Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (Childhood Autism Risks from Genetics and Environment) case-control study

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ABSTRACT
Background: Periconceptional folate is essential for proper neurodevelopment.
Objective: Maternal folic acid intake was examined in relation to the risk of autism spectrum disorder (ASD) and developmental delay (DD).
Design: Families enrolled in the CHARGE (Childhood Autism Risks from Genetics and Environment) Study from 2003 to 2009 were included if their child had a diagnosis of ASD (n = 429), DD (n = 130), or typical development (TD; n = 278) confirmed at the University of California Davis Medical Investigation of Neurodevelopmental Disorders Institute by using standardized clinical assessments. Average daily folic acid was quantified for each mother on the basis of dose, brands, and intake frequency of vitamins, supplements, and breakfast cereals reported through structured telephone interviews.
Results: Mean (±SEM) folic acid intake was significantly greater for mothers of TD children than for mothers of children with ASD in the first month of pregnancy (P1; 779.0 ± 36.1 and 655.0 ± 28.7 µg, respectively; P < 0.01). A mean daily folic acid intake of ≥600 µg (compared with <600 µg) during P1 was associated with reduced ASD risk (adjusted OR: 0.62; 95% CI: 0.42, 0.92; P = 0.02), and risk estimates decreased with increased folic acid (P-trend = 0.001). The association between folic acid and reduced ASD risk was strongest for mothers and children with MTHFR 677 C>T variant genotypes. A trend toward an association between lower maternal folic acid intake during the 3 mo before pregnancy and DD was observed, but not after adjustment for confounders.
Conclusions: Periconceptional folic acid may reduce ASD risk in those with inefficient folate metabolism. The replication of these findings and investigations of mechanisms involved are warranted. Am J Clin Nutr 2012;96:80–9.

INTRODUCTION
Little is known about the modifiable risk factors for autism spectrum disorders (ASDs). Both animal and human studies have shown the essential role of folate during nervous system and brain development (1). Although mechanisms remain unclear, randomized clinical trials in the early 1990s showed that periconceptional folic acid supplements prevented 50–70% of neural tube defects (NTDs) (2). These trials led to recommendations to increase folic acid supplement intake before and during pregnancy (3). Also, because only approximately half of all pregnancies are planned (4), and they may not be recognized until after the early period when folate demand is highest and folic acid supplements are most effective at reducing NTD occurrence, manufacturers in the United States were mandated to fortify enriched grain products with 140 µg folic acid/100 g grain by 1 January 1998 (5). Several studies (6, 7) have shown a declining prevalence of NTDs in the United States since that time.

Autism is a neurodevelopmental disorder that potentially originates during early pregnancy (first month) (8) when folate is known to be critical. Folic acid has been used to treat children with genetic disorders that present with autistic symptoms, including fragile X syndrome and Rett syndrome, with some benefits (9, 10). A subset of children with ASD or autistic symptoms have cerebral folate deficiency (11, 12), possibly resulting from serum folate receptor autoantibodies blocking the folate binding site on receptors and impeding transport across the blood–cerebrospinal fluid barrier.
Administration of supplemental folic acid normalized metabolites and improved ASD symptoms in some instances (12–14). These studies were small and lacked rigorous measures of symptom improvement, but they raise the possibility that supplemental folic acid during early neurodevelopment might have led to greater recovery or even prevention of developmental symptoms.

For some women who do not consume sufficient amounts of supplemental folic acid periconceptionally, current levels of folic acid fortification might have increased blood folate concentrations enough to facilitate neural tube closure and survival of their fetus but not enough to prevent more subtle anomalies of brain development in certain fetuses who could later display neurodevelopmental conditions such as autism. Similarly, as suggested by Rogers (15) and explored by others (16–18), enhanced folate status during pregnancy might protect against these potential effects and reduce the risk of neurodevelopmental disorders including ASD and developmental delay (DD). Our objective was to examine the survival rates of fetuses possessing genetic polymorphisms such as MTHFR 677 C>T, which are associated with high homocysteine and subsequently require higher amounts of folate for proper neurodevelopment. Such polymorphisms have been observed in higher frequencies in children with autism (19, 20), suggesting that these children might be genetically predisposed to less efficient folate metabolism and function. We hypothesize that higher intakes of maternal folic acid before and throughout pregnancy may protect against these potential effects and reduce the risk of neurodevelopmental disorders including ASD and developmental delay (DD). Our objective was to examine the role of maternal folic acid intake in the etiology of ASD and DD, overall and stratified by MTHFR 677 genotype.

