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Research Article

Maternal Use of Folic Acid and Other Supplements and Risk of Childhood Brain Tumors

Elizabeth Milne[1], Kathryn R. Greenop[1], Carol Bower[1], Margaret Miller[2], Frank M. van Bockxmeer[3,5], Rodney J. Scott[7,8], Nicholas H. de Klerk[1], Lesley J. Ashton[9], Nicholas G. Gottardo[1,4,6], and Bruce K. Armstrong[10], for the Aus-CBT Consortium

Abstract

Background: Interest in a possible protective effect of maternal vitamin use before or during pregnancy against childhood brain tumors (CBT) and other childhood cancers has grown over the past decade. Our Australian study of CBTs, conducted between 2005 and 2011, investigated whether maternal use folic acid and other supplements was protective.

Methods: Case children were identified through the 10 Australian pediatric oncology centers and controls were recruited by national random digit dialing. Mothers of 327 cases and 867 control children provided information on supplement use before and during the index pregnancy, including brand name, dose, and timing. Data were analyzed using multivariable unconditional logistic regression.

Results: The OR for any maternal use of folic acid, use of folic acid without iron or vitamins B6, B12, C, or A, and any vitamin use before pregnancy, were: 0.68 [95% confidence interval (CI), 0.46–1.00; 0.55 (95% CI, 0.32–0.93) and 0.68 (95% CI, 0.46–1.01), respectively. The ORs for use of these supplements during pregnancy were also below unity, but generally closer to the null than those for the prepregnancy period. There was some evidence of an inverse dose–response during each time period.

Conclusions: These results suggest that folic acid supplements before and possibly during pregnancy may protect against CBT. Such associations are biologically plausible through established mechanisms.

Impact: This study provides evidence of a specific protective effect of prenatal folic acid supplementation against the risk of CBT that is not attributable to the actions of the other micronutrients investigated. *Cancer Epidemiol Biomarkers Prev; 21(11); 1933–41. ©2012 AACR.*

Introduction

Childhood brain tumors (CBT) are the leading cause of cancer death in children (1). Their only established risk factors are cerebral irradiation and specific hereditary conditions (2). Many CBTs occur in early childhood, implicating prenatal or early postnatal factors. Many environmental factors have been investigated, but the results have mostly been nondefinitive (1, 3).

Interest in a possible protective effect of maternal vitamin use before or during pregnancy against CBT and other childhood cancers has grown recently. In 2001, a case–control study from Western Australia reported a strong inverse association between maternal folic acid supplementation (with or without iron) during pregnancy and risk of acute lymphoblastic leukemia (ALL): OR = 0.40, 95% CI 0.21–0.73 (4). These findings have not been replicated in subsequent ALL studies, although we found weak evidence of a protective association with folic acid use before pregnancy in our national Australian case–control study (5). Protective effects of micronutrient supplementation before and during pregnancy against childhood cancers are biologically plausible. In particular, folate and other B-vitamins are crucial in maintaining genomic stability through their intimate involvement in methylation and the synthesis and repair of DNA (6, 7). With respect to CBT specifically, folate requirements are considerable as the developing fetus's neural crest and brain cells undergo rapid division. Thus, maternal folate insufficiency may disrupt these processes and predispose

Authors' Affiliations: [1]Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia; [2]Child Health Promotion Research Centre, School of Exercise and Health Sciences, Edith Cowan University; Schools of [3]Pathology and Laboratory Medicine and [4]Paediatrics and Child Health, University of Western Australia; [5]PathWest Biochemistry, Royal Perth Hospital, Perth; [6]Department of Haematology and Oncology, Princess Margaret Hospital for Children, Perth; [7]Hunter Medical Research Institute, School of Biomedical Sciences, Faculty of Health, University of Newcastle, New South Wales, Australia; [8]Hunter Area Pathology Service, HNEHealth, Newcastle; [9]Children's Cancer Institute Australia for Medical Research, Lowy Cancer Research Centre, University of New South Wales, Sydney, New South Wales; and [10]Sydney School of Public Health, University of Sydney, Sydney, New South Wales, Australia

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Elizabeth Milne, Telethon Institute for Child Health Research, P.O. Box 855 West Perth, Western Australia 6872, Australia. Phone: 61-08-9489-7756; Fax: 61-08-9489-7700; E-mail: lizm@ichr.uwa.edu.au.

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CNS cells to increased rates of DNA damage which, if unrepaired or misrepaired, can lead to mutation and malignant transformation (8).

Previous studies of CBT have generally examined associations with use of vitamins as a group; 4 reported a protective effect (9–12), and a 2007 meta-analysis of multivitamin use before or early in pregnancy reported a summary OR of 0.73 [95% confidence interval (CI), 0.60–0.88; ref. 13]. Recently, however, a German case–control study found no association with maternal use of vitamins, folate, or iron supplements during pregnancy (14); a Swedish cohort study reported no association with vitamin use, but a weak inverse association with folic acid before pregnancy(15); and a Spanish study reported weak evidence of an inverse association of CBT with use of multivitamins or folic acid in the first month of pregnancy only (16). The variation among these results and their basis in use of different types of supplements in different time periods make them inconclusive.

