

Synthetic folic acid supplementation during pregnancy may increase the risk of developing autism

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Abstract. Persons in developed countries are getting large amounts of folates in the form of folic acid. Folates are now ingested in three ways: as natural folates from food, as synthetic folic acid added to processed grains, and synthetic vitamin supplements. As a result of the supplementation, the circulating level of unmetabolized folic acid as well as total folates has greatly increased over the past generation, probably to levels largely unprecedented in human history. Folic acid has been shown to be able to epigenetically alter the functioning of the genome and to have long term effects on gene expression. The Centers for Disease Control Vaccine Safety Datalink data set compared children with autism to control children on several variables. Here, we report that folic acid supplementation during gestation is associated with an increased risk for autism. The effect remains even when health seeking behaviors and other variables are controlled.

Keywords: Folic acid, excess vitamins, autism, folate, synthetic vitamins

1. Introduction

The last century was marked by increasing scientific awareness that there are necessary nutritional factors that were not fully understood. As a result, expansive research projects have been undertaken to isolate specific food substances thought to prevent disease. For example, scientific understanding of the nature and effects of B vitamins has progressed throughout the past several decades. Of the eight B vitamins now known, in the 1930's, only thiamin, riboflavin and "P-P factor" (pellagra preventing) were officially defined [1]. Vitamin B9 (Folate) was first extracted from spinach leaves in 1941. The term folate comes from the same root as foliage: green and leafy. Folates are vital: they accept carbon atoms and pass them on as needed as the fundamental basis for proper methylation. Folate has

been found to be a key nutrient required for synthesizing DNA. Increased awareness of such food substances and their functions has led to the synthesis of artificial supplements and the prevention of many terrible diseases (rickets, scurvy, pellagra). Such vitamin supplements have also been added to many different types of processed foods. The combined effect of mass food supplementation with ingesting of additional supplements (e.g. via vitamin pills) has not always been fully and systematically investigated, and at least some effects of ubiquitous synthetic supplementation may not be predictable.

According to Smith, Kim and Refsum in their 2008 review, a fundamental difference in the metabolism of synthesized folic acid versus folate is that folic acid must be reduced to tetrahydrofolate before it can be used. This process itself requires the utilization of the key enzyme DHF-reductase (dihydrofolate-reductase, a key component in the entire folate cycle). Because there are individual differences in DHF-reductase activity in humans (e.g. methylenetetrahydrofolate reductase MTHFR C667T variants), and because humans, as

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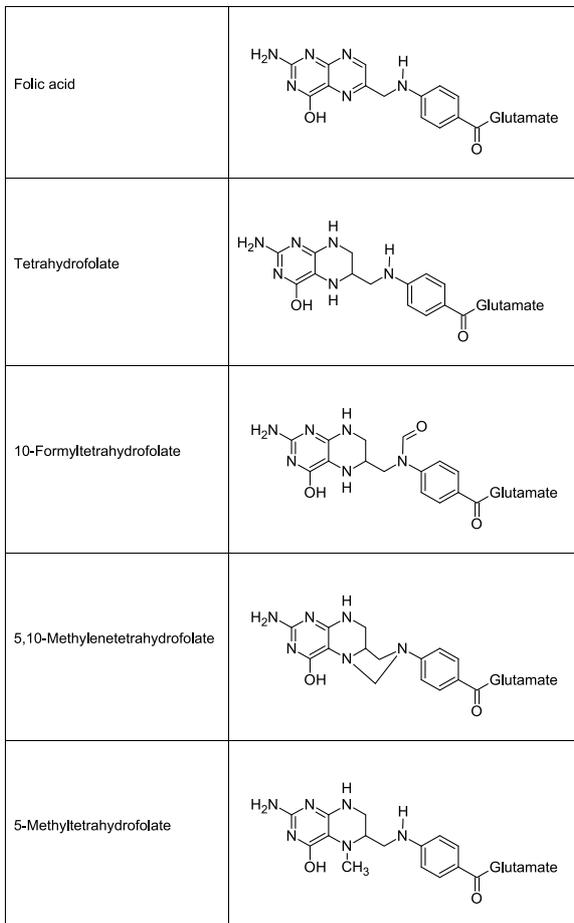


Fig. 1. Differences in folates.

a species, have low levels of this enzyme to begin—the competition for this enzyme may potentially be relevant [2], perhaps especially so in some persons. Overall, there is a body of research on the excesses and safety of synthetic vitamins [3,4]. Folic acid and folate are not identical: recent research has documented biological differences in metabolism and absorption based on the chemical differences in the two substances [2, 5]. Folic acid is nearly 100% bioavailable, whereas natural folates are far less so. Although lay persons are often specifically told that folic acid and folate are identical [6], this oversimplifies and does not fully conform to scientific research (Fig. 1).

