² Significantly different from control children: ² P < 0.001, ³ P < 0.01, ⁴ P < 0.05, ⁵ P < 0.002.

Those in the control children (Table 1). The ratio of SAM to SAH was almost 50% lower in the autistic children than in the control children. The significant reductions in plasma cystathionine and cysteine concentrations observed in the autistic children (Table 1) were consistent with a decrease in CBS-mediated transsulfuration. Associated with the low mean plasma cysteine concentration was a significant decrease in tGSH concentrations. GSSG was increased almost twofold, and tGSH:GSSG was reduced by 70%.

Supplementation with folic acid and betaine (intervention 1)

A subset of 8 autistic children participated in an intervention trial designed to improve their metabolic profile. Oral supplementation with 800 µg folic acid and 1000 mg betaine, both given twice a day, was maintained for a period of 3 mo (intervention 1), and a second blood sample was drawn. Relative to baseline concentrations, mean plasma methionine, SAM, homocysteine, cystathionine, cysteine, and tGSH concentrations and SAM:SAH and tGSH:GSSG in these 8 children were higher (Table 2). In addition, the high SAH and adenosine concentrations observed at baseline decreased with the betaine and folic acid supplements during intervention 1. The mean concentrations of methionine, SAM, SAH, adenosine, and homocysteine were not statistically different from those in the control children, which indicated that intervention with folic acid and betaine had brought these methionine cycle metabolites into the normal range. Although supplementation was effective in normalizing the methionine cycle metabolites to the concentrations in the control subjects, the intervention significantly improved but did not normalize tGSH or GSSG concentrations or tGSH:GSSG.

Supplementation with folic acid, betaine, and methyl vitamin B-12 (intervention 2)

For intervention 2, an injectable form of methylcobalamin (75 µg/kg) was added to the folic acid and betaine regimen for a period of 1 mo, after which the third blood sample was taken for...
subcutaneous injection of 75
not fit that interpretation. The data may be best explained by
activity; however, the observed decrease in homocysteine does
concentrations would suggest a reduction in methionine synthase
increase in adenosine and SAH. The low methionine and SAM
onine, SAM, and homocysteine were associated with significant
cycle, significant decreases in plasma concentrations of methi-
several studies (32–34). The observed imbalance in methionine
metabolites and Down syndrome (15, 28–31), we measured the concentra-
including Alzheimer disease, Parkinson disease, schizophrenia,
Because abnormal folate metabolism and low glutathione con-
research into potential etiologic factors and candidate genes.
(24–27). This increased prevalence of autism has enormous
impedibility genes (21), epigenetic effects (22), and environmental
factors (23). The apparent increase in the diagnosis of autistic-
spectrum disorders from 4–5 in 10 000 children in the 1980s to
30–60 in 10 000 children in the 1990s has raised great concern
Autism is a complex neurodevelopmental disorder that is
thought to involve an interaction between multiple, variable sus-
sceptibility genes (21), epigenetic effects (22), and environmental
factors (23). The apparent increase in the diagnosis of autistic-
spectrum disorders from 4–5 in 10 000 children in the 1980s to
30–60 in 10 000 children in the 1990s has raised great concern
(24–27). This increased prevalence of autism has enormous future public health implications and has stimulated intense
research into potential etiologic factors and candidate genes.
Because abnormal folate metabolism and low glutathione con-
centrations have been reported in other neurologic disorders,
including Alzheimer disease, Parkinson disease, schizophrenia,
and Down syndrome (15, 28–31), we measured the concentra-
tions of methionine methylation and transsulfuration metabolites
in a cohort of autistic children.
The concentrations of metabolites among the control children
in this study were within the range of values previously found in
several studies (32–34). The observed imbalance in methionine
and homocysteine metabolism in the autistic children is complex
and not easily explained by perturbation of a single pathway or
isolated genetic or nutritional deficiency. Within the methionine
cycle, significant decreases in plasma concentrations of methi-
onine, SAM, and homocysteine were associated with significant
increases in adenosine and SAH. The low methionine and SAM
concentrations would suggest a reduction in methionine synthase
activity; however, the observed decrease in homocysteine does
not fit that interpretation. The data may be best explained by
oxidative inactivation of methionine synthase in combination
with a decrease in SAH hydrolase activity secondary to the in-
crease in adenosine (35, 36). Adenosine binds to the active site of
SAH hydrolase, and increased concentrations of adenosine have
been shown to reduce SAHH activity (36, 37). A combined
enzyme deficit would also be consistent with the observed de-
crease in SAM and increase in SAH concentrations. In this case,
the decrease in homocysteine concentrations would reflect an
adenosine-mediated decrease in SAH hydrolysis and homocys-
teine synthesis. The functional consequence of an increase in
SAH is product inhibition of most cellular methyltransferases
(38). Low methionine and SAM concentrations in combination
with increased SAH and adenosine concentrations were shown
previously to be associated with reduced cellular methylation
capacity (39). The twofold decrease in SAM:SAH also suggests
an impaired capacity in these autistic children for cellular meth-
ylation.
The metabolic pattern observed in the transsulfuration path-
way may provide a more cohesive explanation for the unusual
imbalance in methionine cycle metabolites. Low concentrations
of cystathionine, cysteine, and tGSH are consistent with reduced
flux through the transsulfuration pathway. Furthermore, the sig-
nificant increase in GSSG disulfide and the 67% decrease in
tGSH:GSSG indicate chronic oxidative stress. Within the
methionine cycle, methionine synthase, betaine homocysteine
methyltransferase, and methionine adenosyltransferase are all
redox-sensitive enzymes that are down-regulated by oxidative
stress (40–42). A decrease in methionine- and SAM-regulated
CBS activity would increase the requirement for cysteine, effec-
tively making it an essential amino acid in these children.
Because cysteine is the rate-limiting amino acid for glutathione
synthesis, its decrease is consistent with low concentrations of
 glutathione (43, 44). The remarkably consistent decrease in
cysteine and glutathione concentrations and tGSH:GSSG in
the autistic children suggests an increased vulnerability to oxy-
dative stress.
The genetic or environmental factors (or both) that would
initiate oxidative stress and abnormal metabolic profiles in the
autistic children are not clear. It is possibly relevant that, in
autistic children, decreased activity of adenosine deaminase

