Childhood folate, B6, B12 and food group intake and the risk of childhood brain tumors: results from an Australian case-control study.

Short title: Childhood nutrition and risk of brain tumors.

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Abstract

Purpose

The etiology of childhood brain tumors (CBT) is poorly understood but dietary factors could be involved. In this case-control study of CBT, the possible associations of childhood intake of dietary and supplemental folate, B6 and B12 with the risk of CBT were investigated, along with various food groups.

Methods

Cases diagnosed between 2005 and 2010 were identified from 10 pediatric oncology centers in Australia, controls by nationwide random-digit dialling. For study children of ages 3 to 14 years, diet in the year before diagnosis (or recruitment) was assessed using food frequency questionnaires. Folate intake was adjusted for bioavailability, and dietary micronutrient intake was energy-adjusted. Micronutrients and food groups were analysed using logistic regression adjusting for relevant confounders. Principal components analysis was conducted to assess food group intake patterns for analysis.

Results

Food and micronutrient data were available for 216 cases and 523 controls. Folate intake was associated with a reduced risk of CBT overall (odds ratio for highest tertile vs lowest: 0.63, 95% confidence interval: 0.41, 0.97) and particularly low grade gliomas (odds ratio for highest tertile vs lowest: 0.52, 95% confidence interval: 0.29, 0.92). Vitamin B6 and B12 intake were not associated with CBT risk, nor was processed meat.

Conclusions

High folate intake during childhood may reduce the risk of CBT. This potentially important finding needs to be corroborated in other studies. If replicated, these results could have important implications for public health recommendations regarding diet during childhood.

Keywords

Child, brain tumors, folate, neoplasms, micronutrients, processed meat, vitamins

Introduction

Childhood brain tumors (CBT) are a histologically and clinically diverse range of neoplasms, many of which have a poor prognosis with high mortality rate or lifelong disability. Several risk factors for CBT have been implicated through association studies, but causal relationships have yet to be established. Micronutrient intake of the mother (during gestation) or the child are plausible potential risk factors. Several micronutrients, such as folate, vitamin B6 and vitamin B12 have a role in DNA synthesis and integrity, and insufficiency of such nutrients could lead to DNA damage or incorrect methylation [1].

Most research examining the relationship between nutrition and CBT has only considered maternal diet before and during pregnancy; very few studies have looked at nutrition during the child's life. Several studies, including our own, found inverse associations between maternal folate supplementation and/or dietary folate intake before or during pregnancy and risk of CBT [2-4].

Folate during pregnancy might be important as critical developmental periods are reached (including closure of the neural tube), and cells are rapidly dividing, increasing the need for folate for DNA synthesis. It is less certain whether the period after birth is sensitive to diet-influenced carcinogenesis. We have previously investigated vitamin supplementation and dietary folate intake in infants and children under 3 years of age, but no associations were seen [5]. Two other studies investigated childhood dietary folate intake and reported no associations [6,7]. Studies examining childhood fruit/vegetable and supplement intake were reviewed in 2005 [8]; 2 out of 3 studies of multivitamins reported ORs below the null (1 reported no association), while out of 5 studies of fruit and vegetables, 1 reported non-significant ORs above the null, 1 reported no association, 2 reported non-significant ORs below the null and 1 reported a significant inverse association.

Other foods may also influence CBT risk. Cured and processed meats, in particular, have previously been thought to increase cancer risk because nitrites in these foods might react with amines to form *N*-nitroso compounds, which are carcinogenic in experimental animals [8]. Again, most of the evidence for childhood cancer comes from maternal intake during pregnancy [8-10]. The abovementioned review [8] investigating the association between *N*-nitroso compounds and CBT risk concluded there was limited evidence of an association between the child's intake of cured meat and risk of CBT (only 3 out of 7 papers reported a positive association).

As diet is a potentially modifiable risk factor, an understanding of the role that childhood nutrition may play in CBT etiology could be of great value in informing prevention strategies. The primary aim of this analysis was to investigate whether childhood intake of the micronutrients (folate, B6 and B12) were associated with the risk of CBT. Our secondary aim was to assess whether intake of

specific food types or diet patterns (including processed meats, vegetables and fruits) were associated with the risk of CBT.