Over the past generation, there has been major shift in human intake of folic acid. Folic acid was speculated to prevent neural tube defects for many years, and was clearly proven to prevent such birth defects in 1991 [7]. As a result, in 1993, it became the official recommendation in the United States that all women of

child bearing years take folic acid as a supplement. In 1996, the US and Canada took the additional step of requiring that all grain products sold have significant amounts of supplemental folic acid added. Importantly, such folic acid supplementation has drastically reduced the number of neural tube related birth defects such as spina bifida across multiple countries and populations [8–10]. Again, such health benefits cannot be overstated.

Notwithstanding the patent benefit associated with supplying sufficient folic acid during the weeks around conception, continual high levels of folic acid supplementation throughout a pregnancy and lactation may not be needed, and may possibly be harmful. An early study on heavy folic acid supplementation among previously healthy persons by Hunter and colleagues published in the *Lancet* [11] suggested that folic acid was neurotoxic in that sleep and mental status changes were brought on in the majority of those observed; it should be noted that although similar studies have been undertaken, this effect has never been replicated. In general, it does not appear that even large amounts of folic acid taken orally are acutely toxic in adults. However, given the fundamental role of folate levels in synthesizing nucleotides (including RNA and DNA) and in methylation reactions as a methyl donor, high levels may have inadvertent implications for proper methylation of DNA during times of rapid cell division, such as in prenatal development. The idea that adding folic acid to the food supply might have unintended consequences has been speculated as early as 2005, and specifically speculated to be relevant for the increase in autism in 2011 [12,13].

An early review of potential problems with mass folic acid supplementation of the food supply was undertaken by Lucock and Yates [14]. Here, they noted that a drastic increase in folates could lead to a selection for the previously rare MTHFR genetic substitution of T for C at area 677 (MTHFR C677T), and that if folic acid is supplemented at doses above 400 mcg that unmetabolized folic acid will circulate in the blood supply at a level largely consistent with the excess dose. In 2005, Lucock and Yates noted that high levels of folic acid in the blood does not generally occur as a result of ingesting natural folates and that “no work has been done so far to evaluate the biological and genetic consequences of excess long term exposure” to these circulating folic acids (p. 238). After that review, there were two separate findings of unexpected increases in asthma and breathing problems associated with folic acid use [15, 16] leading to speculation that excess methyl donors

during gestation lead to relevant epigenetic changes. This work dovetailed with another review questioning the wisdom of mass folic acid supplementation published in the same year [17]. Smith et al. [17] point out that by supplementing the food supply; several hundred thousands of persons are exposed to greatly increased levels of folic acid. Their overall suggestion was that folic acid supplementation is good for some people, but due to individual differences in consumption of folates and genetic vulnerabilities, such a blanket approach will be harmful to others. These authors noted that prior research had shown that expectant mothers with low vitamin B-12 (which sometimes happens with strict vegetarians) AND high levels of folic acid were associated with offspring having an unexpected increased risk for insulin resistance [18].

Troen et al. [19] found that some women past child bearing age subjected to high folic acid supplementation may be at risk for reduced immune system functioning [19]. As would be expected, women with low folate levels (from the diet) benefitted from folic acid supplementation in terms of markers of immune system functioning. However, women with already high total folate from the diet were harmed by additional folic acid supplementation: markers of immune activity were lower (reduced natural killer cell cytotoxicity) in these women than if they had no supplementation. Additionally, folic acid's role in dementia and Alzheimer's disease is still unclear. One study which followed 965 persons (over age 65) for approximately six years found that self-reported total folic acid intake was protective against the development of Alzheimer's disease [20]. In contrast, Morris et al. followed a much larger sample ($n = 3718$) for approximately six years and reported that the incidence of Alzheimer's disease onset among elderly persons was increased among persons with the highest amounts of folic acid [21]. Total B12 was still associated with a decrease in Alzheimer's disease risk. A more recent study [22] directly measured maternal levels of total folates in the blood of women at 35 weeks gestation, and then looked at birth outcomes. Results showed that high levels of folic acid in the blood were associated with a reduction in fetal length (39.3 inches versus 40.2 inches, $p < 0.03$). Also, those who were taking folic acid supplements were more likely to give birth at under 40 weeks gestation ($p < 0.05$). Overall, there was a reduction in birth weight (2894 gr versus 3154, $p = 0.01$). In contrast, there was a positive association between another B vitamin (B12 levels) and fetal length. This research also obtained measures of homocysteine and vitamin B6 as well as total folate

and B12. In mothers with folic acid supplementation, folate levels were not predictive of homocysteine levels, although in non-folic acid supplementing mothers, folate was negatively associated with homocysteine, as would be expected. Generally speaking, the results of recent research on supplementation with folic acid suggest that folic acid supplementation may have unintended negative consequences, and that selective excess intake of one vitamin type may have the potential to negatively alter metabolic activities.