In our study, the largest conducted to date, detailed information was collected about dose and timing of maternal use of specific micronutrient supplements, including folic acid, iron, and vitamins A, B6, B12, and C, at specific times before and during pregnancy. Here, we present the results of our study and seek to distinguish possible effects of folic acid from those of other vitamins.

Materials and Methods

The Australian Study of Childhood Brain Tumors (Aus-CBT) was a national population-based case–control study conducted between 2005 and 2010 to investigate nutritional, environmental, and genetic risk factors for CBT. Incident cases were identified through all 10 pediatric oncology centers in Australia; cases were eligible if they were diagnosed between 2005 and 2010, resident in Australia, and had an English speaking biological parent available. Controls were recruited by national random digit dialing (RDD) and frequency matched to cases by age, sex, and state of residence in a ratio of 3:1. Controls matched to CBT cases diagnosed in 2005 and 2006 were originally recruited as controls for our national study of childhood leukemia (2003–2007). Identical recruitment methods, described elsewhere (5, 17–19), were used in both studies. Aus-CBT was approved by the Human Research Ethics Committees at all participating hospitals.

Mailed questionnaires were used to obtain medical and residential histories, demographic characteristics, and exposure data, including whether mothers took 'folate supplements' (yes or no) in the month before the index pregnancy (prepregnancy), during trimester 1, and during trimesters 2/3. Mothers were also mailed food frequency questionnaires (FFQ), which included the open-ended question "Did you take any vitamin, mineral, or other dietary supplements regularly for at least a month" for each of these periods. The FFQ also asked for detailed information on supplement brand details, dose, and frequency of use for each period. Mothers who did not complete these questionnaires were asked to provide information about supplement use during a telephone interview. Information about the constituents of reported supplements was obtained from manufacturers and combined with reported frequency and duration of use to estimate the average daily dose of folic acid. In addition to parent-reported measures of socioeconomic status (SES), each participant's address was linked to an Australian Bureau of Statistics Census Collection District (CD). Each CD was assigned a score for the area-based index of relative socioeconomic disadvantage (IRSD; ref. 20).

Statistical analysis

Unconditional logistic regression was used to estimate OR for the association between folic acid use and CBT risk, with "no folic acid before or during pregnancy" as the reference category. Data from the FFQ were also grouped into categories of folic acid use with and without iron, with the reference category "no folic acid or iron" before or during pregnancy. These reference categories were used to avoid "contamination" by women who had used supplements in a different time period. Folic acid dose was analyzed using both categorical and continuous methods. ORs were also estimated for use of vitamins B6 or B12 (they were almost always taken together), C, A, "any" vitamins, and use of folic acid without any of these vitamins or iron. Dietary folate intake was excluded from our analysis because supplementation with folic acid has a much greater effect than dietary intake on folate status (21), and folic acid supplementation was the primary focus of this analysis.

All analyses included the frequency matching variables age, sex, and State of residence, and variables that met the classical definition of confounding: they were associated with case–control status and folic acid use among control mothers. Variables included on this basis were ethnicity, maternal age, child's birth year, maternal education, and source of data (written questionnaire or telephone interview). Use of directed acyclic graphs endorsed our selection of covariates.

Results

We were notified of 794 CBT cases, of which 64 were ineligible (36 with no English-speaking parent, 23 nonresidents, and 5 with no biological parent available). Of the 730 eligible cases, 568 (77.8%) were invited to take part by a physician. A physician chose not to invite the other 162 for medical or psychosocial reasons. Parents of 374 cases consented (65.8% of invited, 51.2% of eligible). Information on folic acid use was available for 331 (88.5%) consenting case mothers, 4 mothers provided only demographic information, and another 39 provided no data. Detailed information about dose and other vitamins was available for 327 (87.4%) case mothers. Two clinicians independently assigned each case to a CBT subtype; there was complete agreement. The numbers of cases in each subtype, and their recorded diagnoses, are shown in the Supplementary Table.

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Table 1. Characteristics of cases and controls in the Australian study of childhood brain tumors 2005–2010