METHODS

Study design and Participants

Cases were eligible for inclusion if they had: a diagnosis of any brain tumor (malignant or benign) between 2005 and 2010 and before the age of 15 years, were resident in Australia and had an English-speaking biological parent available to participate. Cases were identified through all 10 pediatric oncology centers in Australia. Controls were recruited by national random digit dialling (RDD) and frequency matched to cases in a ratio of approximately 3:1 by age at diagnosis (within 1 year), sex and State of residence. Controls matched to CBT cases diagnosed in 2005 and 2006 were originally recruited as controls for our national case-control study of childhood leukemia (Aus-ALL; 2003-2007) using identical recruitment methods [11]. Both Aus-CBT and Aus-ALL were approved by the Human Research Ethics Committees at all participating hospitals. Written informed consent was obtained from parents of all participating children; informed consent was also sought from children whose parents thought they were old enough to provide it.

Data collection and preparation

Mothers completed a general exposure questionnaire to ascertain pregnancy, birth and demographic information. In addition to self-reported demographics, we also obtained an area-based Index of Socioeconomic Disadvantage (IRSD) score for each household based on census data [12]. Food frequency questionnaires (FFQs) were completed if children were ≥ 3 years of age; these children were likely to be completely weaned and have an established solid food eating pattern. The FFQ was based on the Australian Commonwealth Scientific and Industrial Research Organization's FFQ [13], but with age appropriate portion size and reduced to 109-items that contribute significantly to folate, vitamin B6 and/or vitamin B12 intake. The FFQ was completed by a parent in most cases (usually the mother); a small number of older children (2 cases/6 controls) filled in their own FFQs. The FFQ requested respondents to report the child's usual eating habits in the previous 12 months (or the 12 months before diagnosis for case children). For each food type, a standard serve size was given and respondents were asked to list the frequency of consumption and the number of standard serves usually consumed on eating occasions. Additional questions asked for the usual brands of foods that were permitted to be fortified with folic acid during the study period (e.g. breads, cereals, some drinks). The FFQ also asked if the child had used any supplements regularly for at least a month; the use of supplements containing folic acid, vitamin B6 or vitamin B12 was determined

based on the labelled ingredients of the preparations taken, with doses of folic acid from supplements and food fortification multiplied by 1.7 to adjust for bioavailability and create dietary folate equivalents [14].

Nutrient intake calculations have been described previously [3]; briefly, nutrients were quantified using a customized computer program that merged data from Australian food composition databases AUS-NUT 07 [15] for folate and energy, and NUTTAB 2006 for B6 and B12 [16]. Mandatory folic acid fortification of bread-making wheat flour was introduced in Australia in September 2009 [17]; this was taken into account for children whose FFQ reporting period overlapped this date.

Energy intake was normally distributed after excluding 6 children with extreme high values thought implausible for their age. Folate, B6 and B12 values were log transformed to correct right skew. These values were then energy-adjusted using methods previously described [18]. Energy-adjusted folate, B6 and B12 values were added to the respective amounts from supplements. Total folate, B6 and B12 intake (diet plus supplement) was then grouped according to tertiles of intake among controls, with the lowest tertile in each forming the reference group.

The 109 food items in the FFQ were organized by nutritional similarity with the assistance of the study nutritionist (MM) [19] and collapsed into 13 main food groups for analysis (see results section for group types).

Statistical analysis

Odds ratios (OR) and 95 percent confidence intervals (95% CI) for each exposure of interest were calculated using unconditional logistic regression in SPSS (IBM SPSS for Windows, Version 22.0, Armonk, NY, IBM Corp, 2011). All models were adjusted for study matching variables: child's age, sex and State of residence. Additional variables were assessed as potential confounders of the association between dietary variables and CBT risk: child's year of birth/diagnosis, ethnicity, maternal education, household income and IRSD, maternal age, maternal alcohol use and smoking during pregnancy, maternal folic acid supplementation and dietary folate intake during pregnancy. Covariates meeting the empirical criteria for confounding (ie were associated with case/control status and child's dietary variable in controls) were included in the analytical models; these are shown in table footnotes. Linear trend *P*-values were calculated by fitting ordinal frequency categories to the model as continuous variables with 1 degree of freedom. Results were analyzed for all CBT cases combined and by the main tumor subtypes.