The tolerable safe upper limit of folic acid has been suggested to be 1000 mcg per day. According to the Office of Dietary Supplements, "Folate intake from food is not associated with any health risk," the risk of toxicity comes from the synthesized supplements and foods artificially supplemented with synthetic folic acid that do not normally have folate in them. Folic Acid "intakes above this level increase the risk of adverse health effects" [23]. For children under the age of 8, the tolerable upper limit was set at 300–400 mcg. To put this in perspective, one cup of breakfast cereal alone has 400 mcg of supplemented folic acid. Data from the National Health and Nutrition Examination Survey (NHANES 1999–2000) documents that the increase in folate levels is not limited to women of child bearing years, and suggests that folate levels have rapidly climbed. For example, since an earlier NHANES study (conducted between 1988–1994), the average blood folate levels increased nearly threefold from 12.5 nmol/L to 32.2 nmol/L. The prevalence of high blood folate levels (which was defined as too high for valid results without additional dilution) increased from 7% to 43%. The mean for children under 5 was 45.5 nmol/L. Finally recent surveys suggest that more than 10% of pregnant women are taking folic acid dietary supplements in excess of 1000 mcg per day while pregnant [24], not counting the supplementation they are ingesting if they eat cereal, bread, or pasta.

Beard et al. [13] specifically speculated that increased folic acid supplementation could be relevant in understanding the increased risk for autism spectrum disorder (ASD). Beard and colleagues examined the rate of autism in a county for which prevalence data was available (Olmstead County, Minnesota) and compared this to the published levels of folic acid supplementation. Specifically, prevalence increases across time was highly correlated ($r = 0.87$) with the published percent of prenatal vitamins that contained folic acid (using the Physician Desk Reference for each year). The authors conclude their article by stating, "A case-control study would be useful to assess folic acid exposure among

newly-diagnosed patients with autism and controls using medical record information.” (p. 17). The temporal correlation is of interest, but not compelling standing alone. The authors suggested that were a case-control study done (persons with autism compared to controls), it could document whether an increased risk of autism is, in fact, associated with folic acid supplementation.

2. Methods

2.1. Center for disease control (CDC) dataset

In 2010 Christofer Price et al. published their article entitled, “Prenatal and infant exposure to thimerosal from vaccines and immunoglobulins and risk of autism” [25]. The data for the article was obtained via a large scale CDC sponsored Vaccine Safety Datalink project that collected immunization records across 3 health maintenance organizations within the United States. This research included 256 case children classified as having an ASD and 752 controls (children matched to cases but without a diagnosis of ASD) born between January 1, 1994 and December 31, 1999. In addition to the vaccine-related variables, this data set was rich with other variables. Of significant interest – one variable that was queried regarded the use of folic acid containing prenatal vitamins. For some analyses, Price et al., included folic acid supplementation as a covariate. Such supplementation, however, was not a primary variable of interest nor were there any predictions made regarding a possible link to an ASD.

The current authors requested and were granted access to the public-use data set which included many of the variables used in the Price et al. Pediatrics publication. This data allowed one to test the replicability of many of the findings in the two associated technical reports based on the larger research project [25,26]. Request for access was made directly to the CDC. Several public-use data files were provided to the authors of the current manuscript including: 1) Main Public Use Analysis File, 2) Prenatal Exposures Data File, 3) Child Body Weight File, and 4) Vaccine History Data File.

The primary purpose of the current analyses was to further investigate the nature and correlates of folic acid supplementation during pregnancy. According to section 17.3 of Technical Report II (25, pp. 100–101), “Data on the use of folic acid during pregnancy was obtained primarily from maternal report as part of the parent interview.” As described in the Technical Re-

port, the majority of both case (96%) and control (91%) mothers reported having used folic acid supplements while pregnant. Additionally, the authors of the Technical Report noted a significant relation between folic acid supplementation and diagnosis such that mothers of children diagnosed with an ASD were more likely to report folic acid supplementation ($p = 0.011$) (see Technical Report II, p. 100). Although such a finding has theoretical and practical implications, this finding, along with the links between folic acid supplementation and other health related indicators has not been widely disseminated to the scientific community. To our knowledge, this finding has not been elaborated upon or formally published in any peer-reviewed journal. Assuming there is no reason to suspect systematic reporting-accuracy differences between mothers of case and control children, these findings highlight the need to further investigate folic acid as a contributing factor to an ASD.

Here, we focus on the relation between folic acid supplementation and other health related variables including gender and ASD, Autistic Disorder (AD), and ASD with regression. Prior to data collection an extensive stratification procedure was employed such that participants were stratified by gender, HMO, and year of birth. So, while it is not possible to examine the main effects associated with these “nuisance” variables within a conditional logistic regression framework, it is possible to examine their interaction with folic acid supplementation on an ASD diagnosis.

