19 January 2011

Folate status, nutrition and maternal transmission in Irish families with neural tube defects

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Abstract

Background: Maternal relatives in neural tube defect (NTD) families have elevated rates of NTDs and of other birth defects. Folic acid supplementation raises blood folate levels and prevents most new cases of NTDs. There is little information on blood folate levels among distant relatives, and on the potential relationship of serum folate to maternal transmission.

Methods: A cross-sectional study assessed serum folate levels among relatives in Irish families with NTDs to evaluate factors associated with blood folate, including matrilineal effects. 324 relatives gave a blood sample for analysis of serum folate and completed two questionnaires concerning lifestyle and dietary folate. Quartiles of the standardized serum folate distribution were evaluating for statistical significance with chi-square tests.

Results: Maternal uncles/aunts were more likely to fall into the lowest quartile of serum folate compared to all other relatives (34.1% vs 22.5%, odds ratio=1.68, 95% confidence interval (CI) 0.83, 3.45, p=0.15), after controlling for current alcohol use and intake of fortified foods. Factors that were associated with higher folate levels included intake of enriched/fortified foods (p=0.001) and supplemental folic acid (p<0.0001). Naturally-occurring food folate was weakly associated with serum folate (p=0.14). Increasing amounts of dietary folate were directly associated with levels of serum folate in this cohort of relatives in Irish NTD families.

Conclusions: The weak association of low levels of serum folate with maternal line probably did not explain the excess risk for birth defects seen among maternal relatives in this cohort.

Introduction

Neural tube defects are thought to result from the combined effects of an inherited genetic susceptibility, the basis for which is unknown, and an environmental agent -- a relative deficiency of folic acid in the diet -- whose mode of action is also unknown [1]. Evidence for the genetic susceptibility comes from the raised risk of recurrence of NTDs among both close and distant relatives [2]. Low levels of first trimester blood folate are associated with increased risks for an NTD-affected pregnancy [3]. Clinical trials confirmed the role of folic acid in protecting pregnancies against NTDs [4]. Public health authorities recommend that all women between the ages of 15 and 44 who can become pregnant should take a folic acid tablet daily. However, most women in their reproductive years, despite good knowledge of the benefits of folic acid, were not taking supplemental folic acid [5] [6]. Food fortification was undertaken in countries, such as the USA , to ensure that their populations received additional folic acid in their diets [7]. Subsequently, increases in blood folate levels of the US and Canadian populations were accompanied by declines in the birth prevalence of NTDs [8] [9].

Distant relatives in families where an individual has been born with an NTD are more likely than the general population to have excess rates of all birth defects combined; maternal relatives have higher rates of all birth defects combined than paternal relatives and higher rates of NTDs [10, 11]. Although some studies have shown relative deficiencies among mothers close to the time of delivery [12], there is little information on the blood folate status of distant relatives to help explain their increased susceptibility. To address this issue we carried out a study of serum folate levels among relatives, that was intended to assess factors associated with serum folate among relatives, and to compare average blood folate levels between maternal relatives and paternal relatives. Relatives may have an innate folate disorder [13] making it difficult for them to access additional food folate; if so, other strategies would be needed to ensure maximum protection for their pregnancies.

Materials and methods

Study design and participants

The participants in this cross-sectional study were drawn from Irish families with NTDs. These families had previously participated in epidemiologic studies of the Boyne Research Institute [10]. To be eligible for this study, male and female participants had to be 15 years of age or older, resident in the North-East region of Ireland, or Northern Ireland, either the proband or a blood relative of the proband. In terms of relationship, relatives (defined according to their relationship to the proband -- the affected individual) had to be one of the following: proband, parents, siblings, nieces and nephews, offspring (of the proband), grandparents, uncles and aunts, first cousins and first cousins once removed (the offspring of the first cousins). Participants gave written informed consent and assent as appropriate. From 47 families 331 participants came to the clinic, for a response rate among eligible, locatable relatives of 49.3%. From the 331 participants, 314 had data on serum folate, and completed the lifestyle and dietary folate questionnaires. Six participants could not contribute a blood sample due to veins too small or collapsed, or participant was unwilling/unable. Ten participants did not complete the dietary questionnaire because of a computer problem, and one blood sample was discarded as incorrect due to an error during the sampling procedure. The study was conducted in a phlebotomy clinic setting between June and November, 2007.