Statistical interactions with child's age, sex and socioeconomic status (maternal education, household income) were formally tested by fitting product terms in the models and assessing the Wald *P*-value of the term, as well as examining stratification of results.

To examine potential patterns in dietary intake, principal components analysis (PCA) was undertaken using the above 13 food groups using continuous 'daily servings' variables. Pattern extraction from controls only was performed with orthogonal varimax rotation and Kaiser normalization, and factors retained based on Eigenscores >1 and interpretability. For the components extracted, a score was allocated to each participant based on conformity to that pattern (regression method), which were then divided into tertiles (based on cut-offs in controls) for analysis.

RESULTS

Between 2005 and 2010, 730 eligible case families were identified. In 573 families, the child was 3 years of age or more and suitable for analysis of food and nutrient intake. Of these 573, 450 (78%) were invited to participate by their treating physicians and 290 (64% of invited, 51% of eligible) consented to participate. In the same period, 3,624 eligible control families (with a child <15 years) were identified through RDD, of whom 2,255 (62.2%) consented to take part. Quotas for frequency matching by age, sex and state meant that 1,467 of these were recruited to the study; of these, 1,144 control children were 3 or more years of age at recruitment and thus eligible for the current analysis. Ultimately, 261 case families and 1,065 eligible control families returned the minimum demographic information, and child FFQs were returned for 223 (85% recruited) case and 543 (47% recruited) control children.

The mean age of the 573 case children aged 3 years or older who were eligible for participation in Aus-CBT was 8.2 (SD 3.6), and 57.6% were boys; these characteristics were similar to the final group of 223 available cases (mean age 8.6 (SD 3.8), 58.3% boys). For ethical reasons, no other information was available for case children where the family did not consent to participate.

Demographic characteristics of those children involved in the current analysis are shown in Table 1.

The proportion of males was higher among cases than controls, and a higher proportion of controls were in the youngest age group. Control mothers tended to be older and have better education levels than case mothers, and control children were more likely to be of European ethnicity.

The percentiles for raw micronutrient and energy intake are shown in Online Resource 1 for 216 cases and 523 controls (after exclusion of children with missing covariates and extreme energy values). Supplements containing folic acid, B6 or B12 were taken by 39 (18%) cases and 100 (19%) controls (~90% of children who took one of these nutrients took all of them).

Table 2 shows the results of each tertile of micronutrient intakes for all CBT cases and for low-grade gliomas and embryonal tumors separately (the only two groups with sufficient number of cases to analyse separately). Models were mutually adjusted for each nutrient and included all relevant confounders (see Table footnote). The highest tertile of folate intake was associated with a reduced risk of CBT overall with a significant linear trend P -value (0.03). The association between folate and low-grade gliomas was slightly stronger than that for embryonal tumors, but the estimates for the latter were imprecise (Table 2). No associations between B6 and B12 intake and risk of CBT were seen.

Online Resource 2 shows the results of sensitivity analyses for each micronutrient and CBT overall, where the first model includes only the matching variables and the second includes relevant confounders but not mutual adjustment for nutrients. These results were similar to the fully adjusted results for CBT overall (Table 2).

The results for folate intake and risk of CBT were similar for older and younger children and for males and females (Online Resource 3). Results were also stratified by socioeconomic status; there was no interaction between folate intake and household income, but there was a significant interaction with maternal education ($P = 0.02$): the inverse association with folate intake appeared to be stronger among children whose mother had no tertiary education (Online Resource 4).

The associations between food group intake and risk of CBT are shown in Table 3: the only major food groups to be associated with CBT risk was milk and dairy foods that showed a negative association ($P_{\text{trend}} = 0.03$), and juice intake showed a positive association with CBT risk ($P_{\text{trend}} = 0.01$). The results were similar when food groups were measured as a percentage of total daily serves reported, i.e. relative rather than absolute intake (data not shown).

PCA extracted four distinct dietary patterns, together accounting for 47.8% of the variance (Online Resource 5). The four dietary patterns extracted were characterized by 1) high intake of fruit, vegetables, fish and eggs, 2) high intake of meats and potatoes, 3) high intake of unfortified bread and cereals, nuts/pulses, juice and processed meats, and 4) high intake of folic acid fortified cereals and dairy products. There was no relationship observed between these dietary patterns and with CBT risk (Online Resource 6).