To more fully assess the relation between folic acid and other theoretically related health variables (mother age, birth order, poverty ratio, health seeking behavior, mothers prenatal alcohol use, prenatal viral infections) we first assessed the zero-order correlations among these variables. Next, we computed a conditional logistic regression model (similar to that employed by Price et al.) to examine the relation between folic acid supplementation and ASD diagnoses after controlling for several other study variables that may relate to a decision to use folic acid containing vitamins and/or an ASD diagnosis. Also, in order to further assess differences between controls and cases we explored the interaction between gender and folic acid supplementation. This interaction term was not included in either the technical reports published by the CDC or in the analyses reported by Price et al. Finally, we employed a stepwise Discriminant Function Analysis (DFA), to determine how well participants could be classified into their respective case and control groups as a function of folic acid and several other measured variables

noted above. Here we wanted to determine: a) which set of variables/characteristics can be used to best discriminate between cases and controls and b) if the same set of variables/characteristics generalizes across each of the three ASD groups: all ASD, the subgroup with evidence of regression, and/or the subgroup with full autistic disorder (AD).

2.2. Statistical methodology

Because both ASD diagnoses and several other measured variables, including folic acid supplementation, were coded as dichotomies (1 = ASD, 0 = control; 1 = folic acid supplementation, 0 = No folic acid supplementation) a Phi coefficient is used to assess the degree of relation between variables (r_ϕ). Also, for those relations where one variable is a dichotomy and the other variable is continuous (such as between an ASD diagnoses and poverty ratio) the appropriate index of association is the point-biserial coefficient (r_{pb}). As such, the point-biserial symbol is used in place of the traditional Pearson product moment correlation coefficient symbol (r) when assessing such relations.

A conditional logistic regression was computed using SAS 9.2 proc phreg procedure to assess the relation between folic acid supplementation and an ASD diagnosis while adjusting for several covariates related to child and family characteristics (e.g., maternal age, birth weight, poverty ratio, birth order, breast feeding duration), maternal prenatal health care/seeking behavior (e.g., adequacy of prenatal care, cholesterol screen, pap smear, prenatal alcohol use, prenatal viral infections), and child medical conditions (e.g., anemia, pica). Additional adjustments were included within strata for those variables on which children were matched during participant selection: gender, HMO, and year of birth. Moreover, to examine the combined effects of gender and folic acid on an ASD diagnosis, the interaction term between folic acid supplementation and gender was also entered into the model.

Because of the stratified case-control design method used to collect data by the CDC, conditional logistic regression represents one of the best approaches for analyzing such data. Yet, this approach does not provide an easily interpretable index of whether each variable in the model is accounting for unique variance in the prediction of an ASD diagnosis. As a result, we also computed a stepwise DFA. The DFA approach allows one to determine which set of predictors contribute most to discriminating between case and control groups, as well as providing information on which

variables make unique contributions to between group discrimination (i.e., significantly improve the ability to correctly classify children as either cases or controls).

To model those predictors (covariates within the conditional logistic regression model) that most contribute to distinguishing between cases and controls, a stepwise DFA was performed using the variables as a predictor of group membership. Overall, two separate discriminate function classification models were computed. The first assessed which variables contribute most to distinguishing between the ASD group and controls; whereas, a second DFA assessed which variables contribute most to maximally distinguishing between AD and controls.

3. Results

3.1. Correlational analyses

Inspection of the zero-order correlations among the three ASD diagnoses and other variables indicated that ASD was positively related to folic acid supplementation, r_ϕ (1008) = 0.08, $p = 0.015$, childhood pica within first 3 years after birth, r_ϕ (1008) = 0.14, $p < 0.01$, if the child's mother had a cholesterol screen within the previous 3 year prior to giving birth, r_ϕ (1008) = 0.08 $p < 0.01$, and marginally related to when prenatal care was initiated, r_ϕ (1008) = 0.06, $p = 0.06$. These relations indicate that ASD children are more likely to have pica, have mothers who took folic acid supplements during pregnancy, and also more likely to have mothers who had a health screening within the previous three years (for cholesterol) but whom were also less likely to initiate prenatal care earlier in their pregnancy. In addition, relations emerged between ASD and poverty ratio, r_{pb} (1008) = -0.09 , $p < 0.01$, mothers age when the child was born, r_ϕ (1008) = -0.06 , $p = 0.04$, and birth order, r_ϕ (1008) = -0.07 , $p = 0.02$. These indicate that ASD diagnosis was also associated with families having a lower income (higher poverty ratio), mothers being younger than 20 years old when their child was born (compared to mothers aged between 20–24 years), and when the child of interest was a first born child (when contrasted with children were third or later in the birth order).