Measurements

The biospecimens consisted of three tubes of non-fasting blood totalling 24 ml. One tube was sent to a commercial laboratory for immediate serum folate and vitamin B12 analysis; two tubes were frozen for future molecular studies. Two questionnaires sought information on lifestyle and dietary folate intake. The self-administered lifestyle questionnaire collected basic demographic information, such as date of birth, current height and weight, and a brief reproductive history. In addition, for the past three months only, information was sought on types of diet (whether vegetarian, vegan, etc) medications, and intake of folic acid supplements and multivitamins. Questions about alcohol, cigarette and drug (marijuana, cocaine, heroin) use, medications and health conditions, and conditions that might affect absorption of folic acid, such as coeliac disease, gluten intolerance or digestive problems were included.

The second questionnaire was a modification of the Block Folic Acid/Dietary Folate Equivalents screener (DFE) consisting of a series of 22 questions about specific foods and their frequency. The original questionnaire was validated in a US setting [14]. The Block screener was modified for an Irish diet as follows: four new questions were added to capture folate-fortified foods available at the time in Ireland (dairy spreads, milk, energy bars, peanut butter/Nutella). Additions were made to other questions (porridge, scones, croissants, panini, lentils, take-aways, sweet corn). One question was dropped (breakfast burrito). Fifteen questions on the original screener were unchanged. Portion sizes were not asked. The questions are asked in the clinic by a trained interviewer. The resulting data were analyzed by the company and returned for incorporation into the study results. The screener measured the following sources of dietary folate which are included in this report: naturally-occurring food folate (in μg, micrograms; mean=173.6, SD (standard deviation) 63.3, range 0.04 – 367.0), fortified/enriched folic acid from food (μg; mean=334.8, SD 141.0, range 39.6 – 833.9), folic acid from supplements for those who took supplements, measured as DFE, or dietary folate equivalent (mean=668.2, SD=346.3, range 54.4 – 1360.0), and total folate from food, measured as DFE (mean=946.0, SD=460.0, range 154.9 – 2582.2). The term "Dietary Folate Equivalents" (DFEs) was adopted in the US to help account for the differences in absorption of naturally occurring dietary folate and the more bioavailable synthetic folic acid. The quantity of

dietary folate equivalents occurring naturally in food equals the micrograms of folate as reported; the dietary folate equivalents provided by fortified foods equal the micrograms of food folate plus 1.7 times the micrograms of added folic acid [15].

Serum folate in nanograms per ml was measured using an Elecsys folate immunoassay (Roche Diagnostics, Indianapolis, IN, USA). Vitamin B12 was measured using a Chemoluminescent Microparticle Immunoassay (Architect System, Abbott Laboratories, Abbott Park, IL, USA). During the course of the study the lab changed its standards, resulting in two different distributions of serum folate. The first group of 302 participants had a mean serum folate value of 10.7 (SD 4.1, range 3.8 – 20.0), while the mean value for the second group of 22 was 6.9 (SD 3.3, range 2.0 – 13.5). Serum folate values were significantly different from each other ($p<0.05$), necessitating a Z-transformation (see below). Results pertaining to vitamin B12 are not reported in this paper. A number of polymorphisms associated with the metabolism of folic acid were evaluated; the results will be reported in a separate paper.

Statistical Analysis

Data handling was done in EXCEL, and statistical analysis in SAS 9.1 (SAS Institute, Cary, NC, USA) and STATA. The dependent variable was serum folate measured in nanograms/ml. The fact that the two distributions of serum folate (see above) were significantly different from each other meant that for the analysis the data had to be transformed to result in a single z distribution with a mean of 0.0 and a standard deviation of 1.0. The data transformation was done using EXCEL, and the results were checked in SAS. The sensitivity of the test did not extend beyond 20 ng/mL; as a result a number of (N= 18) participants had folate levels of 20 ng/ml. Transformed values equivalent to 20 ng/ml or more were dropped from analyses that used continuous variables. Principal analyses looked at quartiles of each of the two distributions of the original serum folate measures combined as well as transformed z-scores. Because no participant who took either multivitamins with folic acid or folic acid by itself had blood folate levels in the lowest quartile, a pooled variable was created assigning YES to any participant who took either multivitamins with folic acid, or folic acid alone, or a multivitamin preparation in which it was unknown whether folic acid was included, and NO otherwise (called “folic acid”). Measures of association were evaluated with t-tests for continuous variables and chi-square tests for categorical variables with the level of significance set at 0.05. All statistical tests were two-tailed. The Mantel-Haenszel chi square test was used to evaluate potential confounding of the association between serum folate and line by a third factor. Paternal relatives were related to the proband via the father. They were pooled from paternal uncles/aunts, paternal first cousins and paternal first cousins once removed (the children of the paternal first cousins); maternal relatives were defined in the same way. For the analysis, paternal and maternal relatives were further subdivided into maternal and paternal uncles/aunts and paternal and maternal first cousins. Of the 324 relatives total 22 were paternal uncles/aunts, 44 were maternal uncles/aunts, 41 were paternal first cousins and 65 were maternal first cousins. Analyses of relatives compared each group to all other relatives.