SUBJECTS AND METHODS

Participants

Individuals included in this study were recruited through the CHildhood Autism Risks from Genetics and Environment (CHARGE) population-based, case-control study as described previously (21). Eligible children included those who met the following criteria: aged between 24 and 60 mo, living with at least one English- or Spanish-speaking biological parent, born in California, and residing in the catchment areas of a specified list of California Regional Centers that coordinate services for persons with developmental disabilities. Children with autism, intellectual disability, or DD were identified through the California Regional Center System, and general population controls were identified from state birth files and frequency matched to the age and catchment area distribution of the autism cases and a 4:1 male-to-female ratio. Children were included in these analyses if they were born after 1999 and were the first child within the family recruited into the study. Children with known genetic syndromes were not excluded. Data from CHARGE Study interviews completed from 2003 through July 2009 were included. The CHARGE Study protocols were approved by the University of California–Davis Institutional Review Board and the State of California Committee for the Protection of Human Subjects. No data were collected without informed consent of the parent or parents.

Diagnostic classification

All children were assessed for cognitive function by using the Mullen Scales of Early Learning (22) and for adaptive function by using the Vineland Adaptive Behavior Scales (23). The children of families recruited from the general population or with DD were screened for evidence of ASD by using the Social Communication Questionnaire, and if they scored ≥15, they were evaluated for autism.

The diagnoses of children identified with autism by the regional center were confirmed by study personnel by using the Autism Diagnostic Interview–Revised (ADI-R) (24, 25) and the Autism Diagnostic Observation Schedule–Generic (ADOS) (26, 27). Two subgroups (autism and ASD) were initially defined. Autism case status is defined as meeting criteria on the communication, social, and repetitive behavior domains of the ADI-R and scoring at or above the total cutoff for autistic disorder on the ADOS module 1, 2, or 3. A broader definition of impairment encompasses ASD as defined by Risi et al (28) (ASD2), which requires the child to meet the following criteria: 1) meet the cutoff for ASD on ADOS module 1, 2, or 3; 2) meet the cutoff for Abnormality of Development at age ≤36 mo in ADI-R; and 3) meet the criteria for both Social and Communication domains in ADI-R, or meet the criteria for the Social domain and be within 2 points of the criteria for the Communication domain, or meet the criteria for the Communication domain and be within 2 points of the Social domain criteria, or be within one point for both Social and Communication domains. Autism and ASD are widely conceptualized as similar conditions on the same continuum of overlapping symptoms that differ only in severity (28). All analyses were first conducted on the autism (n = 288) and ASD (n = 141) groups separately; when no substantial differences were found between the groups’ maternal folic acid intake, they were collapsed, and results were presented for the combined ASD group, which also fits with how the disorder is seen clinically (29).

Folic acid quantification

Data on the intake of multivitamins, prenatal vitamins, folic acid–specific vitamins, cereals (breakfast cereals, granolas, and hot cereals), and other supplements (including breakfast shakes and bars) were collected through parental telephone interviews. The questions were provided previously (30). These data included information on whether or not each item was consumed; and if so, what brand and dose, during which months of an index period (beginning 3 mo before and throughout each month of pregnancy), and how frequently the item was consumed.

From this information, we calculated a value of folic acid intake on the basis of the brand, dose, and frequency of consumption of each product and summed these into a total value for each month for each woman. The same brands and frequencies were used for each month with reported consumption. For example, in the first month of pregnancy, if a woman reported taking a multivitamin with 400 μg folic acid once per day, 2 servings of a breakfast cereal containing 120 μg folic acid each (ie, 240 μg) per day, and no other supplements, her average total daily folic acid intake for that month would be calculated as 640 μg/d. In the same example, if she reported taking a prenatal vitamin with 800 μg folic acid once per day, the same amount of breakfast cereal, and no other supplements (including multivitamins) in the next month, her total average intake would be calculated as 1040 μg/d for month 2.

Amounts of folic acid were assigned to each brand or product on the basis of information from the USDA National Nutrient Database for Standard Reference (31) or the manufacturer’s
intake during P1. Variables were then excluded by using back-
ward selection while retaining in the model variables that caused
≥10% change in the exposure variable estimates. Maternal ed-
ucation, child’s birth year, and certain other nutrients were the
only variables that met criteria as confounders; adjustment for
most other potentially confounding variables changed the esti-
imated associations for folic acid–containing supplements very
little (typically <5%). Preeclampsia, type of delivery (sched-
uled, unscheduled or emergency cesarean delivery, vaginal),
vaginal bleeding during pregnancy, induced labor (yes, no), and
pregnancy intention were examined as mediators of the associ-
ation between folic acid intake and ASD (36). Sensitivity
analyses using multiple imputation via the Markov chain Monte
Carlo algorithm (37) assessed the impact of missing data. The
frequency and dose of folic acid intake within the develop-
mentally relevant period were examined to delineate thresholds
for the protective effect.