Considering only full AD, positive relations remained between an AD diagnosis and folic acid supplementation, r_ϕ (939) = 0.07, $p < 0.03$, and childhood pica, r_ϕ (939) = 0.14, $p < 0.01$; with a marginal relation between AD and health screening behavior (cholesterol

screening), $r_{\varphi}(939) = 0.02, p = 0.07$. Children with an AD diagnosis had mothers who indicated taking folic acid supplements. Additionally, children with an AD were more likely to have had pica within their first three years of life, and were more likely to be born to mothers who obtained health screening (cholesterol) within the previous three years prior to giving birth. Negative relations emerged between AD and poverty ratio, $r_{pb}(939) = -0.10, p = 0.002$, and between AD and birth order, $r_{\varphi}(939) = 0.07, p = 0.02$. AD diagnosis was more likely when poverty ratio was higher, and more likely among first born children (when contrasted with children were third or later in the birth order).

For ASD-regression, positive relations emerged between ASD-regression and folic acid supplementation, $r_{\varphi}(701) = 0.08, p = 0.029$, and between ASD-regression and childhood pica, $r_{\varphi}(701) = 0.20, p < 0.01$. Similar to those findings noted above, children whose mothers supplemented with folic acid during pregnancy and those children who had pica within the first three years after birth were more likely to be diagnosed with ASD-regression compared to those without folic acid supplementation and children who did not develop pica early in their development. Negative relations emerged between ASD-regression and poverty ratio, $r_{pb}(701) = -0.10, p = 0.012$, and advanced maternal age, $r_{\varphi}(701) = -0.09, p = 0.020$. Also consistent with those findings noted above, children were more likely to be diagnosed with ASD-regression when poverty ratio was higher and when a mother was younger than 20 years (when compared to those children who were born when their mother was between 20–24 years old).

3.2. Conditional logistic regression

Tables 1 (ASD model) and 2 (AD model) display the parameter estimates, standard errors of estimates, chi-squares, p values, odds ratio, and 95% CI for the odds ratios for each model parameter. The overall Likelihood Ratio, $\chi^2(25) = 77.11, p < 0.0001$ and Wald Test, $\chi^2(25) = 66.34, p < 0.0001$ were significant indicating that the covariate adjusted model represented a significant improvement in fit over a null model (AIC model fit statistics: 1014.34 without covariates and 987.23 with covariates). Overall, for ASD, after adjusting for other covariates, maximum likelihood estimates indicated a significant effect of folic acid supplementation on ASD diagnosis ($\chi^2 = 5.37, p = 0.020$, OR: 2.34, 95% CI: 1.14–4.82). These results indicate that mothers who self-report using folic acid supplementation are

over twice as likely to have had a child with ASD compared to those mothers who did not report using folic acid supplements. The interaction between gender and folic acid supplementation did not contribute significantly to the fit of the model ($p = 0.71$) suggesting that males and females were equally impacted by folic acid supplementation.

A similar pattern emerged on AD diagnosis: Likelihood Ratio, $\chi^2(22) = 73.44, p < 0.0001$; Wald Test, $\chi^2(22) = 60.55, p < 0.0001$. Table 2 provides the maximum likelihood parameter estimates. Again, folic acid supplementation increased the odds of having a child diagnosed with an AD by more than 2.5 times that of mothers who did not supplement ($\chi^2 = 4.55, p = 0.033$, OR: 2.52, 95% CI: 1.08–5.91). The interaction term between gender and folic acid supplementation was not significant ($p = 0.96$). A similar model on ASD-regression lead to unstable parameter estimates for folic acid supplementation, possibly due to the relatively small sample size: only 49 children with ASD-regression (resulting in a lower case to control ratio for some strata and fewer strata being analyzed).

3.3. Discriminant function analysis

Results from the first DFA yielded a significant discriminant function maximally separating ASD cases from controls, $\Lambda = 0.94, \chi^2(8) = 62.10, p < 0.001$, canonical correlation = 0.25). Examination of the structural coefficients, which represent the correlations between each of the predictors (i.e., covariates within conditional logistic regression models) and discriminant functions, indicated that the best predictors for distinguishing between groups were childhood pica, poverty ratio, health screenings, birth order, maternal age, adequacy of prenatal care, and folic acid supplementation. In addition, the standardized canonical discriminant function coefficients provide information on which predictors have the greatest impact on the discriminant function scores.

Table 3 displays the standardized canonical discriminant function coefficients, structural coefficients, significance of F to enter, significance of F to remove, exact F-ratio for each step, and significance value associated with the change in F at each step. F to enter provides initial information on the importance of each predictor, by itself, in distinguishing between groups (i.e., predicting group membership). F to remove reflects the reduction in prediction if a predictor was removed from the model and provides information on the unique contribution each predictor makes to distin-

Table 1
Covariate adjusted conditional logistic regression model results comparing ASD cases to controls