Ethical Approval

The study was approved by the Ethics Board of the Boyne Research Institute, and by the Ethics Committee of the Health Service Executive of the Irish Government’s Department of Health and Children.

Results

Study characteristics

This report describes results from 324 participants whose blood samples were tested for serum folate. First cousins were the most numerous class of relatives, with 106 participating, followed by uncles/aunts (N=66) and siblings (N=59). Thirteen nuclear families (proband, mother and father) took part (Table 1). Nearly three-quarters of the women (72.4%) had had a pregnancy with the number of pregnancies ranging up to 13. 22.7% of participants said that they had taken folic acid in the last 3 months. Heavy alcohol consumption (more than 3 glasses per day on average) was reported by 16.4%; 23.9% were current smokers. Eleven (3.5%) participants had diabetes. Age at study ranged from 15 to 85 (median 41 years); body mass index (BMI) ranged from 17.5 to 39.6 (median 25.6). Coeliac disease, gluten intolerance and lactose deficiency affected few participants (2, 4 and 3 respectively). No participant had phenylketonuria (PKU) or cystic fibrosis.

Serum folate levels

The distribution of quartiles of serum folate among individual types of relatives is shown in Table 2. The two small groups of probands (N=13) and first cousins once removed (N=7) were each significantly lower in serum folate than all other relatives combined (p=0.03). These were the youngest groups of relatives in age (28.7 and 22.3, respectively). There was some suggestion that they were lower on dietary folate intake: probands were more likely to fall into the lowest quartile of total folate compared to other relatives (p=0.07); first cousins once removed also tended to be low on total folate (p=0.18). It is possible that the low serum folate of these two groups of relatives is explained by dietary folate intake. Further analysis was impossible due to small numbers.

To evaluate the principal hypothesis of this study we compared quartiles of serum folate between maternal and paternal relatives (Table 3). There were no significant differences between paternal and maternal relatives. Among paternal relatives 31.3% fell into the lowest quartile of serum folate, compared to 24.4% of maternal relatives. To extend this analysis, maternal and paternal relatives were evaluated according to whether they were uncles/aunts or first cousins. Some significant differences emerged. Maternal uncles/aunts were more likely than other relatives to be low in serum folate; 34.1% fell into the lowest quartile compared to 22.5% of other relatives (p=0.03). Maternal first cousins were less likely to be low on serum folate (13.9% vs 26.2%, p=0.10; Table 3). In a multiple logistic regression analysis including terms for alcohol use and food fortification, the odds ratio describing the association between low serum folate in maternal uncles/aunts was 1.68 (95% confidence interval (CI) 0.84, 3.45, p=0.15). Another logistic regression model evaluated the association between serum folate and maternal first cousins (including terms for alcohol use and intake of fortified foods), and resulted in an odds ratio of 0.53 (95% CI,0.24, 1.14, p=0.10). Thus, these two groups of relatives show a weak association with serum folate independently of other powerful potential confounders, and in the opposite direction.