RESULTS
Families of 278 TD, 429 ASD, and 130 DD children who
participated in the CHARGE Study from the start of enrollment
in 2003 through July 2009 met diagnostic criteria and were included
in these analyses. Children with ASD and their mothers tended to
be sociodemographically similar to TD children and their
mothers. DD children were more likely to be female than were
TD children (who were matched on sex to the projected sex
distribution of the ASD group) and their mothers were less
educated, less likely to have private health insurance, and more
likely to be born in Mexico and to have not intended to become
pregnant (Table 1).

Total folic acid from all sources during the index period was
determined for mothers of 89.6% TD, 85.8% ASD, and 89.1%
DD children after exclusion of women reporting intake of un-
known or irregular frequency for any source; results did not differ
if these women were included and assigned a low frequency value
(eg, 2 times/wk, or 2/7 per day) under the assumption that intake
that was not remembered well would be more limited. After
summing all of the measured sources, mean (±SEM) daily total
folic acid intakes reported by mothers of DD children for the 3
mo before pregnancy (399.7 ± 46.8 μg) tended to be lower than
those of the TD group (494.0 ± 36.6 μg; P = 0.08) but were not
significantly lower than those of the ASD group (439.6 ± 24.6 μg;
P = 0.30) (Figure 1). Mothers of DD children reported intakes
closer to the amounts reported by mothers of TD children in P1
(716.5 ± 64.2 μg and 779.0 ± 36.1 μg, respectively; P = 0.17)
and reported average intakes above those of the TD group
throughout the rest of pregnancy (Figure 1).

Mean estimated daily total folic acid intakes were greater for
mothers of TD children than for mothers of ASD children throughout
the entire index period, with the greatest difference observed for P1
(Figure 1). Mothers of TD children consumed, on average, 123.9 ±
46.4 μg more folic acid daily during P1 than did mothers of chil-
ren with ASD (655.0 ± 28.7 μg; P < 0.01). In addition, the
percentage of women meeting the guidelines for supplemental folic
acid intake during pregnancy (≥600 μg/d) in P1 was higher for
mothers of TD children (68.5%) than for mothers of children with
ASD (53.9%) (P = 0.001) or DD (54.4%) (P = 0.02). After ad-
justment for maternal education and child’s birth year, the associa-
tion between ≥600 μg folic acid and reduced risk remained
significant for ASD (OR: 0.61; 95% CI: 0.41, 0.89) but was

Genotyping
Blood was collected from all family members as part of the
CHARGE Study protocol, and genomic DNA was isolated from
peripheral blood leukocytes by using standard procedures
(Puregene kit; Gentra Inc). MTHFR 677C>T (rs1801133) was
genotyped by using the TaqMan system (Assay on Demand)
from Applied Biosystems (ABI). Population structure bias was
assessed as described previously (32, 33).

Statistical analyses
All data were reviewed for outliers, out-of-range values, and
logical inconsistencies by using univariate descriptive analyses.
Total folic acid intake was not normally distributed, so tests of
group differences were conducted by using the exact Wilcoxon
nonparametric test with Monte Carlo estimates of exact P values
to account for numerous ties in our data. All tests for differences
were 2-sided. Multinomial logistic regression was used to cal-
culate ORs as measures of association between categories of folic
acid intake and case status [ASD or DD compared with
typical development (TD), simultaneously] and their respective
95% CIs. Unadjusted and adjusted trend tests for folic acid in-
take with case status were performed by using the Cochran-
Armitage trend test and the Wald test for linear trend in the log-
odds of case status, respectively (34, 35). For trend tests, folic
acid intake categories were scored from lowest to highest by
using consecutive integers. All analyses were conducted by

Stratified analyses and interaction terms were used to examine
effect modification of folic acid intake by other factors including
child sex and birth year, maternal and child race-ethnicity (non-
Hispanic white, Hispanic, other), maternal age, maternal education
(high school graduate or less, some college or 2-y degree, bach-
elor’s degree or higher), prepregnancy BMI (in kg/m2), maternal,
child race-ethnicity (non-

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label; if this information was not available, a standard amount
was assigned on the basis of the amount of folic acid most
commonly found in similar products: for example, values of 400
and 800 μg folic acid/tablet were used for unknown multivita-
min and prenatal vitamin brands, respectively. If multiple brands
were reported, we averaged the values across all brands to create
a value per serving. The CHARGE Study did not collect com-
plete diet information for the pregnancy because of the lengthy
period of recall; thus, total dietary folate was not examined.