Supplied demographic data	Case 335 *n* (%)	Control 1,363 *n* (%)
Child's sex		
Female	138 (41.2)	643 (47.2)
Male	197 (58.8)	720 (52.8)
Age group of child at diagnosis or recruitment		
0—1	35 (10.4)	144 (10.6)
2—4	95 (28.4)	422 (31.0)
5—9	101 (30.1)	444 (32.6)
10–14	104 (31.0)	353 (25.9)
Child's year of birth group		
1990–1997	91 (27.2)	332 (24.4)
1998–2003	139 (41.5)	679 (49.8)
2004–2010	105 (31.3)	352 (25.8)
Child's state of residence		
New South Wales/ Australian Capital Territory	114 (34.0)	404 (29.6)
Victoria/Tasmania	97 (29.0)	378 (27.7)
South Australia/Northern Territory	20 (6.0)	111 (8.1)
Western Australia	44 (13.1)	148 (10.9)
Queensland	60 (17.9)	322 (23.6)
Child's year of diagnosis or recruitment		
2005–2006	115 (34.3)	617 (45.3)
2007–2008	116 (34.6)	392 (28.8)
2009–2010	104 (31.0)	354 (26.0)
Maternal age group		
≤24	55 (16.5)	134 (9.9)
25–34	205 (61.4)	843 (62.3)
35+	74 (22.2)	376 (27.8)
Maternal Education		
Did not complete secondary school	83 (24.9)	305 (22.6)
Complete secondary or trade qualification	114 (34.2)	463 (34.2)
University or college	136 (40.8)	584 (43.2)
Household Income (AUD/annum)		
Up to $40, 000	57 (17.3)	226 (16.9)
$40,001–$70,000	91 (27.7)	359 (26.9)
$70,001–$100,000	83 (25.2)	350 (26.2)
>$100,000	98 (29.8)	400 (30.0)
Birth order		
1	150 (45.0)	557 (40.9)
2	109 (32.7)	483 (35.5)
3+	74 (22.2)	321 (23.6)
Gestation (prematurity)		
Term (37+ weeks)	292 (89.3)	1003 (91.5)
Preterm (<37 weeks)	35 (10.7)	93 (8.5)
Ethnicity		
European	210 (62.7)	1023 (75.1)
At least 50% European	76 (22.7)	203 (14.9)
At least 50% nonEuropean and unknown if 50% European	15 (4.5)	59 (4.3)
Indeterminate	34 (10.1)	78 (5.7)
Multiple birth		
No	322 (96.7)	1317 (96.7)
Yes	11 (3.3)	45 (3.3)
Birth defect		
No	289 (95.4)	903 (96.0)
Yes	14 (4.6)	38 (4.0)
Folic acid data source (exposure questionnaire)		
Written questionnaire	301 (90.9)	939 (69.8)
Phone interview	30 (9.1)	407 (30.2)

AUD: Australian Dollar

Between 2005 and 2010, 3,624 families of eligible control children were identified by RDD, of whom 2,255 (62.2%) agreed to participate. Taking account of the presumed loss of eligible families in residences that never answered the RDD call, we estimate the true percentage agreement to be 47% (17). In accordance with our frequency-matching quotas, 1,467 of these families were recruited to the study. Mothers of 1,346 controls (92% of recruited) provided information about folic acid use, 17 provided only demographic information, and another 104 recruited families provided no data. Detailed data on dose and use of other vitamins were provided by 867 (59.1%) control mothers.

Demographic and other characteristics of cases and controls were similar, with some exceptions (Table 1); controls were slightly more likely to be female, have European ethnicity, have a mother older than 35 years at their birth, and have their mother provide information by telephone rather than in written questionnaires. A higher proportion of controls than cases were recruited in 2005–2006, as controls from our national leukemia study were frequency matched to CBT cases diagnosed in those years. The use of the 2005 and 2006 leukemia study controls also resulted in a higher percentage of controls than cases born between 1998 and 2003. Thus, the child's age and year of recruitment were related.

Both cases and controls lived in more socially advantaged CDs than the Australian population as a whole. The mean IRSD scores were 1,024.3 for case CDs, 1,024.7 for control CDs, and 1,006.0 for all Australian CDs (P value <0.001 for case and control CDs compared with all

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Australian CDs). When characteristics of case and control mothers who completed a FFQ were compared, the distributions were similar to all participants shown in Table 1 and as described above for ISRD, except that a higher proportion of control than case mothers had a tertiary education (48.8% vs. 40.7%; data not shown in tables).

The ORs for the association between risk of CBT and maternal use of supplements containing folic acid before or during pregnancy were all below unity for both the exposure questionnaire and FFQ (Table 2). The lowest ORs were for prepregnancy use— OR 0.65 (95% CI: 0.43–0.96; ascertained by exposure questionnaire) and OR 0.68 (95% CI, 0.46–1.00; ascertained by FFQ). ORs for use in Trimester 1 and in Trimesters 2/3 were somewhat closer to the null. The FFQ OR for folic acid use during Trimester 1 (with or without ongoing use into later pregnancy) but not prepregnancy was somewhat higher at 0.84 (95% CI, 0.58–1.23; not shown in tables). ORs for use prepregnancy, but not in Trimester 1, could not be calculated, as all but 3 case and 13 control mothers who took folic acid before pregnancy also took it in Trimester 1. Use of a less stringent reference group ("no folic acid in the same time period") allowed inclusion of many more subjects, particularly for the prepregnancy period, but did not materially alter the effect estimates (data not shown). There was evidence of a dose–response relationship for folic acid supplements taken prepregnancy and in Trimester 1 (linear trend $P = 0.01$ and 0.03, respectively) and, to a lesser extent, in Trimesters 2 and 3 ($P = 0.08$; Table 2). When children from multiple births or with birth defects were excluded, the results did not change (data not shown); they were therefore included in all analyses.

Associations between risk of CBT and use of folic acid in combination with iron or other vitamins, and use of iron and vitamins B6 or B12, C, and A, individually, were analyzed in mothers who completed the FFQ (327 case and 867 control mothers). The ORs for combinations of folic acid and iron taken before or during pregnancy were all below unity (Table 3). The lowest OR was for folic acid taken without iron before pregnancy: 0.56 (95% CI, 0.35–0.89). There was little evidence of any association between CBT and use of iron or vitamins B6 or B12, C, or A in any time period (Table 3). The relatively low numbers in these analyses precluded mutual adjustment, but adjustment for folic acid use did not alter the results (not shown). Maternal use of "any vitamins" before pregnancy was inversely associated with CBT risk, and there was evidence of a similar, albeit weaker, association for use during pregnancy (Table 3). Note that all but 3 control and 13 case mothers who took vitamins before pregnancy also took folic acid. Finally, we estimated the ORs for use of folic acid without iron or vitamins B6, B12, C, or A. The OR for prepregnancy use was 0.55 (95% CI, 0.32–0.93), whereas there was little evidence of a similar association with use during pregnancy. This analysis, though, was based on only about a third of all folic acid users, as most women who took folic acid took preparations that contained at least one of the other micronutrients.