We evaluated the distribution of serum folate in quartiles by levels of a number of other factors, including those in Table 1. Age at the time of study was not associated with level of serum folate. Factors that were associated with folate levels in the lowest quartile were heavy alcohol intake, that is, drinking more than three glasses per day (p=0.01), current smoking (p=0.04) and (illegal) drug use (p=0.03). In the opposite direction, we evaluated the protective effect of different sources of dietary folate in bivariate comparisons. The degree of protection varied with the folate source (Table 4). Naturally-occurring food folate conferred the least protection: the proportion of participants with the highest quartile of serum folate ranged from 19.2% to 30.3% with increasing

quartiles of naturally-occurring food folate. Fortified/enriched foods provided more protection (p=0.001). Here the percentage of participants in the highest quartile ranged from 15.4% to 38.2% according to increasing quartiles of fortified food intake. Any type of supplemental folic acid conferred the most protection (p<0.0001), with 50% of supplemented participants falling into the highest quartile of serum folate, compared to 18.9% of unsupplemented participants. We compared the odds ratios for the direct association between serum folate and the highest quartile of food folate as follows: for naturally-occurring folate it was 2.25 (95% CI 0.91, 5.60), for folate from enriched/fortified foods it was 4.33 (1.80, 10.39), and for supplemental folate it was 15.8 (4.58, 54.51; Table 6). This means that a participant whose intake of supplementary folate fell into the highest quartile was 15 times more likely to have a serum folate level in the highest quartile compared to all other quartiles. This comparison is unadjusted for other factors, including different folate sources. The distribution of serum folate levels in z-scores was plotted for participants who did not take any form of supplemental folic acid and for those who did (Figure 1). The shape of the distribution for the supplemented group is shifted to the right compared to the total group; the upturn in the right tail possibly reflects the insensitivity of the test. Most (12/18) participants with blood folate levels of 20 ng/ml were taking supplemental folic acid. We evaluated the dose-response relationship between serum folate and total dietary folate in Figure 2. The box plots show an increasing level of serum folate according to quartiles of total dietary folate.

The question arose if, overall, participants might have lower blood folate levels than expected, since our hypothesis involved lower folate levels. Since the study did not include a control group, to address this question the clinical laboratory provided a dataset consisting of all (N=5,284) serum folate tests, excluding the study tests, done during the same time period. From this dataset we eliminated values below and above the limits of detection (0.0 and $\geq$20.0 ng/mL), and limited the comparison to tests done with a single set of control standards. Thus, the clinical laboratory group of N=4767 was compared to 285 NTD relatives. The mean blood folate level of the clinical laboratory group was 9.12 ng/mL compared to a value of 10.10 ng/mL for the participants. A test of whether the two groups had the same distribution was rejected (p=0.001). Thus, the mean folate level of the study participants was significantly higher than that of the clinical laboratory group.

Discussion

This is the first study to evaluate serum folate levels among a number of different relatives in NTD families. We found weak evidence of serum folate among maternal uncles/aunts. Maternal first cousins were not lower in folic acid than expected. The study provided only weak support for our hypothesis that maternal relatives had significantly lower serum folate levels than paternal relatives. It seems unlikely that low serum folate could explain the observations of increased rates of birth defects among maternal relatives in these families. The rationale for this study was based in previous observations that 1) linked blood folate with NTDs, and 2) linked birth defects and NTDs with the maternal line (uncles/aunts, first cousins and first cousins once removed) [3] [11] [16].. Likewise, mothers' levels of serum folate, while tending to be low, were not significantly lower than the other participants. Neither does the cohort, overall, have lower serum folate levels than expected based on a large sample from the clinical laboratory. Our study provides only weak evidence of low folate among maternal relatives. We conclude that current levels of serum folate do not explain a persistently higher rate of birth defects among subsequent generations of maternal relatives in these families.

Other studies that evaluated blood folate levels before or close to the time of delivery of a child with a neural tube defect found lower levels of plasma folate among mothers compared to controls [12], other studies found no association [17][18]. A systematic review concluded that on balance serum folate was significantly lower in affected pregnancies compared to controls, particularly in the first trimester [19]. The difference in serum folate levels associated with having a child with an NTD seems to lessen after birth. This would be consistent with the results of our study, which show no, or minor differences, between groups of relatives. Others have evaluated folate absorption in women with a history of a NTD-affected pregnancy and found small differences in the range of 20-25% in measures of excretion after challenge [20][21]. We have no measures of absorption of serum folate in our study.

Factors significantly associated with the lowest quartile of blood folate in this study were heavy drinking, smoking, drug use and poor self-reported health status. While alcoholism has long been associated with low levels of blood folate [22]probably mediated through deranged function of the protein reduced folate carrier (RFC) in the intestine [23]it is not clear that heavy drinking is so clearly associated with low folate, since other studies did not see this association [24]. Smoking and drug use was also related to lower levels of serum folate, consistent with other studies [24]. This study did not find an association between serum folate and either age or BMI, in contrast to previous reports. In one study [24]age was positively correlated with serum folate in a Greek population and attributed to higher intake of fruit and vegetables.