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## TABLE 1
Characteristics of children with ASD, DD, or TD and their mothers in the CHARGE Study, 2003–2009

<table>
<thead>
<tr>
<th></th>
<th>ASD (n = 429)</th>
<th>Value</th>
<th>P</th>
<th>DD (n = 130)</th>
<th>Value</th>
<th>P</th>
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<td>Male</td>
<td>228 (82.0)</td>
<td>372 (86.7)</td>
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<td>Female</td>
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<td>57 (13.3)</td>
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<td>44 (33.8)</td>
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<td><strong>Child race-ethnicity</strong></td>
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<td></td>
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<td>Non-Hispanic white</td>
<td>141 (50.7)</td>
<td>221 (51.5)</td>
<td>0.13</td>
<td>59 (45.4)</td>
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<td>Hispanic</td>
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<td>49 (37.7)</td>
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<td>Non-Hispanic black</td>
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<td>7 (1.6)</td>
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<td>4 (3.1)</td>
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<td>Mixed and other</td>
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<td>45 (10.5)</td>
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<td>15 (11.5)</td>
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<td><strong>Maternal age at child’s birth</strong></td>
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<td>≤19 y</td>
<td>14 (5.1)</td>
<td>11 (2.6)</td>
<td>0.12</td>
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<td>20–29 y</td>
<td>296 (69.1)</td>
<td>326 (44.2)</td>
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<td>73 (54.6)</td>
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<td>30–34 y</td>
<td>107 (24.8)</td>
<td>141 (25.1)</td>
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<td>34 (24.5)</td>
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<td></td>
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<tr>
<td>≥35 y</td>
<td>49 (11.7)</td>
<td>78 (10.8)</td>
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<td>29 (21.9)</td>
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<td></td>
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<td>United States</td>
<td>223 (80.2)</td>
<td>323 (75.3)</td>
<td>0.31</td>
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<td>Mexico</td>
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<td>35 (8.2)</td>
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<td>21 (16.2)</td>
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<td></td>
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<tr>
<td>Other</td>
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<td>71 (16.6)</td>
<td></td>
<td>9 (6.9)</td>
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<td></td>
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<td><strong>Maternal education</strong></td>
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<tr>
<td>High school graduate or less</td>
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<td>62 (14.5)</td>
<td>0.15</td>
<td>37 (28.5)</td>
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<td>Some college or technical, vocational, or associate degree</td>
<td>86 (30.9)</td>
<td>163 (38.1)</td>
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<td>54 (41.5)</td>
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<tr>
<td>Bachelor, master’s, professional, or doctorate degree</td>
<td>148 (53.2)</td>
<td>203 (47.4)</td>
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<td>39 (30.0)</td>
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<td>Private</td>
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<td>353 (82.3)</td>
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<td>90 (69.2)</td>
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<td>Government program</td>
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<td>76 (17.7)</td>
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<td>Intended to become pregnant when they did</td>
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<td>261 (64.3)</td>
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<td>Indifferent about becoming pregnant at that time</td>
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<td>50 (12.3)</td>
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<tr>
<td>Intended to become pregnant later</td>
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<td>63 (15.5)</td>
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<td>14 (11.3)</td>
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<td>Did not intend to become pregnant at all</td>
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<td>32 (7.9)</td>
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<td>16 (12.9)</td>
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<td><strong>Maternal cigarette smoking</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>No</td>
<td>236 (89.4)</td>
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<td><strong>Maternal alcohol consumption</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>124 (48.6)</td>
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<td>184 (46.4)</td>
<td></td>
<td>50 (41.3)</td>
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<td><strong>Prenatal vitamins during P1</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>9 (7.4)</td>
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<td>Yes</td>
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<td>16 (12.2)</td>
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<td><strong>Cereal during P1</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>20 (16.8)</td>
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<td>Yes</td>
<td>225 (88.2)</td>
<td>327 (82.4)</td>
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<td>99 (83.2)</td>
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<td><strong>Other supplements during P1</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>252 (95.8)</td>
<td>384 (94.1)</td>
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<td>11 (4.2)</td>
<td>24 (5.9)</td>
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<td>3 (2.5)</td>
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</table>

<sup>1</sup> Values are n (%). P values were derived from chi-square tests comparing category proportions between the ASD or DD group and the TD group. ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and Environment; DD, developmental delay; TD, typical development.

<sup>2</sup> Any reported for the period 3 mo before pregnancy through the end of pregnancy.

<sup>3</sup> Any reported for P1.
attenuated to a trend for an association for DD (OR: 0.61; 95% CI: 0.37, 1.02). In stratified analyses, the association between folic acid and reduced risk of ASD was significant only when the mother and/or child had a MTHFR 677 C>T variant genotype (Figure 2); ORs did not differ across strata of MTHFR 677 C>T genotype for DD (see Supplemental Table 1 under “Supplemental data” in the online issue). Results were similar when analyses were limited to non-Hispanic white mothers (data not shown).