Variable	Parameter estimate	Standard error	Chi-Square	Chi-Square probability	Hazard ratio	95% CI lower limit	95% CI upper limit
Mom 20–24	−0.40	0.69	0.33	0.56	0.67	0.17	2.59
Mom 25–29	0.29	0.64	0.20	0.66	1.33	0.38	4.69
Mom 30–34	0.59	0.64	0.84	0.36	1.81	0.51	6.38
Mom > 35	0.70	0.65	1.17	0.28	2.02	0.57	7.19
Birth order 2	−0.13	0.17	0.56	0.46	0.88	0.63	1.24
Birth order 3	−0.75	0.23	10.65	0.001	0.47	0.30	0.74
BF1-6month	−0.28	0.20	1.81	0.18	0.76	0.51	1.13
BF > 6 month	−0.33	0.20	2.70	0.10	0.72	0.49	1.07
Poverty ratio	−0.10	0.03	12.23	< 0.0001	0.90	0.85	0.96
Inadequate prenatal care	0.70	0.44	2.54	0.11	2.02	0.85	4.78
Cholesterol 1	−0.17	0.32	0.29	0.59	0.84	0.45	1.58
Cholesterol 2	0.44	0.24	3.45	0.06	1.55	0.98	2.46
PAP 1	0.44	1.23	0.13	0.72	1.55	0.14	17.38
PAP 2	−0.21	1.21	0.03	0.86	0.81	0.08	8.64
Prenat. alcohol	0.28	0.24	1.33	0.25	1.32	0.82	2.12
Prenat. viral infection	0.29	0.32	0.82	0.36	1.34	0.71	2.50
Prenat. lead	−0.19	0.18	1.14	0.29	0.82	0.58	1.18
Birth weight 1–1.499 kg	−2.60	1.47	3.14	0.08	0.08	< 0.01	1.32
Birth weight 1.5–2.499 kg	−1.25	1.00	1.55	0.21	0.29	0.04	2.05
Birth weight 2.5–3.99 kg	−1.29	0.97	1.77	0.18	0.28	0.04	1.84
Birth weight \geq 4 kg	−1.15	0.99	1.37	0.24	0.32	0.05	2.18
Anemia	−0.69	0.57	1.46	0.23	0.50	0.16	1.54
Child pica	1.49	0.35	17.83	< 0.0001	4.46	2.23	8.92
Folic acid/prenatal multivitamin	0.85	0.37	5.37	0.02	2.34	1.14	4.82
Gender x folic acid supplementation	−0.04	0.10	0.13	0.71	0.96	0.78	1.18

Table 2
Covariate adjusted conditional logistic regression model results comparing AD cases to controls

Variable	Parameter estimate	Standard error	Chi-Square	Chi-Square probability	Hazard ratio	95% CI lower limit	95% CI upper limit
Mom 20–24	−0.70	0.71	0.98	0.32	0.50	0.12	1.99
Mom 25–29	−0.15	0.66	0.06	0.81	0.86	0.24	3.10
Mom 30–34	0.19	0.66	0.08	0.78	1.21	0.33	4.37
Mom > 35	0.31	0.66	0.22	0.64	1.36	0.37	4.97
Birth order 2	−0.08	0.20	0.18	0.67	0.92	0.63	1.35
Birth order 3	−0.79	0.27	8.89	0.003	0.45	0.27	0.76
BF1-6 month	−0.25	0.23	1.17	0.28	0.78	0.49	1.23
BF > 6 month	−0.23	0.23	1.03	0.31	0.79	0.51	1.24
Poverty ratio	−0.13	0.04	13.18	< 0.0001	0.88	0.82	0.94
Inadequate Prenat. care	0.29	0.53	0.30	0.59	1.33	0.47	3.77
Cholesterol 1	−0.46	0.37	1.51	0.22	0.63	0.30	1.32
Cholesterol 2	0.41	0.26	2.44	0.12	1.51	0.90	2.52
PAP 1	0.30	1.26	0.06	0.81	1.36	0.12	15.87
PAP 2	−0.48	1.23	0.16	0.69	0.62	0.06	6.81
Birth weight 1–1.499 kg	−2.78	1.46	3.65	0.06	0.06	< 0.01	1.08
Birth weight 1.5–2.499 kg	−1.78	1.02	3.05	0.08	0.17	0.02	1.24
Birth weight 2.5–3.99 kg	−1.85	0.98	3.56	0.06	0.16	0.02	1.07
Birth weight $t \geq$ 4 kg	−1.80	1.00	3.21	0.07	0.17	0.02	1.19
Anemia	−1.90	1.05	3.30	0.07	0.15	0.02	1.16
Child pica	1.57	0.39	16.20	< 0.0001	4.79	2.23	10.26
Folic acid/prenatal multivitamin	0.93	0.43	4.55	0.03	2.52	1.08	5.91
Gender x folic acid supplementation	0.01	0.11	< 0.01	0.96	1.01	0.81	1.24

Table 3
Discriminant function analysis (DFA) results for those variables found to maximally distinguish ASD cases from controls