The existence of a positive dose-response relationship between total dietary folate intake and serum folate seen in our study is present in other studies [25]. Of the various components of dietary folate, we found that supplemental folate was the strongest contributor to serum folate, while naturally occurring food folate was the weakest component, in line with other reports [26][27]. Much of the folate in food is lost during cooking [28]and alcohol can inhibit the absorption of folate from the intestine [23].

Blood folate levels were significantly higher among relatives than among the reference group providing more evidence against our hypothesis. There may be some possible explanations. First, the reference group had blood folate tests for indications likely related to ill health, which could result in low folate. Second, it is possible that relatives were more likely to take folic acid tablets than the reference group. In this study 7.2% were taking folic acid tablets, and a further 5.5% were taking folic acid as part of a multivitamin tablet. In earlier surveys of knowledge and use of folic

acid, we found that female relatives were more likely to take supplemental folic acid than other females in the reproductive ages. Among 100 female relatives from these same families, 9% were taking folic acid compared to 5.5% from a survey of 500 women from the general population [29]. It is also likely that the involvement by relatives in intervention studies increased awareness and folic acid intake. In two separate intervention studies carried out among female relatives a mailed-out package of information increased folic acid intake; some relatives indicated that their knowledge about the benefits of folic acid was due to the communication from the researchers [30, 31]. A survey of red cell folate levels among selected subgroups of the Irish population in 2007 [32]showed a significant increase (more than doubling) in blood folate levels due to voluntary fortification of food products by manufacturers.

Strengths and limitations of this study

Our study is the first time that distant relatives in NTD families have been evaluated for blood folate levels, focusing on matrilineal effects. We found only weak evidence for this association. In addition, the data were enriched by comprehensive dietary information on folate intake as well as other covariates. There are a number of observations relevant to these results: one, the samples were obtained some time since the affected (proband) pregnancies occurred: maternal blood folate levels could have been low at the time of conception and early development of an affected child, but alterations in the diet since then, specifically increased folic acid intake, could have eliminated or reduced any differences. However, this is unlikely to explain the lack of matrilineal association. The study largely concerned healthy individuals; relatives with serious birth defects, aside from probands, were not represented. A third observation is that our study numbers are small and may not be sensitive enough to pick up differences affecting subgroups. In addition, serum folate was the measurement of choice due to cost considerations. Others have used red cell folate [32]. However, a bench-marking exercise suggested that each method yielded equivalent information when attempting to determine if folate deficiency was present [33]. Our study showed good correlation between serum folate and total dietary folate.

This study describes serum folate levels among a group of relatives in NTD families. Since this was a not a random sample, the response rate was about half of those invited to participate, and the group consisted of family members, it is not clear that our findings are generalizable. However, it is reassuring that the shape of the distribution of folate values greatly resembles data published for the US [8]. The relatively small number of subjects studied made it difficult to detect hypothesised differences between relatives. However, participants provided considerable information on their health habits, which enriched the analysis. It is also positive that the total dietary folate intake is associated with serum folate level and falls within the range of values for studies of red cell folate [14].

In these relatives serum folate levels are sensitive to changes in dietary and lifestyle factors, including both positive and negative influences. This makes it difficult to detect differences that might be due to subtle genetic factors within families. Future studies of genetic polymorphisms may help to dissect out underlying differences contributing to risk for birth defects.

Summary

There was some suggestion that maternal uncles/aunts were lower in serum folate than expected. It seems unlikely that low folate levels can explain the relative excess of birth defects among maternal

compared to paternal relatives. However, serum folate levels in these families were directly related to supplemental folate intake, less strongly to intake of folate-fortified foods, and only slightly affected by naturally occurring food folate. Since serum folate levels fell largely at the low end of the range for participants, it is possible that protection against neural tube defects is not optimal. Pre-pregnancy counseling should include recommendations about supplemental folic acid to prevent birth defects and adverse pregnancy outcomes in this vulnerable population.

