**Paternal dietary folate, B6 and B12 intake and the risk of childhood brain tumors.**

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Abstract (194 words)

It is biologically plausible that paternal pre-conception diet low in nutrients related to DNA integrity could affect sperm DNA and subsequently risk of cancer in the offspring. The aim of this analysis was to investigate whether paternal preconception dietary folate, B6 or B12 intake was associated with risk of childhood brain tumors (CBT) in an Australian case-control study. Cases aged <15 years were recruited from 10 Australian pediatric oncology centres between 2005 and 2010, and controls from random-digit dialling, frequency-matched to cases on age, sex and state of residence. Paternal dietary information was obtained by food-frequency questionnaires. Nutrient values were energy adjusted, and divided into tertiles for analysis by unconditional logistic regression. In fathers with relevant data (237 cases and 629 controls), no association with dietary folate and B6 and risk of CBT was seen; high B12 intake was associated with an increased risk of CBT (OR highest vs lowest tertile: 1.74, 95% CI: 1.14, 2.66) without an increasing trend. This data does not support the hypothesis that paternal dietary folate intake influences the risk of CBT. The increased OR observed between dietary B12 intake and risk of CBT is without any certain explanation.

Keywords: brain tumors, child, folate, diet, paternal, epidemiological, cobalamin

## Introduction

There are very few known causes of childhood brain tumors (CBT), which are the second-most common type of childhood cancer after the leukemias (1). Parental or childhood diet could influence the risk of CBT, due to the ingestion of potential carcinogens or anti-carcinogens (2-5).

Folate may influence cancer risk because of its key role in DNA synthesis and repair, and gene methylation (6, 7). Previous research by our group has found some evidence of a protective effect against CBT of both supplemental and dietary folate intake by the mother leading up to or during pregnancy (8, 9). To date, there is no published epidemiological research on possible effects of paternal intake of folate and related nutrients in the preconception period on risk of CBT. Paternal folate levels may influence the integrity of sperm DNA (10), and folate deficiency could increase the amount of DNA damage in the resulting fetus, predisposing it to carcinogenic events (7). There is some evidence from both animal (11, 12) and human (13) studies that this can occur.

The Australian Study of Childhood Brain Tumours (Aus-CBT) was a nationwide case-control study conducted between 2005 and 2010 designed to investigate environmental, dietary and genetic risk factors for CBT. The aim of this analysis was to investigate whether paternal dietary intake of folate in the 6 months leading to the pregnancy was associated with a reduced risk of CBT. We also explored associations with folate's cofactors in the one-carbon metabolic cycle: vitamins B6 and B12. We also investigated folate/B6/B12 intake stratified by alcohol intake because of the effect of alcohol on absorption of folate and other vitamins, and the association between paternal alcohol intake and CBT previously shown (14). To our knowledge, this is the first investigation of paternal intake of these nutrients and risk of CBT.

## METHODS

### *Participants and recruitment*

Detailed recruitment methods have been reported previously (8). In brief, incident CBT cases were identified through all 10 pediatric oncology centers in Australia; and were eligible for participation if diagnosed between 2005 and 2010, were resident in Australia and had an English speaking biological parent available. Control children were recruited by nationwide random digit dialling (RDD), frequency matched to cases on age (within 1 year), sex and State of residence. Controls matched to CBT cases diagnosed in 2005 and 2006 were matched to cases from our concurrent study of childhood leukemia (Aus-ALL; 2003-2007), using identical recruitment methods (15). Both Aus-CBT and Aus-ALL were approved by the Human Research Ethics Committees at all participating hospitals.

### *Data collection*

Fathers of cases and controls completed a general exposure questionnaire (including demographics and questions about alcohol consumption in the 12 months before the pregnancy) and a 126-item food frequency questionnaire (FFQ) modified from the FFQ of the Australian Commonwealth Scientific and Research Organization (16) to focus on intake of folate, vitamin B6 and vitamin B12. Standard serving sizes were given for each food type, and fathers were asked to list the frequency of consumption and the number of standard servings consumed per occasion in the 6 months before his partner's pregnancy with the case or control child. Additional questions collected information about usual brands of foods, particularly those potentially fortified with folic acid. Fathers were also asked to report any vitamin or mineral supplements taken in the 6 months before the index pregnancy. In

addition, we obtained an area-based SES indicator corresponding to the child's home address (Index of Relative Socioeconomic Disadvantage, IRSD)(17).

*Assessment of nutrient intake*

The calculation of dietary folate, vitamin B6, vitamin B12 and energy has been described elsewhere (9). Briefly, these values were calculated using Australian food composition databases (AUS-NUT 07 (18) for folate and energy, and NUTTAB 2006 (19) for B6 and B12), with additional information about foods fortified with folic acid obtained from manufacturers and food labels added to the program (the latter values being multiplied by 1.7 to allow for greater bioavailability (20)). The labelled ingredients on any reported supplement preparation consumed by fathers was used to determine intake of supplemental folic acid, vitamin B6 or vitamin B12.