We also investigated the use of folic acid and any vitamin supplements among the 2 largest CBT subtypes—low-grade gliomas and medulloblastoma/primitive neuroectodermal tumors (PNET). The results were similar to the overall results but there was stronger evidence for an inverse trend with increasing folic acid intake

Table 2. Risk of childhood brain tumors associated with maternal use of folic acid supplements before or during the index pregnancy

	Prepregnancy		Trimester 1		Trimesters 2/3	
Took folic acid	Cases/ controls	OR[a] (95% CI)	Cases/ controls	OR[a] (95% CI)	Cases/ Controls	OR[a] (95% CI)
Exposure questionnaire						
No[b]	103/358	1.00	103/358	1.00	103/358	1.00
Yes	96/453	0.65 (0.43–0.96)	211/907	0.83 (0.61–1.15)	162/724	0.73 (0.53–1.03)
Food frequency questionnaire						
No[b]	106/227	1.00	106/227	1.00	106/227	1.00
Yes	108/357	0.68 (0.46–1.00)	198/579	0.83 (0.59–1.15)	160/444	0.80 (0.57–1.14)
Folic acid dose (FFQ)						
0.1–300 μg	42/91	1.01 (0.62–1.65)	66/149	1.05 (0.70–1.60)	74/184	0.92 (0.62–1.37)
300.1–450 μg	23/115	0.49 (0.28–0.85)	66/194	0.82 (0.55–1.23)	41/123	0.74 (0.46–1.19)
>450.1 μg	43/151	0.60 (0.38–0.98)	66/236	0.67 (0.44–1.00)	45/137	0.68 (0.43–1.09)
P of linear trend		*0.01*		*0.03*		*0.08*
Per 100 μg		0.93 (0.88–0.99)		0.95 (0.91–1.00)		0.94 (0.89–0.99)

[a]Adjusted for frequency matching variables (age, sex, state of residence), ethnicity, maternal age group, child's year of birth group, maternal education level, and data source.

[b]Reference level: no folic acid prepregnancy or during pregnancy.

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Table 3. Risk of childhood brain tumors associated with maternal use of any supplements and specific supplements before or during the index pregnancy[a]

	Prepregnancy		Trimester 1		Trimesters 2/3	
Supplements	Cases/ controls	OR (95% CI)	Cases/ controls	OR (95% CI)	Cases/ Controls	OR (95% CI)
No folic acid or iron[b]	97/202	1.00	97/202	1.00	97/202	1.00
Folic acid with iron	54/133	0.81 (0.48–1.37)	100/289	0.78 (0.52–1.19)	105/317	0.78 (0.52–1.18)
Folic acid without iron	54/224	0.56 (0.35–0.89)	98/290	0.83 (0.56–1.22)	55/127	0.91 (0.58–1.43)
No iron[b]	188/464	1.00	188/464	1.00	188/464	1.00
Any iron	55/140	0.98 (0.65–1.47)	104/305	0.87 (0.63–1.20)	116/357	0.86 (0.63–1.17)
No vitamin B6 or B12[b]	225/575	1.00	225/575	1.00	225/575	1.00
Any vitamin B6 or B12	49/124	1.03 (0.68–1.56)	78/234	0.89 (0.63–1.26)	85/253	0.92 (0.66–1.29)
No vitamin C[b]	228/575	1.00	228/575	1.00	228/575	1.00
Any vitamin C	47/127	0.96 (0.64–1.46)	73/233	0.83 (0.59–1.18)	83/258	0.87 (0.62–1.21)
No vitamin A[b]	258/655	1.00	258/655	1.00	258/655	1.00
Any vitamin A	29/72	1.17 (0.72–1.90)	48/161	0.81 (0.56–1.19)	59/184	0.88 (0.62–1.26)
No iron or vitamins[b]	95/189	1.00	95/189	1.00	95/189	1.00
Any iron or vitamins	114/384	0.68 (0.46–1.01)	204/609	0.80 (0.57–1.12)	178/500	0.79 (0.56–1.12)
No folic acid, iron or other vitamins[b]	97/201	1.00	97/201	1.00	97/201	1.00
Any folic acid without iron or vitamins B6, B12, C, A	32/125	0.55 (0.32–0.93)	81/222	0.83 (0.56–1.24)	49/114	0.87 (0.54–1.39)

[a]Based on FFQ data, adjusted for frequency matching variables (age, sex, state of residence), ethnicity, maternal age group, child's year of birth group, maternal education level, and data source. ORs were not adjusted for other micronutrients as there were too few subjects taking some vitamins.

[b]Reference level: no supplement of this type in any period.

for low-grade gliomas than for medulloblastoma/PNET (Table 4). The other brain tumor subgroups were too small to allow separate analyses.