Step	Predictor	Standardized DFA coefficient	Structural coefficient	Sig. of F to enter	Sig. of F to remove	Exact F-ratio	p-value
1	Pica	0.55	0.55	< 0.001	< 0.001	8.51	< 0.001
2	Poverty ratio	-0.45	-0.37	0.003	0.001	12.14	< 0.001
3	Cholesterol	0.39	0.33	0.009	0.003	10.11	< 0.001
4	Birth order	-0.38	-0.29	0.019	0.004	14.05	< 0.001
5	Mom 20–24	-0.34	-0.25	0.044	0.010	19.60	< 0.001
6	Folic acid/ Multivitamin	0.29	0.31	0.015	0.026	7.98	< 0.001
7	PAP	0.28	0.20	0.117	0.036	9.14	< 0.001
8	Inadequate Prenat. Care	0.26	0.23	0.062	0.043	10.90	< 0.001

Table 4
Discriminant function analysis (DFA) results for those variables found to maximally distinguish AD cases from controls

Step	Predictor	Standardized DFA coefficient	Structural coefficient	Sig. of F to enter	Sig. of F to remove	Exact F-ratio	p-value
1	Pica	-0.58	-0.56	< 0.001	< 0.001	19.06	< 0.001
2	Poverty ratio	0.49	0.39	0.002	< 0.001	14.13	< 0.001
3	Cholesterol	-0.37	-0.29	0.024	0.007	11.62	< 0.001
4	Birth order	0.36	0.28	0.032	0.007	10.33	< 0.001
5	PAP	0.32	0.22	0.085	0.020	9.34	< 0.001
6	Anemia (6–30 months)	0.29	0.25	0.050	0.030	8.64	< 0.001
7	Mom 20–24	0.28	0.21	0.102	0.041	8.02	< 0.001
8	Folic acid/ multivitamin	-0.27	-0.28	0.027	0.046	7.54	< 0.001

guishing between groups. Childhood pica was found to be the strongest contributor to distinguishing between ASD cases and controls followed by poverty ratio, frequency of health screening (cholesterol), birth order, maternal age at child birth, folic acid supplementation, health screening (PAP), and adequacy of prenatal care. The group centroids for the control and ASD groups were -0.147 and 0.433, respectively. The remaining predictors did not significantly contribute to the prediction of group membership. Mothers of ASD children were more likely to be younger (< 20 years – when compared to the 20–24 maternal age groups), live below the poverty line, have health screenings but inadequate prenatal care, and take folic acid supplements. In addition, ASD group of children were characterized by having pica and being born earlier than their siblings (at least when first born children were compared to those born third or later). Examination of the F to remove indices indicated that the prediction of group membership would be significantly reduced if any predictors were removed from the model.

A second DFA was computed to examine which predictors maximally distinguish between AD cases and controls. The predictors for this analysis were the same as described above, with the exception that mother's prenatal alcohol use, prenatal viral infections, and prenatal lead exposure were not included due to unstable parameter estimates for these variables.

Results yielded a significant discriminant function maximally separating AD cases from controls, $\Lambda = 0.94$, $\chi^2(8) = 58.64$, $p < 0.001$, canonical correlation = 0.25). Examination of the structural coefficients indicated that the best predictors for distinguishing between groups were childhood pica, poverty ratio, health screenings, birth order, anemia, maternal age, and folic acid supplementation (see Table 4). Again, childhood pica and poverty ratio were found to be the strongest predictors distinguishing between AD cases and controls followed by frequency of health screening (cholesterol), birth order, health screening (PAP), anemia, maternal age, and folic acid supplementation. The group centroids for the control and AD groups were 0.127 and -0.510, respectively. In contrast to the ASD findings noted above, prenatal care did not distinguish significantly between AD and control groups; whereas, anemia was found to account for unique variance in the prediction of group membership. Further examination of the pattern of F to Enter and F to remove suggest that pica, poverty ratio, cholesterol health screening, folic acid supplementation, and anemia are those predictors most likely to provide separation between AD and control groups.

4. Discussion

Every factor that increases risk provides an important etiological clue and advances our understanding

in this area. Whatever factors are causing ASD, it is some combination of genetic vulnerability and environmental triggers. Neither the exact genes nor the exact triggers are known. But there are things that are known. There seems to be a somewhat decreased ability to detoxify the body in persons with ASD [27–29]. Researchers have reported that specific genetic variations that are involved in detoxification are seen more commonly in ASD individuals [30–32]. Research has focused on differences in methylation processes and oxidative stress in persons with ASD. Oxidative stress is likely to play a role in the majority of brain-based developmental disorders [33]. Recently it has been reported that persons with ASD have more markers of anti-neuron autoimmunity and lower levels of glutathione in circulation [34]. Persons with ASD have been shown to have both lower glutathione levels and low levels of S-adenosine methionine [35], a situation that would be expected to greatly impair effective methylation.