*Statistical analysis*

Folate, B6 and B12 values were log transformed to correct right skew. These values were then energy-adjusted using methods described elsewhere (21). For quality control reasons, we excluded fathers where the reported daily energy intake was implausible: below 4,000 or above 20,000 kilojoules. Energy-adjusted folate, B6 and B12 values were categorized into tertiles (based on the distribution in controls), with the lowest tertile forming the reference group. Odds ratios (OR) and 95 percent confidence intervals (95% CI) were estimated for energy-adjusted dietary intake of folate, B6 and B12 using unconditional logistic regression in SPSS (IBM SPSS for Windows, version 20.0, Armonk, NY, IBM Corp, 2011). All three nutrients were included in the same model, and all models were adjusted for study matching variables: child's age, sex and state of residence. In addition, variables associated with both case/control status and control father's dietary nutrient intake were included: paternal age, year of diagnosis or recruitment, parental education, child's ethnicity, and level of paternal

alcohol consumption in the year prior to the pregnancy. Maternal dietary intake of folate, B6 and B12 was also assessed for inclusion in the models but made no difference to the ORs for paternal dietary intake and were not used in the final models.

Only about 7% of fathers used supplemental folic acid or B6/B12 (B6 and B12 were almost always taken together), so no further analysis of supplement use was undertaken. Moreover, paternal supplementation did not meet the conditions for confounding, so was not included in the final models. Stratified analyses were undertaken by paternal alcohol consumption, age of the child, and age of the father. For these stratification analyses, alcohol intake was categorized as low or high consumption; cut-offs (<21 drinks/week/≥21 drinks/week) were based on the level of intake previously shown to be associated with CBT risk (14). Dietary nutrient intake was also analysed for the two largest CBT subtypes: low-grade gliomas and embryonal tumors.

RESULTS

Details of recruitment and participation have been previously described (8). Briefly, 730 eligible CBT cases diagnosed between 2005 and 2010 were identified, of whom 568 (78%) were invited to participate by their treating physicians. Of these, 374 (66% of invited, 51% of eligible) consented to take part. We identified 3,624 eligible controls via random-digit dialling; 2,255 of these agreed to participate. In accordance with the study's age and sex frequency matching quotas, 1467 of these were recruited. Of those who agreed to participate, 335 case families and 1,363 control families returned at least basic demographic data, while 242 case fathers and 641 control fathers completed an FFQ. One case and 3 control fathers were missing covariate data, and 4 case and 9 control fathers' FFQ data was excluded due to implausible total energy intakes, leaving a final total of 237 case and 629 control fathers for analysis.

Table 1 shows the characteristics of case and control families included in this analysis. Control fathers tended to be slightly older than case fathers, and control parents had higher levels of educational attainment and were more likely to be of European ethnicity. Supplemental Table 1 shows the median raw values of folate, B6, B12 and energy intake for the fathers in our study.

There was little evidence that folate or B6 intake was associated with the risk of CBT (Table 2). The ORs for the second and third tertiles of B12 intake were elevated, but there was no clear dose-response relationship.

When the results for folate, B6 and B12 intake were analysed separately by level of paternal alcohol intake, the ORs for folate intake were below the null among fathers with higher alcohol consumption ($P_{trend}$ = 0.30), and the increased ORs seen for B12 in the main analysis appeared to be present only among fathers with lower alcohol consumption. However, the numbers were small in the high-alcohol group and the *P*-values for the interaction terms were 0.39 and 0.53 respectively (Supplemental Table 2).

When the analyses were stratified by the age of the child at diagnosis/recruitment and by the father's age at the child's birth, the elevated ORs for B12 intake were seen mainly among younger children, and showed some evidence of a dose-respond trend in this group ($P_{trend}$ for young children: 0.001; interaction term *P*-value: 0.05) (Table 4), and in older fathers (interaction *P*-value = 0.32) (Supplemental Table 3). For each of the micronutrients, the results for low-grade gliomas were consistent with the results for CBT overall, while none of the nutrients appeared to be associated with risk of embryonal tumours (Supplementary Table 4).

To further investigate the elevated ORs observed for B12 intake, terms for several foods rich in B12 were added to the model one at a time to assess if they could account for this

association. However, the ORs for B12 did not change materially with addition of these foods to the models (fish, chicken, processed meat, beef products, pork/lamb products, yeast extract spreads, dairy products), and these foods were not independently related to risk of CBT (data not shown).