Acknowledgements

We thank the participants for their collaboration, Claymon Laboratory for their technical assistance, NutritionQuest for dietary folate measurements, and assistance from the Drogheda Volunteer Service, the Health Service Executive of Ireland and the staff of the Boyne Research Institute.

Funding Sources

For their grant support we thank the Joseph E. and Marjorie B. Jones Foundation of Washington, DC and the Drogheda and District Charity Chest.

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Authors' Contributions

JB conceived and designed the study, carried out the data analysis and interpretation and wrote the manuscript. RL assisted in study concept and design and acquisition of data. SM assisted in study concept and design and acquisition of data.

Competing interests

The authors declare that they have no competing interests.

Table 1: Characteristics of 324 Participants in Serum Folate Study

Characteristics	**N**	**%**
Proband gender Female	203	62.7
Relationship within the family Proband Offspring (of proband) Father Mother Sibling Uncle/Aunt First cousin First cousin once removed Nieces & nephews Maternal grandparents	 13 2 30 37 59 66 106 7 2 2	 4.0 0.6 9.3 11.4 18.2 20.4 32.7 2.2 0.6 0.6
Paternal vs Maternal Relationship to Proband Paternal Maternal	 64 115	 35.8 64.2
Ever pregnant, females only Yes	 147	 72.4
Number of pregnancies, females only None 1-2 3-4 5-13	 57 54 62 30	 28.1 26.6 30.5 14.8
Vegetarian diet in the last 3 months Yes	 11	 3.4
Diet to lose weight in the last 3 months Yes	 52	 16.4
Diet to gain weight in the last 3 months Yes	 3	 0.9
Took tablets for anemia in the last 3 months Yes	 6	 1.9
Took birth control pills in the last 3 months, females only	39	19.2
Took multivitamins with folic acid in the last three months Yes	 18	 5.8
Took multivitamins without folic acid in the last three months Yes	 17	 5.4

Took multivitamins, don't know if they contained folic acid in the last three months Yes	26	8.2
Took folic acid tablets by themselves in the last three months Yes	23	7.2
Took any type of supplemental folic acid in the last three months Yes	46	22.7
Took iron tablets in the last three months Yes	15	4.7
Alcohol, >3 glasses per day on average Yes	51	16.4
Cigarettes, on average more than 5 daily Yes	76	23.9
Drugs, any such as marijuana, cocaine or heroin Yes	15	4.8
Illnesses in the last three months Flu Cold Diabetes	 19 40 11	 6.0 12.5 3.5
Age at study participation Mean Range	 41.8 15.5 – 85.7	
BMI, body mass index Mean Range	 26.2 17.4 – 39.7	

Table 2: Quartile Distribution (%) of serum folate according to relationship within family

Type of Relationship	Quartiles of Serum Folate								
	Lowest Quartile		Second Quartile		Third Quartile		Highest Quartile		
	N	%	N	%	N	%	N	%	P value*
Proband, N=13	5	38.5	6	46.2	1	7.2	1	7.2	0.03
Sibling, N=59	6	10.2	19	32.2	22	37.3	12	20.3	0.16
Father, N=30	11	36.7	5	16.7	8	26.7	6	20.0	0.33
Mother, N=37	10	27.2	12	32.4	7	18.9	8	21.6	0.42
Uncles/aunts, N=66	21	31.8	11	16.7	10	15.2	24	36.7	0.57
First Cousins, N=106	23	21.7	25	23.6	30	28.3	28	26.4	0.24
First cousins once removed, N=7	4	57.1	2	28.6	1	14.3	0	0	0.03
All other relatives, N=6	2	33.3	1	16.7	2	33.3	1	16.7	0.73

*P value compares serum folate quartile distribution of each type of relationship to all other relatives

Table 3: Quartile Distribution (%) of serum folate according to maternal and paternal relationships

Type of Relationship	Quartiles of Serum Folate								P value*
	Lowest Quartile		Second Quartile		Third Quartile		Highest Quartile		
	N	%	N	%	N	%	N	%	
Paternal Line (N= 64)	19	29.7	14	21.9	10	12.4	21	32.8	0.14
Maternal Line (N =117)	27	23.1	23	19.7	34	29.1	33	28.2	0.33
Paternal Uncles/aunts (N =22)	5	22.7	5	22.7	3	13.6	9	40.9	0.36
Maternal Uncles/aunts (N=44)	15	34.1	5	11.4	8	18.2	16	36.4	0.03
Paternal first cousins (N=41)	13	31,7	9	22.0	7	17.1	12	29.3	0.45
Maternal First Cousins, N=65	9	13.9	17	26.2	22	33.9	17	26.2	0.11