DISCUSSION

The primary aim of this analysis was to investigate whether childhood dietary intake of folate, B6 and B12 was associated with the risk of CBT. We found evidence that higher intake of dietary folate during childhood was associated with a decreased risk of CBT, particularly among children

whose mother had no tertiary education. No association was observed for B6 or B12. This is the first study to show an association between childhood intake of folate and decreased risk of CBT.

Previous studies have investigated the child's intake of fruits and vegetables – one found no association with any dietary factor [7], the other found a possible protective association with fruit (some of which are high in folate) [20], but these studies looked at diet in the first year of the child's life when food intake would be largely dependent on breast and other milks and intake of solid foods would still be irregular. Another study looked at childhood diet before diagnosis in 300 case and 574 control children (ages 0-18 years) with a 100-item FFQ, and found no association with dietary folate (or fruit or vegetable intake) [6]. In the current study, we did not see any associations between vegetable, fruit, or folate-fortified cereal intake and risk of CBT; folate intake in these children seems to comprise a wide range of sources each contributing a small amount to the total (intake from supplements only contributed about 20% of the total in the small number of children who took these). This suggests that the source of folate is not important as long as overall intake is high. It is plausible that a high folate diet could influence cancer risk because of folate's role in DNA synthesis; low folate could lead to incorporation of uracil into DNA that can lead to strand breaks and DNA damage [1]. Low folate levels are also associated with increased oxidative stress [21] and inappropriate DNA methylation [22] which may play a role in tumor pathogenesis [23,24].

In this analysis, few other food groups or dietary measures (including processed meats) were associated with CBT risk. The two significant associations that were observed - milk and dairy foods were associated with a decreased risk, and fruit juices with an increased risk of CBT – lack plausible biological explanations and may be chance observations.

As with all case-control studies, our sample may be affected by selection bias since the provision of data was less complete for controls than for cases, and controls who participated tended to have higher SES measures than cases. This could result in an overestimation of folate intake (which is associated with SES). While we have adjusted for maternal education and IRSD in all models, some residual confounding may remain. Stratification of folate intake by household income showed little difference across groups, but when stratified by maternal education, the negative association for folate was only seen in the lower maternal education group. It may be that children of mothers with a tertiary education have a healthier lifestyle generally and, as a result, that the biological advantages of a diet high in folate have less influence on disease risk. If that were the case, our findings may only be generalizable to families with lower parental education.

Alternatively, it is possible that some other unmeasured factor – only correlated with folic acid in less-educated parents – may underlie the observed association.

Our FFQ did not cover all possible foods (especially ‘junk food’) as it was designed to be a compromise between getting maximum information about key micronutrients (folate, B6 and B12) while not overburdening the respondents. However, by excluding some foods, there may be participants whose diets and energy intakes are poorly represented, and this limits our ability to energy-adjust micronutrient values, or to investigate total energy or intake of specific nutrient-poor, energy-dense foods. Additionally, the focus on folate and healthy foods in the FFQ limit our ability to detect dietary patterns that are characterized by the intake of nutrient-poor, energy-rich foods. There is likely to be some error in the reporting of dietary intake in the child’s early life. If this error were non-differential between cases and controls, it would tend towards under-estimation of the association. However, response bias might be present if case or control parents differentially tended to over-report perceived ‘good’ foods in favour of other foods. In our study, and in case-control studies generally, this is more likely to occur among controls who tend to be more highly educated than cases; however, we adjusted for maternal education in all analyses. Moreover, the potential for response bias would be limited by the fact that micronutrient values were derived from multiple food items in the FFQ (and were energy-adjusted); and since individual food groups were generally not associated with CBT, maternal response bias seems unlikely.

While case parents had been asked to report the child’s diet in the year prior to becoming ill, it is possible this was not enough ‘lag time’ and that diets may have changed when early symptoms of CBT appeared. In this case, it could be that any differences between cases and controls are a product of confounding by indication. However, this would mean a selective change in foods high in folate, which seems unlikely given the diverse range of sources. The period of FFQ reporting was for the 12 months prior to diagnosis; while this period is probably insufficient on its own to influence tumor development, dietary patterns in children tend to be fairly stable [25,26], and the average intakes reported for the 12 months before diagnosis are likely to reflect longer-term dietary patterns.