Discussion

We observed an inverse association between maternal use of folic acid before pregnancy and CBT, with evidence of an inverse dose–response. Similar, but weaker, associations were observed for folic acid use during pregnancy suggesting that prepregnancy might be a more biologically relevant time period. The findings were similar for low-grade gliomas and medulloblastoma/PNET, the commonest types of CBT.

The only previous study to investigate maternal folic acid use before pregnancy reported an OR of 0.34 (95% CI, 0.10–1.06) for CBT with maternal intake of $\geq$ 400 μg folic acid per day (16). A reduction in risk was also observed for use in the first 36 days of gestation, OR = 0.57 (95% CI, 0.33–0.99), but not "before or during pregnancy", OR = 0.94 (95% CI, 0.78–1.14). However, the control group for this study composed of children with mesodermal tumors (e.g., leukemia, lymphoma, kidney cancer). Given the evidence of associations between folic acid and these other tumors (14, 22), interpretation of these results is not straightforward. One previous study investigated any vitamin use before pregnancy and risk of CBT and reported an OR of 1.2 (95% CI, 0.8–1.8; ref. 12). Thus, our findings add substantially to the evidence that folic acid use before pregnancy reduces risk of CBT, and suggest that the association is specific to folic acid as its association seemed to be independent of any effect of iron, and the ORs for iron and vitamins B6, B12, C, and A were all close to unity. The evidence of an inverse dose–response relationship also supports an association specific to folic acid.

Two previous studies reported inverse associations between folic acid use during pregnancy and risk of CBT. Preston-Martin and colleagues (12) reported an OR of 0.7 (95% CI, 0.6–1.0) for use during pregnancy, and inverse dose–response relationships for use of folic acid and vitamins C, E, and A by mothers of cases diagnosed before age 5. Most mothers in their study took multivitamins and it was difficult to separate the association with folic acid from other vitamins. Stålberg and colleagues (15) reported an OR of 0.6 (95% CI, 0.3–1.1) for use during pregnancy, although only 15 (2.9%) case mothers and 27 (5.1%) control mothers had "folic acid use" documented in their medical records, and the investigators could not determine folic acid use from multivitamin preparations. Five other studies have investigated maternal prenatal vitamin use: 3 suggested a protective effect (9, 12, 16) and 2 reported no association (14, 15).

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Table 4. Risk of low-grade gliomas and medulloblasoma/PNET with use of folic acid or any vitamins

	Prepregnancy		Trimester 1		Trimesters 2/3	
	Cases/ controls	OR[a] (95% CI)	Cases/ controls	OR[a] (95% CI)	Cases/ controls	OR[a] (95% CI)
Low-grade gliomas						
No folic acid[b]	53/227	1.00	53/227	1.00	53/227	1.00
Any folic acid	56/357	0.64 (0.38–1.08)	92/579	0.74 (0.47–1.15)	72/444	0.73 (0.46–1.17)
0.1–300 μg	27/91	1.18 (0.64–2.18)	36/149	1.11 (0.65–1.89)	32/184	0.82 (0.48–1.40)
300.1–450 μg	11/115	0.42 (0.20–0.89)	26/194	0.63 (0.36–1.10)	19/123	0.71 (0.37–1.35)
>450.1 μg	18/151	0.44 (0.22–0.89)	30/236	0.57 (0.33–1.00)	21/137	0.63 (0.34–1.17)
P of linear trend		*<0.01*		*0.01*		*0.12*
Per 100 μg increase		0.92 (0.84–1.00)		0.94 (0.88–1.00)		0.94 (0.87–1.01)
No iron or vitamins [b]	49/189	1.00 (ref)	49/189	1.00 (ref)	49/189	1.00 (ref)
Any iron or vitamins	57/384	0.59 (0.35–1.00)	95/609	0.70 (0.44–1.09)	79/500	0.69 (0.43–1.09)
Medulloblastoma/PNET						
No folic acid[b]	22/227	1.00	22/227	1.00	22/227	1.00
Any folic acid	25/357	0.71 (0.33–1.52)	48/579	0.89 (0.48–1.67)	38/444	0.87 (0.45–1.71)
0.1–300 μg	7/91	0.70 (0.25–1.98)	11/149	0.84 (0.36–1.95)	19/184	1.04 (0.49–2.22)
300.1–450 μg	7/115	0.63 (0.23–1.69)	21/194	1.08 (0.53–2.20)	9/123	0.68 (0.27–1.70)
>450.1 μg	11/151	0.80 (0.31–2.02)	16/236	0.73 (0.34–1.57)	10/137	0.81 (0.33–1.95)
P of linear trend		*0.58*		*0.57*		*0.44*
Per 100 μg increase		0.93 (0.82–1.06)		0.96 (0.89–1.05)		0.94 (0.84–1.05)
No iron or vitamins[b]	19/189	1.00 (ref)	19/189	1.00 (ref)	19/189	1.00 (ref)
Any iron or vitamins	26/384	0.77 (0.34–1.70)	49/609	0.93 (0.48–1.78)	42/500	0.91 (0.46–1.79)

[a] Adjusted for matching variables, ethnicity, maternal age group, child's year of birth group, maternal education level, and data source.
[b]Reference level: no supplement of this kind in any period.