*P value compares serum folate quartile distribution of each type of relationship to all other relatives

Table 4: Quartile distribution (%) and odds ratios comparing serum folate levels according to amount of dietary folate intake

	Quartiles of serum folate levels				P Value	Odds Ratio*	95% CI	P Value
	Lowest Quartile	Second Quartile	Third Quartile	Highest Quartile				
Amount of naturally-occurring food folate in the diet								
Quartile 1	26.0	27.3	27.3	19.5				
Quartile 2	27.9	22.8	25.3	24.1	0.11	2.25	0.91, 5.60	0.08
Quartile 3	30.4	27.9	17.7	24.1				
Quartile 4	18.0	22.5	29.1	30.3				
Amount of folate from fortified/enriched food								
Quartile 1	37.7	28.6	18.2	15.6				
Quartile 2	20.3	26.6	29.1	24.1	<0.00	4.33	1.80,10.3	0.0008
Quartile 3	22.8	30.4	27.9	19.0	1		9	
Quartile 4	21.4	15.7	24.7	38.2				
Took any type of supplemental folic acid								
Yes	5.1	20.3	23.7	50.9	<0.00	15.8	4.38,	<0.000
No	29.8	26.0	25.3	18.9	01		54.51	1
Amount of total folate in the diet								
Quartile 1	34.6	28.2	18.0	19.2				
Quartile 2	25.3	25.3	32.9	16.5				
Quartile 3	23.1	25.6	24.4	26.9	<0.00	3.38	1.38, 7.80	0.007
Quartile 4	19.1	21.4	24.7	34.8	1			

**Odds ratios compare the probability of being in the highest quartile of serum folate and the highest quartile of dietary folate intake with being in the highest quartile of serum folate and the lowest quartile of dietary folate intake.*

Figure 1. Distribution of serum folate levels in z-scores for all participants (N=325), and separately for participants taking supplemental folic acid (N=59) and not taking supplemental folic acid (N=266).