Our analysis had limited power as only children in the AUS-CBT study over 3 years of age (223 cases and 543 controls) were included and many variables were assessed. CBT is also a heterogeneous condition and due to small patient numbers in specific subgroups it is difficult to see if our results generalize to all tumor subtypes. It is possible that some of our findings, particularly in sub-groups, are due to chance.

Finally, eligible case children who were not invited by their physicians for medical or psycho-social reasons, or whose parents chose not to participate in our study, are likely to have more severe disease and/or a poorer prognosis than participating cases; this may limit the generalizability of our findings.

Conclusion

A higher dietary intake of folate during childhood may protect against CBT. However, because previous studies of associations with childhood fruit and vegetable intake have produced inconsistent results and we did not observe an association with food groups containing fruit and vegetables or cereal foods fortified with folic acid, our finding for folate needs to be corroborated in other studies. If replicated, these results could have important implications for public health recommendations regarding diet during childhood.

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Conflict of Interest Disclosure: The authors declare that they have no conflict of interest.

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Table 1. Characteristics of children in analysis of child's diet, Aus-CBT

Variable	Category	Cases		Controls	
		N	%	N	%
Child ≥3 years old with FFQ data		223		543	
Tumor type	Low-grade glioma	105	47.1		
	High-grade glioma	21	9.4		
	Embryonal tumor	56	25.1		
	Germ cell tumor	19	8.5		
	Ependymoma	12	5.4		
	Other	10	4.5		
Child’s age	3-5	59	26.5	184	33.9
	5-9	75	33.6	169	31.1
	10-15	89	39.9	190	35.0
Child sex	Female	91	40.8	273	50.3
	Male	132	59.2	270	49.7
State of residence	NSW/ACT	78	35.0	160	29.5
	Victoria/Tasmania	62	27.8	146	26.9
	SA/NT	10	4.5	45	8.3
	WA	31	13.9	63	11.6
	Queensland	42	18.8	129	23.8
Child’s year of birth	1990-1998	84	37.7	178	32.8
	1998-2003	117	52.5	302	55.6
	2004-2010	22	9.9	63	11.6
Year of diagnosis/ recruitment	2005-2006	82	36.8	234	43.1
	2007-2008	76	34.1	168	30.9
	2009-2010	65	29.1	141	26.0
Mother’s age at child’s birth	<25	37	16.7	39	7.2
	25-34	141	63.5	347	63.9
	35+	44	19.8	157	28.9
Mother’s education	<full high school	58	26.1	99	18.2
	Full high school/	70	31.5	197	36.3

	Technical school				
	University/college	94	42.3	247	45.5
IRSD	Population quartile 1	45	20.3	78	14.4
	Population quartile 2	53	23.9	125	23.1
	Population quartile 3	54	24.3	176	32.6
	Population quartile 4	70	31.5	161	29.8
Household income (AUD/pa)	≤\$40,000	36	16.3	61	11.3
	\$40,001-\$70,000	58	26.2	156	28.8
	\$70,001-\$100,000	54	24.4	147	27.1
	>\$100,000	73	33.0	178	32.8
Ethnic group	European/half European	195	87.4	512	94.3
	Half non-European/ mixed/indeterminate	28	12.6	31	5.7

ACT, Australian Capital Territory; IRSD, Index of relative socioeconomic disadvantage; NSW, New South Wales; NT, Northern Territory; SA, South Australia; WA, Western Australia.