Folic acid intake during P1 reported by mothers of ASD children did not differ significantly on the basis of whether the child experienced developmental regression, was developmentally delayed, was nonverbal, or experienced seizures (see Table 2 under “Supplemental data” in the online issue). Prenatal vitamin folic acid contributed the greatest amount to total estimated folic acid intake for all groups and was the source that differed the most between groups (Table 2). Associated risk of ASD decreased as maternal folic acid intakes increased during P1 (P-trend = 0.001); this trend remained after adjustment for maternal educational level and child’s birth year (P-trend = 0.01) (Table 3). Odds ratios became stronger and the associated reduction in risk was even greater after adjustment for total amounts of other nutrients quantified from these sources.

The unadjusted risk of DD decreased according to the highest amount of maternal folic acid intake during the 3 mo before pregnancy (P-trend = 0.03), but the trend was nonsignificant after adjustment for maternal education and child’s birth year (P-trend = 0.16). The associations reversed direction after adjustment for iron and vitamin E intake quantified from supplements; however, collinearity among the variables produced relatively unstable estimates, as evidenced by the wide CI (Table 4).

We attempted to distinguish the effects of the total estimated amount compared with the frequency at which folic acid was consumed by examining amounts of intake within set frequencies (eg, twice daily) and frequencies of intake within each folic acid intake range (quartiles, 100–200-µg intervals); unfortunately, the two were too highly correlated (data not shown).

The concept of a hypothesized link between folic acid and autism became more widespread in the more recent years of this study, in published literature and on websites directed toward parents of children with autism. We compared results based on interviews conducted before the publication of the first folic acid–autism hypothesis article in 2008 (15) with results from interviews conducted afterward, when reporting bias was more likely to have an impact. We found that mothers of children with ASD reported increased mean folic acid intake after 2008, in

![FIGURE 1](https://academic.oup.com/ajcn/article-abstract/96/1/80/4571464/2)

**FIGURE 1.** Mean (±SEM) maternal folic acid intake by pregnancy month for mothers of typically developing children and mothers of children with autism spectrum disorder or developmental delay. Maternal folic acid intake (µg/d) represents total intake reported from vitamins, supplements, and cereal. *Two-sided P < 0.01, Wilcoxon 2-sample test. B, month before pregnancy; P, month of pregnancy.

![FIGURE 2](https://academic.oup.com/ajcn/article-abstract/96/1/80/4571464/3)

**FIGURE 2.** ORs (95% CIs) for associations between mean maternal daily folic acid intake (≥600 µg compared with <600 µg) during the first month of pregnancy and autism spectrum disorder by maternal and child MTHFR genotype. ORs were adjusted for maternal educational level and child’s birth year. Categories of folic acid intake were created on the basis of the recommended intake during pregnancy (600 µg/d). Analyses were based on 272 children with autism spectrum disorder and 275 of their mothers, and 154 children with typical development and 163 of their mothers with MTHFR 677 genotype and folic acid intake data. The frequencies of participants in each category of folic acid intake and MTHFR 677 genotype are presented in Supplemental Table 1 under “Supplemental data” in the online issue.

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contrast to the mothers of TD and DD children who reported decreased mean intake. As a result, the difference in values comparing ASD with TD was attenuated and nonsignificant for this later period (see Supplemental Table 3 under “Supplemental data” in the online issue).

Sensitivity analyses with imputed values for missing total folic acid intake during P1 similarly showed significant differences between ASD and TD (P < 0.0001) and a significant trend for decreased ORs with increasing intake of folic acid (P-trend < 0.0001) (data not shown).

DISCUSSION

This study assessed reported intakes of folic acid and other nutrients during the preconception and prenatal period for mothers of children with ASD, DD, and TD. We showed that despite average reported folic acid intakes being in excess of amounts recommended during pregnancy, mothers of TD children reported higher average intakes and were more likely to meet these recommendations during the periconception period than were mothers of ASD children. These findings were adjusted for sociodemographic and other nutritional factors.

We observed a trend toward decreased risk of DD with higher doses of folic acid in the 3 mo before pregnancy, but these differences were attenuated after adjustment for maternal education and child’s birth year and were not observed after adjustment for other nutrients. Precision was substantially reduced in the nutrient-adjusted model, and therefore these results should be viewed with caution. Still, the attenuation by confounders and the fact that the association between folic acid and DD did not differ meaningfully across MTHFR 677T genotypes could suggest that overall supplement intake was more important for DD than

### TABLE 2

Maternal folic acid intake by source for the first month of pregnancy estimated for mothers of children with TD, ASD, or DD