### TABLE 2

Results of intervention trials

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 8)</th>
<th>Intervention 1 (n = 8)</th>
<th>Intervention 2 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine (μmol/L)</td>
<td>19.2 ± 3.5</td>
<td>25.7 ± 3.6</td>
<td>30.9 ± 7.7</td>
</tr>
<tr>
<td>SAM (nmol/L)</td>
<td>75.5 ± 5.0</td>
<td>112.9 ± 20.8</td>
<td>101.6 ± 20.5</td>
</tr>
<tr>
<td>SAH (μmol/L)</td>
<td>27.6 ± 6.1</td>
<td>16.9 ± 6.5</td>
<td>14.3 ± 7.5</td>
</tr>
<tr>
<td>SAM:SAH</td>
<td>2.9 ± 0.8</td>
<td>7.4 ± 4.1</td>
<td>8.9 ± 4.5</td>
</tr>
<tr>
<td>Adenosine (μmol/L)</td>
<td>0.30 ± 0.2</td>
<td>0.18 ± 0.04</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Homocysteine (μmol/L)</td>
<td>5.4 ± 0.9</td>
<td>6.7 ± 0.7</td>
<td>7.4 ± 1.7</td>
</tr>
<tr>
<td>Cystathionine</td>
<td>0.10 ± 0.02</td>
<td>0.22 ± 0.08</td>
<td>0.25 ± 0.08</td>
</tr>
<tr>
<td>Cysteine (μmol/L)</td>
<td>166 ± 11.4</td>
<td>180 ± 11</td>
<td>199.3 ± 15</td>
</tr>
<tr>
<td>tGSH (μmol/L)</td>
<td>4.0 ± 0.7</td>
<td>5.0 ± 0.9</td>
<td>6.7 ± 1.6</td>
</tr>
<tr>
<td>Oxidized glutathione (nmol/L)</td>
<td>0.59 ± 0.2</td>
<td>0.38 ± 0.1</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>tGSH:GSSG</td>
<td>7.5 ± 2.3</td>
<td>13.8 ± 3.9</td>
<td>28.7 ± 7.1</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; tGSH, total glutathione; GSSG, oxidized glutathione. Intervention 1: 800 μg folic acid and 1000 mg betaine were administered twice a day from immediately after the baseline blood draw for a period of 3 mo; intervention 2: subcutaneous injection of 75 μg methylcobalamin/kg was added to folic acid and betaine supplementation for an additional month.
2 Intervention 1 compared with baseline.
3 Intervention 2 compared with intervention 1.
increased frequency of adenosine deaminase polymorphisms have been shown to be associated with low adenosine deaminase activity (14, 45). The observed increase in adenosine could be due to either an inhibition of adenosine kinase or an increase in 5-nucleotidase, both of which have been shown to occur with oxidative stress (46, 47). Elevated intracellular adenosine has been shown to inhibit glutathione synthesis (48, 49). Alternatively, a genetic predisposition to environmental agents or conditions that promote oxidative stress could contribute to the abnormal metabolic profile observed in the autistic children.