There is lot of important information in the literature. Children with ASD have various markers of epigenetic alterations such as MeCP2 underexpression in the absence of actual genetic mutations for the controlling gene [36]. This is interesting. MeCP2 mutations in the genetic code cause disorders whose manifest behaviors overlap with those associated with an ASD disorder (e.g., Rett Syndrome, MeCP2 duplication syndrome) [36,37]. The promoter region for this gene is normally unmethylated in males and active [36], but in a sample of nine frontal cortex samples, there was increased promoter methylation, an epigenetic effect that seems to result in lesser amounts of the MeCP2 protein (another path to the same problem of low MeCP2 protein). Although beyond the scope of this paper, we note evidence that brain changes in persons with ASD appear to be related to epigenetic changes and improper methylation for neurally relevant genetic functioning. Overall, persons with ASD appear to often have altered expression of genes that can be regulated by epigenetic mechanisms, there is increasing support for the contention that these differences in expression are originating from early epigenetic changes and impact levels of various important proteins and enzymes in the body. The path to this state is likely to include some genetic vulnerability to various kinds of insults leading to oxidative stress, perhaps in tandem with gestational events that slightly alter the programming to be further sensitive to certain kinds of insults.

Here, we report that maternal supplementing with a substance known to epigenetically alter the developing offspring is associated with an increased risk for lat-

er development of ASD in a matched case-control design. It should be noted and that the matching design of dataset can be expected to underestimate the true size of the effect if level of folic acid supplementation changed over the birth cohort years, since this effect (any effect associated with birth year) was matched out. Folate itself is fundamental to normal brain functioning, the methylation cycle, and overall development. Folic acid supplementation somehow prevents neural tube defects when taken around conception. Yet, it appears that high level of unmetabolized synthetic folic acid circulating alters the functioning of hundreds of genes [38], and, unfortunately some of the effects may not be positive (e.g., lower birth weight) [22,39]. Contrary to popular belief, synthetic folic acid is not identical to natural folates, can lead to unmetabolized folic acid in circulation at high doses [14].

Folic acid supplementation can be expected to epigenetically alter the offspring. The ability of folic acid to do so is beyond dispute and has been well established in both animal and human models [40,17]. Recent research has employed human lymphoblastoid cells and controlled amounts of folic acid to quantify the effects on the cell: folic acid supplementation alone can lead to the dysregulation of over 1000 genes [38]. The various effects of such epigenetic changes during development cannot be predicted. Of interest, some of these changes occur at amounts lower than folic acid levels commonly found in supplemented human's circulating blood (15 ng/mL). In sum, persons with ASD were more likely to have mothers taking folic acid supplements during pregnancy. This effect was not explainable by health-seeking behaviors or other variables, including child birth conditions and early childhood health conditions.

The results here reported are limited primarily due to the self-reporting methodology utilized by Price et al. In addition, matching cases to controls on the basis of birth year will obviously have the effect of removing the variance associated with changes in folic acid supplementation over time; in as much as prenatal folic acid supplementation changed over the years 1999–2001, these effects would be lost due to the matching design. If anything, the folic acid affect would be underestimated.

As reviewed above folic acid and folates alter the methylation cycle and excess folic acid can epigenetically alter offspring as reviewed above [17,38,40]. Further, methylation/oxidation problems have been shown to play a role in ASD [41–44]. The a-priori prediction that excess folic acid could be increasing the rate of ASD [13], and that nutritional surveys indicate that a

significant number of persons taking supplement will be well above the RDA [24] imply that these results of a large case-control study documenting higher ASD in those whose mothers took folic acid supplements should be noted. Although the biochemical mechanisms require further study, it appears to be a genuine effect.

If the effect is replicated, it is not clear what the implications would be. The benefit of folic acid supplementation on preventing birth defects is real and not questioned. It is unlikely, even if replicated, that women of child bearing years or women in early stages of pregnancy would ever be advised to lower their folate intake below the RDA. However, it could be that the increased risk associated with folic acid might be limited to persons who

- a. Already have especially high dietary intake either by fortified foods or natural folates.
- b. Have some pre-existing tendency towards methylation errors, susceptibility to oxidative stress or limited ability to reduce folic acid to tetrahydrofolate.
- c. Have high folic acid throughout pregnancy. The protection of spinal tube birth defects is likely limited to folic acid intake during the early part of the first trimester.

It is suggested that these results be replicated, along with a dietary intake survey to estimate total folates consumed with respect to outcomes from genetic profiling for MTHFR C667T variants. It seems possible that high levels of synthetic folic acid supplementation has some risk which may not be outweighed by the benefits for at least some persons.

Acknowledgement

This work was partly funded by a grant from Safeminds to the second author. We thank the CDC and Eric Weintraub for their assistance with providing the public use data set and associated documentations [25,26]. We are grateful to Wayva Lyons for her help with references and to Dr. Jeff Elbert for providing the sketches of folate chemical structures.

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