<table>
<thead>
<tr>
<th>Source</th>
<th>TD</th>
<th>ASD</th>
<th>DD</th>
<th>Difference from TD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal vitamins</td>
<td>372 (44.8)</td>
<td>130 (15.9)</td>
<td>716 (81.6)</td>
<td>-232 (27.2)</td>
</tr>
<tr>
<td>Folic acid–specific vitamins</td>
<td>368 (47.8)</td>
<td>140 (17.3)</td>
<td>716 (81.4)</td>
<td>-219 (25.5)</td>
</tr>
<tr>
<td>Cereal</td>
<td>390 (42.5)</td>
<td>134 (15.4)</td>
<td>716 (81.8)</td>
<td>-223 (26.6)</td>
</tr>
<tr>
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<td>-232 (27.2)</td>
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<tr>
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<td>716 (81.8)</td>
<td>-223 (26.6)</td>
</tr>
</tbody>
</table>

### TABLE 3

Adjusted ORs and 95% CIs for associations between categories of mean maternal folic acid intake for the first month of pregnancy and risk of children with ASD compared with children with TD

<table>
<thead>
<tr>
<th>Folic acid intake</th>
<th>TD</th>
<th>ASD</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg/d</td>
<td>9</td>
<td>28</td>
<td>0.95 (0.75, 1.21)</td>
<td>0.35</td>
<td>0.56</td>
</tr>
<tr>
<td>≤500 µg/d</td>
<td>53</td>
<td>121</td>
<td>0.78 (0.58, 1.04)</td>
<td>0.57</td>
<td>0.35</td>
</tr>
<tr>
<td>500 to &lt;800 µg/d</td>
<td>20</td>
<td>33</td>
<td>0.65 (0.45, 0.94)</td>
<td>0.39</td>
<td>0.27</td>
</tr>
<tr>
<td>800–1000 µg/d</td>
<td>56</td>
<td>82</td>
<td>0.58 (0.38, 0.86)</td>
<td>0.21</td>
<td>0.25</td>
</tr>
<tr>
<td>&gt;1000 µg/d</td>
<td>62</td>
<td>70</td>
<td>0.42 (0.32, 0.59)</td>
<td>0.048</td>
<td>0.18</td>
</tr>
</tbody>
</table>

1 ASD, autism spectrum disorder; TD, typical development.
2 Categories of folic acid intake were based on the typical amount in multivitamins (400–500 µg), over-the-counter prenatal vitamins (800 µg), and the tolerable upper intake level (1000 µg).
3 Adjusted for maternal educational level and child’s birth year.
4 Adjusted for maternal educational level, child’s birth year, and log-transformed total vitamin A, vitamin B-6, vitamin C, and vitamin D from supplements and cereals; estimates were not substantially different when further adjusted for log-transformed vitamin E, vitamin B-12, iron, or calcium from supplements and cereals or when adjusted for prenatal vitamin use in the first month of pregnancy. Colinearity diagnostics showed high variance inflation factors for the 2 highest categories of folic acid intake; removal of log-transformed vitamin A and vitamin D lowered the variance inflation factors to an acceptable level (<10) and produced ORs (CIs) of 0.59 (0.21, 1.66), 0.49 (0.12, 1.95), 0.50 (0.13, 1.99), and 0.37 (0.09, 1.52) for the lowest to highest categories of folic acid intake, respectively (P-trend = 0.22). Estimates are based on 183 TD and 317 ASD children because of missing data on additional nutrient variables. Developmental delay was included in the model as an outcome but for brevity is not presented here.
5 On the basis of a Wald chi-square test for linear trend (in the log-odds of ASD compared with TD) for folic acid intake category entered as a continuous variable in a logistic regression model adjusted for the same covariates as those listed in footnote 4.
6 On the basis of a 2-sided Cochran-Armitage trend test.
TABLE 4
Adjusted ORs and 95% CIs for associations between categories of mean maternal folic acid intake for the 3 mo before pregnancy and risk of children with DD compared with children with TD.