We had sufficient cases to undertake subgroup analyses of folic acid and vitamin use for the 2 largest CBT subtypes only: medulloblastoma/PNET and low-grade glioma. Their results reflected those for all CBT. The only previous study of folic acid use in a CBT subgroup (medulloblastoma/PNET) reported ORs of 1.1 (95% CI, 0.4–3.2) for prenatal folic acid use and 0.7 (95% CI, 0.4–1.0) for periconceptional multivitamin use (23). Two other studies reported inverse associations between vitamin use during pregnancy and risk of medulloblastoma/PNET: Bunin and colleagues reported an OR of 0.56 (95% CI, 0.32–0.96; ref. 10) and Preston-Martin and colleagues reported an OR of 0.61 (95% CI, 0.44–0.84; ref. 12). A Swedish study reported an OR of 0.8 (95% CI, 0.5–1.3) for prenatal multivitamin use and PNET (15). There is also ecological evidence that maternal vitamin use might protect against medulloblastoma; a UK study reported a halving of the incidence of this tumor following the promotion of periconceptional multivitamin supplementation (24); a trend that may be continuing (25). Our results for maternal vitamin use and risk of low-grade gliomas are consistent with 2 previous studies — Bunin and colleagues (11) and Preston-Martin and colleagues (12) reported ORs of 0.6 (95% CI, 0.2–1.5) and 0.74 (95% CI, 0.59–0.92, respectively) for multivitamin use during pregnancy; whereas a third study by Stålberg and colleagues (15) reported an OR of 0.9 (95% CI, 0.7–1.3).

Aus-CBT was a population-based study designed to address specific hypotheses, including that maternal use of folic acid protects against CBT. Therefore, more comprehensive data were collected about the dose and timing of use of folic acid and other vitamins than in previous studies. Almost 78% of eligible cases were invited to participate by the treating clinician and 66% of invited parents consented, resulting in an overall participation fraction of 51%. Except for age and sex, where the distributions were similar to participating cases, information about eligible cases who did not participate was unavailable, so we were unable to determine whether our cases were representative of all eligible cases with respect to potential risk factors. Control families were recruited by national RDD using state-of-the-art methods and, according to the most recent data available (26, 27), approximately 90% of Australian households had a landline telephone connection during the recruitment period. Therefore, residences contacted are likely to be representative of the wider population. Participation among eligible control families was 62% and, although no individual information was available for those who declined, area-based SES scores were higher among participating controls than among the wider Australian population. Thus, as periconceptional folic acid use is known to be positively associated with SES (28), the inverse associations we observed may have been overestimated.

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However, importantly, participating cases and controls had very similar SES distributions, and about 90% of both case and control mothers provided information about whether folic acid was used. Thus, it is unlikely that our findings can be entirely attributed to selection bias.

While 87% of case mothers completed the FFQ (requesting detailed use of any supplements), only 59% of control mothers did so. However, although a higher proportion of this group of control mothers had a university or college education, household income, and area-based SES measures were similar among cases and controls in this subset. Maternal education was considered *a priori* to be a potential confounder and was included in all analyses. Moreover, the results of the analysis of folic acid use from the exposure questionnaire and the open-ended FFQ questions were similar.

Because of the dependence on self-reported data, there is likely to have been error in exposure measurement, particularly as the index pregnancy was 15 years earlier for some mothers. However, the recall period was 5 years or less for almost 60% of mothers, and the percentages of Aus-CBT control mothers reporting periconceptional folic acid use (14.7% in 1990 increasing to 50.3% in 2010) were similar to those among Australian women of child-bearing age, which varied from approximately 13% in 1993 (ref. 29; the year after supplementation was recommended for the prevention of neural tube defects) to about 50% in 2005 (ref. 30; the latest year for which reliable data are available).

The possibility that mothers of cases ruminate about the causes of their child's cancer and recall exposure more completely than control mothers is inherent in case–control studies and may introduce recall bias. However, case mothers' enhanced recall of a potentially protective exposure would not be expected to produce an inverse association; but it might weaken one. There is evidence of this weakening in our data. Case and control mothers' reporting of periconceptional folic acid use increased with the index child's birth year, which ranged from 1990 to 2010. This increase was greater in control mothers (14.7%–50.3%) than case mothers (16.1%–41.0%). Thus, ORs for CBT with maternal folic acid use in children born earlier were close to unity, whereas these ORs were below unity for children born more recently. We suggest that this trend in ORs is due to more complete (or possibly exaggerated) recall by case than control mothers of folic acid use further in the past. As recruitment occurred over 5 years, while the children were born over a much longer period, it is unlikely to be due to increasing SES' of controls over the recruitment period. Moreover, we have shown that although control participation fractions have decreased over time in Australia, this fall has not been accompanied by a commensurate increase in SES (31). Mandatory fortification of bread-making flour in Australia began in September 2009 and, although fortification was permitted before this date, most millers did not implement fortification until the end of August 2009 at the earliest. Only 1 case and 4 controls were born after that time; therefore, it is unlikely to have affected our findings.