The targeted nutritional intervention trial in a subset of the autistic children was specifically designed to increase methionine concentrations (intervention 1). Betaine homocysteine methyltransferase provides a folate–vitamin B-12–independent pathway in the liver and kidney to remethylate homocysteine to methionine (17). Supplemental betaine (trimethylglycine) has been shown to up-regulate betaine homocysteine methyltransferase expression and activity to increase methionine synthesis (50). Folinic acid (5-formyl tetrahydrofolate) was used rather than folic acid because the former is absorbed as the reduced metabolite and can enter folate metabolism as 5,10-methylene tetrahydrofolate, thereby reducing the possibility of promoting a folate trap (51, 52). As shown in Table 2, the intervention with betaine and folic acid was successful in bringing all the metabolites within the methionine cycle into the normal range and simultaneously improving significantly the metabolites in the transsulfuration pathway. The increase in methionine, SAM, and homocysteine concentrations and the decrease in adenosine and SAH concentrations suggested that the intervention stimulated an increased flux through the methionine cycle. In addition, the significant increase in cystathionine concentrations suggests that the supplements were effective also in increasing CBS activity, most likely because of up-regulation by the increase in SAM. The associated increases in cysteine and glutathione indicate that transsulfuration to glutathione was enhanced by the supplements. The decrease in adenosine is consistent with a concomitant release of SAHH inhibition and decrease in SAH and, possibly, the release of a bottleneck in methionine cycle turnover. The mechanism for the decrease in adenosine concentrations, however, is not clear. One possibility is that the increase in cysteine or glutathione concentration (or both) relieved the need for adenosine as a protective factor against oxidative damage (53, 54).

The addition of injectible methylcobalamin to the protocol (intervention 2) was based on empirical observations of clinical improvement in speech and cognition (by JAN) and the possibility that it might enhance methionine synthase activity under conditions of oxidative stress by replacing oxidized inactive coenzyme B-12 [cob(B12)alamin] or by posttranslational up-regulation of methionine synthase, or both (55, 56). One month after the addition of methylcobalamin, the methionine concentrations were within the control range (Table 1), and further improvements in adenosine and SAH concentrations and SAM: SAH were observed. Unexpectedly, and perhaps most significantly, the addition of methylcobalamin reduced the concentrations of inactive GSSG and increased the GSH concentrations and GSH:GSSG so that they were not different from those in the control children (Table 1). These positive changes in the glutathione redox profile most likely reflect the increase in cysteine as the rate-limiting amino acid for glutathione synthesis (44). Of note, there is a higher demand for cysteine (and, indirectly, methionine) for de novo glutathione synthesis during chronic oxidative stress (43). Low antioxidant enzyme activity in autistic children has been reported in several recent studies (57–59) that provide additional support for oxidative stress as a part of the etiology of autism. If the decreases in plasma methionine, cysteine, and glutathione concentrations in autistic children observed in the current study are confirmed in a larger study, low concentrations of these thiol metabolites could provide metabolic biomarkers for autism.