Table 2. Childhood folate, vitamin B6 and B12 intake and risk of CBT

	All CBT			Low grade gliomas			Embryonal Tumors		
	N case/ Controls (216/523)	OR ^a	95% CI	N case/ controls	OR ^a	95% CI	N case/ controls	OR ^a	95% CI
Folate ^d <457mcg	90/177	1.00	Referent	49/177	1.00	Referent	17/177	1.00	Referent
Folate 457-592	65/171	0.69	0.46, 1.04	23/171	0.43	0.24, 0.76	25/171	1.41	0.69, 2.87
Folate >592mcg	61/175	0.63	0.41, 0.97	28/175	0.52	0.29, 0.92	14/175	0.76	0.33, 1.71
<i>P</i> _{trend}		0.03				0.01			0.62
B6 <1.2mg	70/175	1.00	Referent	33/175	1.00	Referent	20/175	1.00	Referent
B6 1.2-1.4mg	74/176	1.07	0.72, 1.61	38/176	1.13	0.66, 1.94	15/176	0.68	0.33, 1.41
B6 >1.4mg	72/172	1.23	0.80, 1.89	29/172	1.12	0.62, 2.00	21/172	1.03	0.50, 2.14
<i>P</i> _{trend}		0.32				0.74			0.82
B12 <3.7 mcg	74/172	1.00	Referent	35/172	1.00	Referent	16/172	1.00	Referent
B12 3.7-4.8 mcg	66/174	0.90	0.60, 1.36	36/174	1.08	0.63, 1.85	15/174	0.83	0.39, 1.80
B12 >4.8 mcg	76/177	1.00	0.66, 1.52	29/177	0.94	0.53, 1.67	25/177	1.27	0.62, 2.59
<i>P</i> _{trend}		0.97				0.81			0.42

^aMultivariable analysis (all nutrients in model together): ORs also adjusted for child age, sex, state of residence, year of diagnosis, maternal education, IRSD, maternal dietary folate intake during pregnancy.

^bFolate from food sources adjusted for energy intake; folic acid from supplements and food fortification adjusted for bioavailability.

Table 3. Food intakes of children in year before diagnosis/recruitment and risk of CBT

	Daily serves (tertiles)	N case/ control	OR ^a	95% CI	OR ^b	95% CI	<i>P</i> _{trend}
Folate fortified cereals	0-0.2	57/180	1.00	Referent	1.00	Referent	0.80
	0.2-0.9	97/161	1.91	1.28, 2.84	1.94	1.29, 2.90	
	>0.9	64/188	1.00	0.66, 1.53	0.96	0.62, 1.48	
Unfortified cereals	0-2.5	61/175	1.00	Referent	1.00	Referent	0.74
	2.5-3.7	84/180	1.31	0.89, 1.95	1.35	0.90, 2.01	
	>3.7	73/174	1.10	0.73, 1.65	1.08	0.71, 1.64	
Milk and Dairy	0-2.0	81/175	1.00	Referent	1.00	Referent	0.03
	>2.0-3.2	80/174	0.95	0.65, 1.39	0.91	0.62, 1.35	
	>3.2	57/180	0.66	0.44, 1.00	0.63	0.42, 0.95	
Fruit	0-1.5	77/179	1.00	Referent	1.00	Referent	0.73
	1.5-2.4	75/179	0.98	0.67, 1.44	1.03	0.69, 1.53	
	>2.4	66/171	0.87	0.58, 1.29	0.93	0.62, 1.40	
Juices	0-0.3	54/179	1.00	Referent	1.00	Referent	
	>0.3-1.0	84/207	1.34	0.90, 2.01	1.37	0.91, 2.06	0.01
	>1.0	80/143	1.74	1.15, 2.64	1.73	1.13, 2.65	
Vegetables (except potato)	0-1.9	67/178	1.00	Referent	1.00	Referent	0.56
	>1.9-3.2	76/177	1.11	0.75, 1.65	1.13	0.76, 1.69	
	>3.2	75/174	1.10	0.74, 1.64	1.13	0.75, 1.71	
Potato	0-0.4	79/175	1.00	Referent	1.00	Referent	0.68
	0.4-0.6	57/184	0.69	0.46, 1.04	0.66	0.44, 1.00	
	>0.6	82/170	1.02	0.70, 1.50	0.92	0.61, 1.37	
Nuts/pulses/soy	0-0.1	83/189	1.00	Referent	1.00	Referent	0.84
	0.1-0.5	64/165	0.87	0.58, 1.28	0.92	0.62, 1.37	
	>0.5	71/175	0.92	0.63, 1.34	1.05	0.70, 1.56	
Fish & seafood	0-0.1	73/177	1.00	Referent	1.00	Referent	0.88
	0.1-0.2	60/146	1.00	0.66, 1.51	1.02	0.67, 1.56	
	>0.2	85/206	1.02	0.70, 1.49	1.03	0.70, 1.51	
Processed meats	0-0.4	65/168	1.00	Referent	1.00	Referent	0.81
	0.4-0.8	79/187	1.08	0.73, 1.60	1.03	0.69, 1.54	
	>0.8	74/174	1.07	0.72, 1.60	1.05	0.70, 1.59	