A reduced risk of CBT associated with maternal folic acid supplementation is biologically plausible through known mechanisms, and a stronger inverse association with use before pregnancy is consistent with the fact that ovarian stimulation and follicular development are subject to the availability of folate (32). Low levels of folate are associated with increased oxidative stress (33) and inappropriate DNA methylation (34), thereby potentiating disease development. Some CNS tumors involve mutations or aberrant DNA methylation in signaling pathway genes, activation of oncogenes, or inactivation of tumor suppressor genes (2)— all of which may result from folate insufficiency. Histone lysine methylation, in which folate would be involved as a methyl donor, may play a part in the pathogenesis of medulloblastoma (35). The fact that polymorphisms in folate pathway genes causing altered enzyme kinetics are associated with an increased risk of risk of brain tumors in children (36) and adults (37) adds to the biologic plausibility that folate is involved. Interestingly, inverse associations have been reported between folic acid or multivitamin use and other solid tumors in children (14, 22, 38, 39), but the responsible micronutrients have not been identified.

Our study of maternal folic acid supplementation and risk of CBT is the largest and most comprehensive to date. It provides evidence of an inverse association of CBT with folic acid use before pregnancy and, to a lesser extent, during pregnancy. Similar associations were observed with multivitamin supplements, but there was little evidence that other vitamins or iron, with which folic acid supplementation is strongly associated, might explain the inverse association with folic acid. Limited earlier literature provides some support for these findings. We conclude that folic acid supplements before and in early pregnancy may protect against CBT. Potential underlying mechanisms should be further investigated, particularly for individual CBT subtypes as the mechanisms may well differ.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Authors' Contributions

Conception and design: E. Milne, C.I. Bower, F.M. van Bockxmeer, R.J. Scott, N.H. de Klerk, L.J. Ashton, B.K. Armstrong
Development of methodology: E. Milne, C.I. Bower, M. Miller, F.M. van Bockxmeer, N.H. de Klerk, L.J. Ashton, B.K. Armstrong
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N.G. Gottardo, B.K. Armstrong
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E. Milne, K.R. Greenop, M. Miller, F.M. van Bockxmeer, R.J. Scott, N.H. de Klerk, L.J. Ashton, B.K. Armstrong
Writing, review, and/or revision of the manuscript: E. Milne, K.R. Greenop, C.I. Bower, M. Miller, R.J. Scott, N.H. de Klerk, N.G. Gottardo, B.K. Armstrong
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.R. Greenop, R.J. Scott, N.G. Gottardo
Study supervision: R.J. Scott, L.J. Ashton, B.K. Armstrong

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References

1. Baldwin RT, Preston-Martin S. Epidemiology of brain tumors in childhood–a review. Toxicol Appl Pharmacol 2004;199:118–31.
2. Gurney JG, Smith MA, Olshan AF, Hecht SS, Kasum CM. Clues to the etiology of childhood brain cancer: N-nitroso compounds, polyomaviruses, and other factors of interest. Cancer Invest 2001;19:630–40.
3. Bunin G. What causes childhood brain tumors? Limited knowledge, many clues. Pediatr Neurosurg 2000;32:321–6.
4. Thompson JR, FitzGerald P, Willoughby ML, Armstrong BK. Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. Lancet 2001;358:1935–40.
5. Milne E, Royle JA, Miller M, Bower C, de Klerk N, Bailey H, et al. Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukemia in the offspring. Int J Cancer 2009;126:2690–9.
6. Kim Y-I. Folate and carcinogenesis: Evidence, mechanisms, and implications. J Nutr Biochem 1999;10:66–88.
7. Duthie SJ, Hawdon A. DNA instability (strand breakage, uracil misincorporation, and defective repair) is increased by folic acid depletion in human lymphocytes *in vitro*. FASEB J 1998;12:1491–7.
8. Blount BC, Ames BN. DNA damage in folate deficiency. Baillieres Clin Haematol 1995;8:461–78.
9. Sarasua S, Savitz DA. Cured and broiled meat consumption in relation to childhood cancer: Denver, Colorado (United States). Cancer Causes Control 1994;5:141–8.
10. Bunin GR, Kuijten RR, Buckley JD, Rorke LB, Meadows AT. Relation between maternal diet and subsequent primitive neuroectodermal brain tumors in young children. N Engl J Med 1993;329:536–41.
11. Bunin GR, Kuijten RR, Boesel CP, Buckley JD, Meadows AT. Maternal diet and risk of astrocytic glioma in children: a report from the Childrens Cancer Group (United States and Canada). Cancer Causes Control 1994;5:177–87.
12. Preston-Martin S, Pogoda JM, Mueller BA, Lubin F, Holly EA, Filippini G, et al. Prenatal vitamin supplementation and risk of childhood brain tumors. Int J Cancer 1998;11:17–22.
13. Goh YI, Bollano E, Einarson TR, Koren G. Prenatal Multivitamin supplementation and rates of pediatric cancers: a meta-analysis. Clin Pharmacol Ther 2007;81:685–91.
14. Schuz J, Weihkopf T, Kaatsch P. Medication use during pregnancy and the risk of childhood cancer in the offspring. Eur J Pediatr 2007;166:433–41.
15. Stålberg K, Haglund B, Strömberg B, Kieler H. Prenatal exposure to medicines and the risk of childhood brain tumor. Cancer Epidemiol 2010;34:400–4.
16. Ortega-García J, Ferrís-Tortajada J, Claudio L, Soldin O, Sanchez-Sauco M, Fuster-Soler J, et al. Case control study of periconceptional folic acid intake and nervous system tumors in children. Childs Nerv Syst 2010;26:1727–33.
17. Bailey H, Milne E, de Klerk N, Fritschi L, Bower C, Attia J, et al. Representativeness of child controls recruited by random digit dialing. Paediatr Perinat Epidemiol 2010;24:293–302.
18. Milne E, Royle JA, Bennett LC, de Klerk NH, Bailey HD, Bower C, et al. Maternal consumption of coffee and tea during pregnancy and risk of childhood ALL: results from an Australian case-control study. Cancer Causes Control 2011;22:207–18.
19. Milne E, Royle JA, de Klerk NH, Blair E, Bailey H, Cole C, et al. Fetal growth and risk of childhood acute lymphoblastic leukemia: results from an Australian case-control study. Am J Epidemiol 2009;170:221–8.
20. Australian Bureau of Statistics. Information paper: an introduction to socioeconomic indexes, 2006. Canberra, Australia: Australian Bureau of Statistics; 2008.
21. Caudill MA. Folate bioavailability: implications for establishing dietary recommendations and optimizing status. Am J Clin Nutr 2010;91:1455S–60S.
22. Grupp SG, Greenberg ML, Ray JG, Busto U, Lanctôt KL, Nulman I, et al. Pediatric cancer rates after universal folic acid flour fortification in Ontario. J Clin Pharmacol 2011;51:60–5.
23. Bunin GR, Gallagher PR, Rorke-Adams LB, Robison LL, Cnaan A. Maternal supplement, micronutrient, and cured meat intake during pregnancy and risk of medulloblastoma during childhood: a Children's Oncology Group Study. Cancer Epidemiol Biomarkers Prev 2006;15:1660–7.
24. Thorne RN, Pearson AD, Nicoll JA, Coakham HB, Oakhill A, Mott MG, et al. Decline in incidence of medulloblastoma in children. Cancer 1994;74:3240–4.
25. Feltbower RG, Picton S, Bridges LR, Crooks DA, Glaser AW, McKinney PA. Epidemiology of central nervous system tumors in children and young adults (0–29 years), Yorkshire, United Kingdom. Pediatr Hematol Oncol 2004;21:647–60.
26. Australian Bureau of Statistics. Western Australia Statistical Indicators. Canberra, Australia: Commonwealth of Australia; 2008. Available from: http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/1367.5Sep%202008.
27. Dal Grande E, Taylor AW. Sampling and coverage issues of telephone surveys used for collecting health information in Australia: results from a face-to-face survey from 1999 to 2008. BMC Med Res Methodol 2010;10:77.
28. Bower C, Miller M, Payne J, Serna P. Promotion of folate for the prevention of neural tube defects: who benefits? Paediatr Perinat Epidemiol 2005;19:435–44.
29. Marsack CR, Alsop CL, Kurinczuk JJ, Bower C. Pre-pregnancy counselling for the primary prevention of birth defects: rubella vaccination and folate intake. Med J Aust 1995;162:403–6.