Although clinical improvements in speech and cognition were noted by the attending physician (PC), they were not measured in a quantifiable manner and are therefore not reported here. Specific dietary differences between groups could have contributed to our results, but we consider it unlikely that uniform dietary differences within the autistic group existed that could have accounted for the remarkably consistent metabolic alterations. Increased frequency of common polymorphisms in these pathways may have contributed to the observed metabolic phenotype, and studies of that subject, as well as studies to quantify clinical improvement, are currently underway. Our attempts to interpret these preliminary metabolic findings are clearly speculative, and a better understanding of the abnormal one-carbon metabolism in these children will require additional research efforts. Nonetheless, the ability to correct the metabolic imbalance with targeted nutritional intervention implies that certain aspects of autism may be treatable.

Nineteen of the 20 children participating in the study were diagnosed with “regressive” autism (apparently normal development until regression into autism between ages 1.5 and 3 y). On the basis of their abnormal metabolic profiles, we hypothesize that an increased vulnerability to oxidative stress (environmental, intracellular, or both) and impaired methylation capacity may contribute to the development and clinical manifestation of regressive autism.

SJJ was responsible for study design, study coordination, interpretation of data, and manuscript writing. PC was responsible for patient recruitment, obtaining supplements, patient compliance, monitoring clinical status, and methylcobalamin injections. SM was responsible for HPLC quantification of plasma metabolites and data collection and interpretation. SJ was responsible for plasma and DNA extraction and data collection and interpretation. LJ was responsible for patient recruitment, study coordinating and consulting, and data interpretation. DWG was responsible for statistical analysis of data. JAN was responsible for initiating the methylcobalamin treatment in autistic patients, providing consultation, and interpreting data. None of the authors has any financial conflict of interest.

REFERENCES

the National Center for Biotechnology Information. Internet: http://
wwwncbi.nlm.nih.gov/omim.]
Disord 2001;31:433–42.
7. Arrieta I, Nunez T, Gil A, Flores P, Usobiaga E, Martinez B. Autosomal
follate sensitive fragile sites in an autosomal Basque sample. Ann Genet
8. Baieri S, Pavone L, Mela C, Fiumara A, Coleman M. Autism and phe-
9. Jaeken J, Van den BG. An infantile autistic syndrome characterised by the
10. Page T. Metabolic approaches to the treatment of autism spectrum dis-
11. Pesi R, Micheli V, Jacomelli G, et al. Cytosolic 5'-nucleotidase hyper-
activity in erythrocytes of Lesch-Nyhan syndrome patients. Neuropept
12. Rimland B. High dose vitamin B6 and magnesium in treating autism: re-
16. Pogribna M, Pogribina M, Pogribyn IP, Hines RJ, James SJ. Homocys-
teine metabolism in children with Down syndrome: in vitro modula-
18. Finkelstein JD. Pathways and regulation of homocysteine metabolism in
method for the simultaneous determination of oxidized and reduced plasma aminothiols using coulometric electrochemical detection. J Nutr
utilizing coulometric electrochemical detection: alteration with plasma
homocysteine and pyridoxal 5'-phosphate concentrations. Clin Chem
21. Keller F, Persico AM. The neurobiological context of autism. Mol Neu-
23. London EA. The environment as an etiologic factor in autism: a new
direction for research. Environ Health Perspect 2000;108(suppl
25. Destefano F, Bhasin TK, Thompson WW, Yeareg-Allsopp M, Boyle C.
Age at first measles-mumps-rubella vaccination in children with autism in
school-matched control subjects: a population-based study in children
26. Schultz JB, Lendenau J, Seyfried T, Dichgans J. Glutathione, oxidative
27. Steinhausen HC, Gobel D, Breinlinger M, Wohllben B. A community
28. Miller AL. The methionine-homocysteine cycle and its effects on cog-
29. Page T. Metabolic approaches to the treatment of autism spectrum dis-
30. Oho H, Sakamoto A, Sakura N. Plasma total glutathione concentrations
31. Muntjewerff JW, Van der Put N, Eskes T, et al. Homocysteine metab-
olism and B-vitamins in schizophrenic patients: low plasma folate as a
possible independent risk factor for schizophrenia. Psychiatry Res 2003;
121:1–9.