<table>
<thead>
<tr>
<th>Folic acid intake</th>
<th>TD</th>
<th>DD</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg/d</td>
<td>15 (8.5)</td>
<td>13 (12.9)</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>&lt;200 µg/d</td>
<td>56 (31.8)</td>
<td>36 (36.6)</td>
<td>0.67 (0.28, 1.60)</td>
<td>0.35</td>
<td>1.07 (0.36, 3.12)</td>
<td>0.91</td>
</tr>
<tr>
<td>200 to &lt;400 µg/d</td>
<td>25 (14.2)</td>
<td>19 (18.8)</td>
<td>0.76 (0.28, 2.02)</td>
<td>0.77</td>
<td>1.65 (0.40, 6.77)</td>
<td>0.49</td>
</tr>
<tr>
<td>400 to &lt;800 µg/d</td>
<td>19 (10.8)</td>
<td>11 (11.9)</td>
<td>0.63 (0.21, 1.83)</td>
<td>0.61</td>
<td>2.22 (0.43, 11.50)</td>
<td>0.34</td>
</tr>
<tr>
<td>≥800 µg/d</td>
<td>61 (34.7)</td>
<td>22 (19.8)</td>
<td>0.49 (0.19, 1.23)</td>
<td>0.14</td>
<td>2.40 (0.37, 15.47)</td>
<td>0.36</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.03</td>
<td>0.16</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 DD, developmental delay; TD, typical development.
2 Categories of folate acid intake were based on the typical amount in multivitamins (400–500 µg) and over-the-counter prenatal vitamins (800 µg); the lowest category (less than the amount in a multivitamin) was split into 2 categories because this was where the majority of responses were for the time before pregnancy, and the top category [above the tolerable upper intake level (1000 µg)] was merged with the 800–1000 µg/d category to account for small numbers of women reporting high folate acid intake before pregnancy.
3 Adjusted for maternal educational level and child’s birth year.
4 Adjusted for maternal educational level, child’s birth year, and log-transformed total iron and vitamin E from supplements and cereals; estimates were not substantially different when further adjusted for log-transformed vitamin B-12, vitamin B-6, vitamin C, vitamin D, or calcium from supplements and cereals or when adjusted for prenatal vitamin use in the first month of pregnancy. Colinearity diagnostics showed high variance inflation factors for the 2 highest categories of folic acid intake; removal of log-transformed total iron lowered the variance inflation factors to acceptable levels (<10) and produced ORs (CIs) of 0.81 (0.41, 1.63), 0.95 (0.35, 2.61), 1.15 (0.33, 4.09), and 1.11 (0.28, 4.39) for the lowest to highest categories of folic acid intake, respectively (P-trend = 0.73). Estimates are based on 162 TD and 98 DD children because of missing data on additional nutrient variables. Autism spectrum disorder was included in the model as an outcome but for brevity is not presented here.
5 On the basis of a 2-sided Cochrane-Armitage trend test.
6 On the basis of a Wald chi-square test for linear trend (in the log-odds of DD compared with TD) for folic acid intake category entered as a continuous variable in a logistic regression model adjusted for the same covariates as those listed in footnote 4.

was folic acid specifically or that differences in recall accuracy between the 2 groups played a role.

This study is the first to examine folic acid intake before and during pregnancy in relation to ASD risk. Increased peri-conceptional maternal folic acid, especially at or above recommended pregnancy levels, was associated with decreased risk of ASD in the developing child when the mother and/or child possessed the MTHFR 677 C>T variant. Our findings are consistent with research that indicates folic acid’s importance in improving childhood behavioral outcomes, including recent cohort studies showing associations between maternal folic acid supplement intake during early pregnancy and fewer behavioral problems in the offspring at age 18 mo (38); reduced risk of severe language delay at age 3 y (39); improved scores on several neurodevelopmental measures, including verbal, verbal-executive function, attention, and social competence at 4 y of age (40); and lower scores of childhood hyperactivity and peer problems at age 8 y (41).

In addition to the low cerebrospinal fluid folate and folate-related gene variants described previously, elevated homocysteine concentrations, increased oxidative stress, and impaired methylation capacity have been observed in children with autism and their parents compared with TD children and their parents (19, 20, 42). The retrospective nature of these studies precludes determining whether these alterations are causes or effects of ASD, but supplemental folic acid could potentially minimize these variations because it is able to resolve differences in NTD risk across folate-related genotypes (43), reduce homocysteine concentrations (44), lessen effects of environmental toxins and oxidative stress (45), and increase DNA methylation levels (46). In addition to potential promise for folic acid in treatment of children with ASD, our findings suggest that there could exist a critical window during development when rectifying these differences could prevent the occurrence of ASDs. The periconceptional period we found associated with the greatest protective effect of folic acid correlates with the period of neural tube closure, which is consistent with evidence placing the origins of at least some cases of autism around this time (8).

The potential for an epigenetic mechanism involving methylation deserves further consideration, especially given the critical period implied by these results. Epigenetic mechanisms are implicated in ASD etiology (47). Folate is an essential one-carbon group donor and acceptor required for methylation reactions (48). Low folate concentrations lead to decreased methylation of proteins, phospholipids, DNA, and neurotransmitters (49). Dietary influences during the periconceptional period are of primary importance for the establishment of DNA methylation patterns having epigenetic effects that can persist throughout the life span (50). During this period, the mammalian preimplantation embryo undergoes extensive demethylation and then reestablishment of the appropriate methylation patterns after implantation (51). Increasing the methyl content of the periconceptional maternal diet with folic acid–containing supplements increases methylation in specific gene regions of the developing infant (52), which can influence gene expression and the infant’s health (50). The epigenetic effect of methyl metabolism is a mechanism proposed for the prevention of NTDs through periconceptional folic acid supplementation (53) and is
the mechanism behind folic acid’s ability to promote nervous system repair (54). We propose that this mechanism be explored for ASD as well.