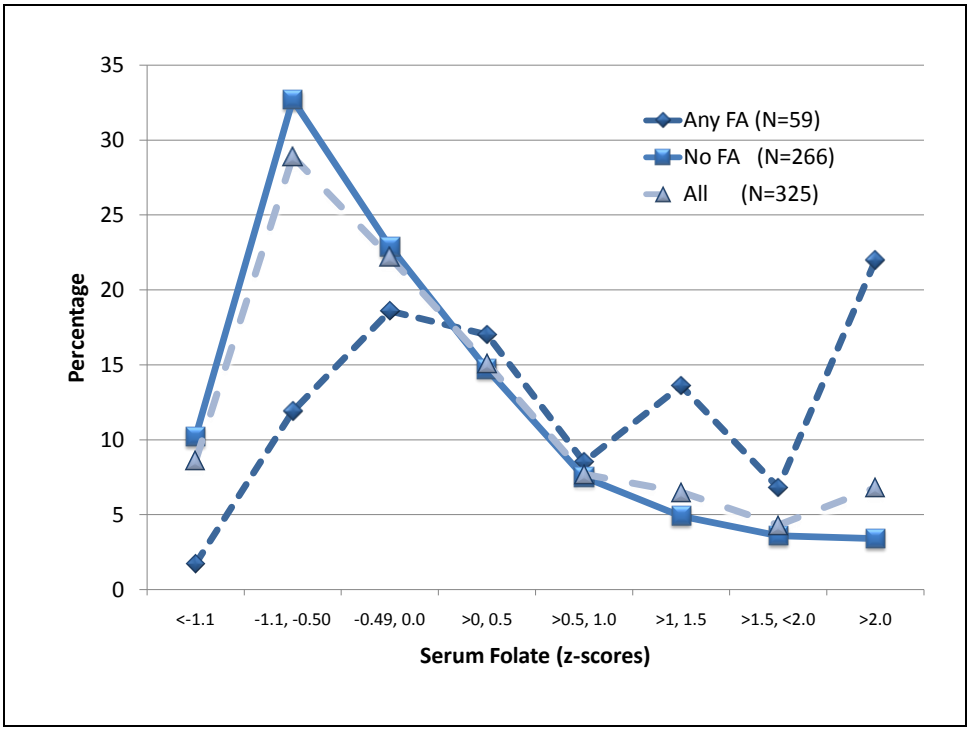
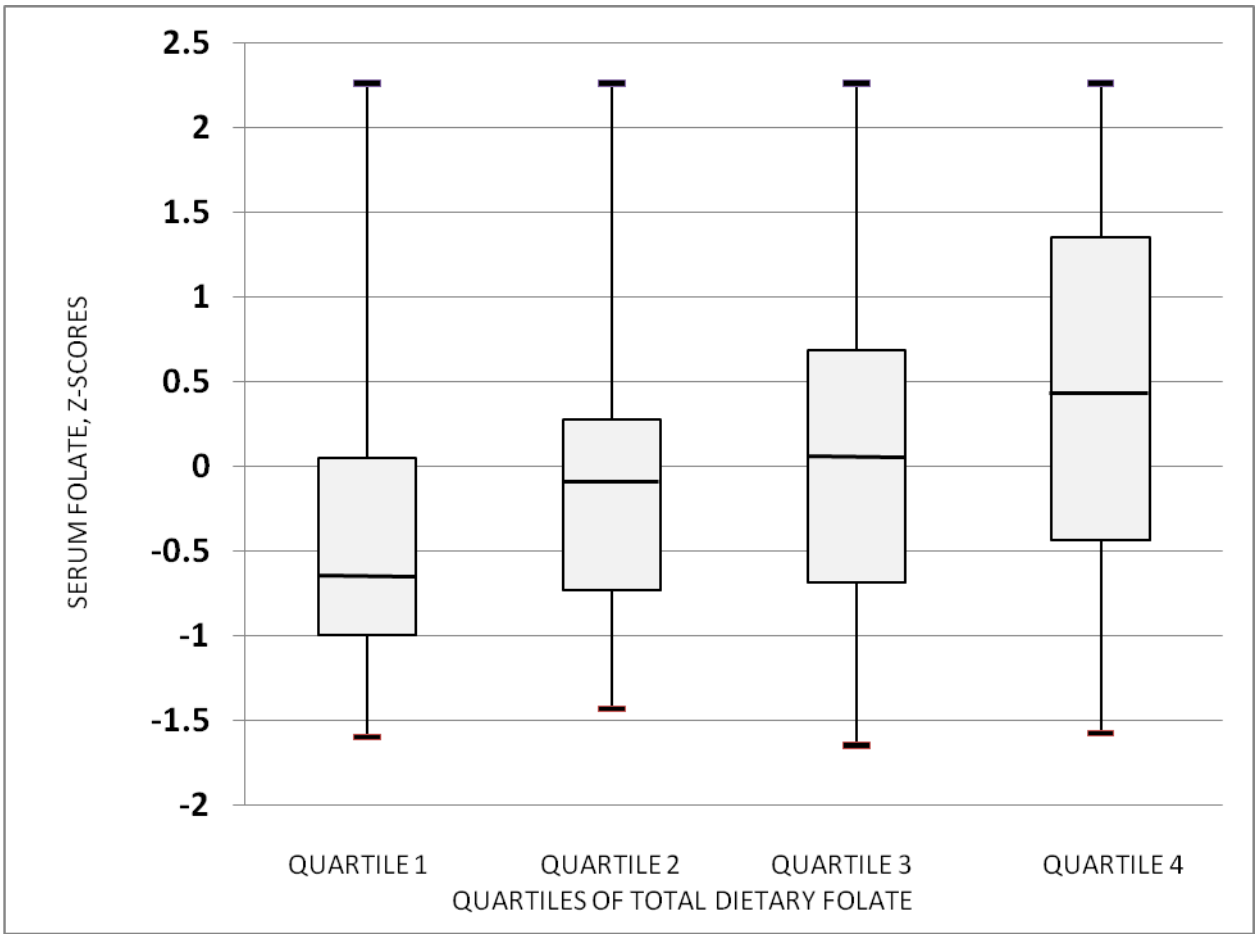


Figure 2. Box plot of levels of serum folate in ng/ml according to increasing quartiles of total dietary folate intake



Median is the line within each box; boxes indicate interquartile range; error bars indicate ranges

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