Red meats	0-0.4	72/183	1.00	Referent	1.00	Referent	0.84
	0.4-0.7	71/176	1.01	0.68, 1.49	1.00	0.67, 1.49	
	>0.7	75/170	1.07	0.73, 1.59	1.04	0.70, 1.56	
Chicken	0-0.2	73/183	1.00	Referent	1.00	Referent	0.18
	0.2-0.3	84/158	1.34	0.91, 1.97	1.34	0.90, 1.98	
	>0.3	61/188	0.78	0.52, 1.17	0.75	0.50, 1.13	
Egg	0-0.1	79/183	1.00	Referent	1.00	Referent	0.84
	0.1-0.3	51/156	0.76	0.50, 1.15	0.69	0.45, 1.06	
	>0.3	88/190	1.05	0.72, 1.52	1.03	0.71, 1.52	

^a ORs adjusted only for matching variables (child's age, sex, state of residence)

^b ORs adjusted for matching variables, year of diagnosis or recruitment, IRSD, maternal education, maternal dietary folate intake during pregnancy.

Online Resources for:

Childhood folate, B6, B12 and food group intake and the risk of childhood brain tumors: results from an Australian case-control study.

Online Resource 1. Raw dietary micronutrient and energy intake percentiles for children 3-15 years of age in the Australian Study of the causes of Childhood Brain Tumors (n cases 216, n controls 523)

		25 th	50 th	75 th
Folate (mcg) ^a	Cases	362	466	637
	Controls	394	496	643
B6 (mg) ^a	Cases	1.0	1.3	1.5
	Controls	1.0	1.2	1.5
B12 (mcg) ^a	Cases	3.0	4.2	5.2
	Controls	3.0	4.2	5.5
Energy (kj)	Cases	6355	7430	8718
	Controls	6230	7543	8802

^aMicronutrient values before adjustment for energy intake.

Online Resource 2. Childhood folate, vitamin B6 and B12 intake and risk of CBT sensitivity analyses

	N case/ Controls (216/523)	OR ^a (95% CI)	OR ^b (95% CI)
Folate ^c <457mcg	90/177	1.00 (referent)	1.00 (referent)
Folate 457-592	65/171	0.73 (0.50, 1.07)	0.72 (0.48, 1.07)
Folate >592mcg	61/175	0.67 (0.45, 0.99)	0.67 (0.44, 1.01)
<i>P</i> _{trend}		0.04	0.05
B6 <1.2mg	70/175	1.00 (referent)	1.00 (referent)
B6 1.2-1.4mg	74/176	1.06 (0.72, 1.57)	1.07 (0.71, 1.59)
B6 >1.4mg	72/172	1.05 (0.71, 1.56)	1.12 (0.75, 1.68)
<i>P</i> _{trend}		0.80	0.59
B12 <3.7 mcg	74/172	1.00 (referent)	1.00 (referent)
B12 3.7-4.8 mcg	66/174	0.92 (0.62, 1.37)	0.91 (0.60, 1.37)
B12 >4.8 mcg	76/177	1.03 (0.70, 1.52)	1.05 (0.70, 1.56)
<i>P</i> _{trend}		0.89	0.82

^aORs adjusted for child age, sex, state of residence.

^bORs adjusted for child age, sex, state of residence, year of diagnosis, maternal education, IRSD, maternal folate intake during pregnancy.

^cFolate from food sources adjusted for energy intake; folic acid from supplements adjusted for bioavailability

Online Resource 3. Child's folate intake and risk of CBT by child's age and sex.