All ASD and DD diagnoses were clinically confirmed and typical social and cognitive development was confirmed for the population-based controls. Detailed information was systematically collected on medical conditions in pregnancy, numerous potential confounding variables, presence of developmental regression and/or seizures, and cognitive status. The large sample size of the study allowed for stratification of results by case subgroups, parent and child characteristics, gene variants, and exposures. Our assessment of exposure included vitamins and fortified cereals, which are the major sources of folic acid intake in the United States (55) and correlate with blood folate concentrations (56). However, a limitation of this study was the lack of information on food folate. For individuals who do not consume vitamins and folic acid–fortified cereals, food folate determines blood folate concentrations, and we were unable to compare folate intake between cases and controls in this small group. Because >95% of US women of childbearing age consume <400 μg dietary folate from foods daily, with a mean of ~151 μg (55), and because the bioavailability of dietary folate is <60% that of folic acid (57), women consuming only food folate would likely fall into the lowest category of available folate. The contribution of food folate to overall folate status, and the corresponding effect on the OR, was likely greatest for women in the lowest category of folic acid intake, which make risk estimates for this category the least reliable.

Another limitation is the retrospective reporting of vitamin and supplement information in which mothers were asked to recall a period several years before the interview and after the child’s developmental status was known. The mean folic acid intake of women in our study before pregnancy was as expected from population estimates in the United States (58), providing evidence that we obtained reasonably accurate estimates of folic acid use. Furthermore, women’s self-reported supplemental folic acid use tends to correlate with blood folate concentrations (38, 59, 60). Still, given the period of recall, inaccuracies would be expected. If the errors were similar for mothers of cases and controls, then the resulting nondifferential misclassification would be expected to have produced ORs closer to the null (61). On the other hand, if the reporting was differential, bias can shift estimates in either direction (recall bias). For recall bias to have explained part of the association between folic acid and ASD, case mothers would have had to have underreported or control mothers would have had to have overreported their intake of folic acid during this period. Recall bias in this direction is contrary to reports showing that mothers of affected children are less likely to underreport most exposures during pregnancy than are mothers of unaffected children (62). In addition, mothers of case and unaffected control children are able to recall information on dietary folate and multivitamin supplements during pregnancy, including initiation and duration of use, equivalently and accurately for periods of >5 y (63). Moreover, our results were stronger in analyses limited to interviews conducted early on, when connections between folate and autism had not received media attention, and bias was less likely to affect reporting. This implies that if recall bias was a factor, it likely would have biased our risk estimates in the direction opposite of the found association, attenuating the observed association. Finally, the differences observed across genotypes are difficult to attribute to recall bias, because the case-control difference in recall would have to be limited to those with high-risk maternal or child genotypes.

We examined parental age, race and ethnicity, maternal smoking and alcohol consumption, and delivery type as confounders, and none appreciably affected the associations of interest. Other vitamins and minerals, including vitamins B-6 and B-12, choline, and iron, play essential roles in proper neurodevelopment (64–66), and their concentrations are correlated with those of folic acid in multivitamin supplements and cereal; however, the inverse association between folic acid and ASD became stronger when we adjusted for the amounts of these nutrients.

Our findings indicate that a sufficient amount of supplemental folic acid in the first month of pregnancy may reduce the child’s susceptibility for ASD. The protective association was limited to children and mothers who had at least one copy of the less efficient MTHFR 677 T allele, which is the majority (~60%) of this population. The fact that the mean reported intake for all groups exceeded the recommended intake for folic acid could suggest that the recommended level, determined for protection against NTDs, is potentially below what is needed to protect against other neurodevelopmental conditions, especially for individuals with certain genetic variants. The importance of these findings is magnified by their implications at a time when policies on folic acid fortification are still being debated. Future research should seek to replicate these results and to enhance understanding of the mechanisms behind any protective effect of folic acid against ASDs, especially in studies with prospectively collected diet and supplement data and/or biomarkers. If replicated, this work could augment previous evidence for the neuroprotective effects of periconceptional folic acid that currently serve as the foundation for public health policy and provide support for recommendations for women of childbearing age to consume folic acid–containing vitamins.

We thank the participants and staff of the CHARGE (CHildhood Autism Risk from Genetics and Environment) Study for their dedication to this research.

The authors’ responsibilities were as follows—RJS and IH-P: designed the research and wrote the manuscript; RJS: conducted the research, performed the statistical analyses, and had primary responsibility for the final content; DJT: provided statistical analysis expertise and support; IH-P: provided databases necessary for the research; RLH and SO: provided clinical diagnoses, and reviewed and revised the manuscript; and JH, HA, LCS, and FT: were responsible for genotyping. All authors read and approved the final manuscript. None of the authors declared a conflict of interest.

REFERENCES