Published OnlineFirst August 31, 2012; DOI: 10.1158/1055-9965.EPI-12-0803

30. Conlin ML, MacLennan AH, Broadbent JL. Inadequate compliance with periconceptional folic acid supplementation in South Australia. Aust N Z J Obstet Gynaecol 2006;46:528–33.
31. Mazloum M, Bailey HD, Heiden T, Armstrong BK, de Klerk N, Milne E. Participation in population-based case-control studies: does the observed decline vary by socio-economic status? Paediatr Perinat Epidemiol 2012;26:276–9.
32. Twigt JM, Hammiche F, Sinclair KD, Beckers NG, Visser JA, Lindemans J, et al. Preconception folic acid use modulates estradiol and follicular responses to ovarian stimulation. J Clin Endocrinol Metab 2010;96:E322–9.
33. Jacobsen D. Cellular mechanisms of homocysteine pathogenesis in atherosclerosis. In: Carmel R, Jacobsen D (editors). Homocysteine in health and disease. Cambridge, UK: Cambridge University Press; 2001, p. 425–41.
34. Ulrey CL, Liu L, Andrews LG, Tollefsbol TO. The impact of metabolism on DNA methylation. Hum Mol Genet 2005;14:R139–47.
35. Northcott PA, Nakahara Y, Wu X, Feuk L, Ellison DW, Croul S, et al. Multiple recurrent genetic events converge on control of histone lysine methylation in medulloblastoma. Nat Genet 2009;41:465–72.
36. Sirachainan N, Wongruangsri S, Kajanachumpol S, Pakakasama S, Visudtibhan A, Nuchprayoon I, et al. Folate pathway genetic polymorphisms and susceptibility of central nervous system tumors in Thai children. Cancer Detect Prev 2008;32:72–8.
37. Bethke L, Webb E, Murray A, Schoemaker M, Feychting M, Lonn S, et al. Functional polymorphisms in folate metabolism genes influence the risk of meningioma and glioma. Cancer Epidemiol Biomarkers Prev 2008;17:1195–202.
38. French AE, Grant R, Weitzman S, Ray JG, Vermeulen MJ, Sung L, et al. Folic acid food fortification is associated with a decline in neuroblastoma. Clin Pharmacol Ther 2003;74:288–94.
39. Olshan AF, Smith JC, Bondy ML, Neglia JP, Pollock BH. Maternal vitamin use and reduced risk of neuroblastoma. Epidemiology 2002; 13:575–80.

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