Age at diagnosis/recruitment						
	N case/ controls	3-7.7 years		N case/ controls	7.8-15 years	
		OR ^a	95% CI		OR ^a	95% CI
Folate <457mcg	37/89	1.00	Referent	53/88	1.00	Referent
Folate 457-592	27/84	0.72	0.37, 1.39	38/87	0.71	0.41, 1.24
Folate >592mcg	27/99	0.68	0.34, 1.36	34/76	0.69	0.38, 1.25
<i>P</i> _{trend}			0.26			0.22
<i>P</i> interaction term						0.99

Sex						
	N case/ controls	Female		N case/ controls	Male	
		OR ^a	95% CI		OR ^a	95% CI
Folate <457mcg	40/98	1.00	Referent	50/79	1.00	Referent
Folate 457-592	29/81	0.82	0.44, 1.51	36/90	0.58	0.33, 1.01
Folate >592mcg	21/89	0.51	0.26, 1.03	40/86	0.70	0.39, 1.24
<i>P</i> _{trend}			0.05			0.27
<i>P</i> interaction term						0.31

^aMultivariable analysis: ORs adjusted for child age, sex, state of residence, year of diagnosis, maternal education, IRSD, maternal folate intake during pregnancy.

Online Resource 4. Child's folate intake and risk of CBT by socioeconomic status.

Household income						
	N case/ controls	≤\$70,000 pa		N case/ controls	>\$70,000 pa	
		OR ^a	95% CI		OR ^a	95% CI
Folate <457mcg	42/75	1.00	Referent	48/102	1.00	Referent
Folate 457-592	27/64	0.65	0.34, 1.25	37/107	0.63	0.36, 1.09
Folate >592mcg	23/67	0.57	0.28, 1.15	37/107	0.60	0.33, 1.08
<i>P</i> _{trend}			0.08			0.11
<i>P</i> interaction term						0.98

Maternal Education						
	N case/ controls	No university/college		N case/ controls	University/college	
		OR ^a	95% CI		OR ^a	95% CI
Folate <457mcg	60/90	1.00	Referent	30/87	1.00	Referent
Folate 457-592	35/108	0.46	0.26, 0.79	30/63	1.29	0.67, 2.48
Folate >592mcg	31/89	0.47	0.26, 0.85	30/86	0.89	0.46, 1.73
<i>P</i> _{trend}			0.01			0.63
<i>P</i> interaction term						0.02

^aMultivariate analysis (adjusting for dietary B6 and B12 intake): ORs adjusted for child age, sex, state of residence, year of diagnosis, maternal education, IRSD, maternal folate intake during pregnancy.

Online Resource 5. Rotated factor loadings for principal component analysis.

	Component			
	1	2	3	4
Variance explained	13.6	13.0	11.5	9.7
Food group				
Fruit	0.67	0.03	0.29	0.10
Vegetables	0.62	0.13	0.25	0.11
Fish	0.62	0.06	-0.17	0.04
Egg	0.54	0.10	-0.08	-0.20
Chicken	0.05	0.74	-0.18	-0.04
Red Meat	0.23	0.72	0.01	0.13
Potatoes	0.10	0.55	0.22	0.01
Processed meat	-0.21	0.45	0.40	0.05
Unfortified cereals	0.01	0.20	0.72	-0.09
Nuts/pulses/soy	0.35	-0.18	0.53	-0.11
Juices	0.01	-0.01	0.44	0.11
Folate fortified cereals	-0.10	-0.05	-0.18	0.82
Milk/dairy	0.12	0.15	0.22	0.69

Online Resource 6. Dietary pattern scores from principal components analysis and risk of CBT

Diet pattern	tertile	N case/ control	OR ^a	95% CI	<i>P</i> _{trend}
1. Fruit, vegetables, fish, egg	1	78/177	1.00	Referent	0.46
	2	76/176	1.03	0.69, 1.52	
	3	64/176	0.85	0.57, 1.29	
2. Meat & potatoes	1	59/177	1.00	Referent	0.82
	2	85/175	1.33	0.88, 2.00	
	3	74/177	1.07	0.70, 1.64	
3. Unfortified cereals, nuts/pulses, juice & processed meat	1	59/179	1.00	Referent	0.30
	2	83/175	1.45	0.97, 2.17	
	3	76/175	1.25	0.83, 1.89	
4. Fortified cereals & milk/dairy	1	76/176	1.00	Referent	0.17
	2	77/174	0.96	0.65, 1.43	
	3	65/179	0.75	0.50, 1.13	

^aORs adjusted for child age, sex, state of residence, year of diagnosis, maternal education, IRSD, maternal folate intake during pregnancy.