## DISCUSSION

To our knowledge, ours is the first study of paternal preconception diet in relation to risk of CBT. Most previous studies have focused on maternal diet during pregnancy (5, 22-24). We have previously reported moderate associations between maternal folate supplementation and/or high dietary folate intake and a reduced risk of CBT (8, 9). It is plausible that a paternal diet low in folate or its cofactors in the one-carbon metabolic cycle during the preconception period could increase susceptibility to cancer in the child by reducing the nutrients needed for successful DNA replication and methylation (7).

However, we found no evidence of an association between dietary folate consumption by fathers and risk of CBT. We did observe elevated ORs for high vitamin B12 intake, and there was evidence that this association was stronger in children diagnosed before age 5, and when the father had lower alcohol consumption or was older; however, a dose response association for B12 was only seen in younger children. ORs for B12 were also more elevated in the low grade glioma subgroup than for embryonal tumors.

The reason for the increased ORs we observed for high B12 intake is not clear. B12 is an essential cofactor with folate in the one-carbon cycle of DNA metabolism, and insufficient intake of these nutrients is associated with increased blood homocysteine levels, increased uracil incorporation in DNA (possibly leading to DNA double strand breaks), and aberrant methylation of DNA (25). Studies of pancreatic (26), prostate (27), and gastric/oesophageal cancer (28) have also reported positive associations with dietary B12. These studies

speculated that B12 may be a proxy for high meat intake, but adjustment for these foods in two studies (26, 27) did not change their results. In our data, no individual product containing high B12 (e.g. meat, yeast spread) was associated with CBT or changed the ORs for B12. However, our results might still be confounded by some unmeasured correlated variable (e.g. cooking method/fat intake).

Seminal plasma B12 levels have previously been associated with increased sperm count and concentration, but not with a reduction in DNA damage in sperm of men undergoing fertility treatments (10). Thus, it is biologically plausible that increased fertility, in the presence of sperm with accumulated DNA damage (attributable to a range of potential factors), could be associated with germline mutations in the embryo and the subsequent development of cancer in the child. This mechanism is speculative and would require confirmation in other studies.

This study has several strengths; recruitment of both cases and controls was nationwide, and controls were selected from the population using the best methods available in this country. We used an FFQ containing a wide variety of foods with a particular focus on folate-rich foods, and we appropriately accounted for food fortification and energy intake. We also had access to information about factors such as alcohol and SES that could potentially confound the association between these micronutrients and CBT risk.

Nevertheless, this study also has several limitations. The participation rate of controls was fairly low, and since our eligibility criteria focused on the availability of the mother, we only had paternal FFQ data from a proportion of recruited families: 63% of cases and 43% of controls. Controls recruited into the study had higher area-based SES indicators than the population as a whole (8), and control fathers who provided FFQ data had higher area-based SES scores than those who did not. We found that higher SES fathers tended to have higher folate and B6 intake and lower B12 intake. Therefore, it is likely that control fathers with high B12 intake were under-represented in our study; this would bias our ORs for B12

upwards. Although we aimed to address this by adjusting for SES (parental education) in all models, residual bias is possible. There is likely to have been some exposure measurement error, since fathers were asked to recall their diet from between 0 and 15 years earlier (although in approximately 60% of fathers the recall period was 6 years or less). If this error were non-differential, the ORs would be biased to the null. If, however, case fathers recalled their intake of individual food items more accurately than control fathers (i.e. recall bias), the impact on ORs is difficult to predict, particularly after adjustment for energy intake. The FFQ contained over 100 food items, with no indication of what particular foods or nutrients we were studying. If recall bias played a part in our findings, similar findings might be expected for all nutrients, but this was not the case. We believe that recall bias is unlikely to explain our findings.

In conclusion, we found no evidence that paternal dietary folate or B6 are associated with the risk of CBT. The elevated ORs seen for vitamin B12 are novel but without any certain explanation; in the absence of any prior hypothesis that paternal vitamin B12 levels might increase CBT risk, chance, selection bias and confounding cannot be ruled out as possible explanations for it.

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Table 1: Demographics of families in Aus-CBT paternal dietary folate and B vitamin analysis.

| Variable | | N case | % case | N control | % control |
|---|---|---|---|---|---|
| Paternal FFQ available | | 237 | | 629 | |
| Child's age | 0-1 | 26 | 11.0 | 76 | 12.1 |
| | 2-4 | 70 | 29.5 | 211 | 33.5 |
| | 5-9 | 73 | 30.8 | 189 | 30.0 |
| | 10-15 | 68 | 28.7 | 153 | 24.3 |
| Child sex | Female | 102 | 43.0 | 303 | 48.2 |
| | Male | 135 | 57.0 | 326 | 51.8 |
| State of residence | NSW/ACT | 88 | 37.1 | 189 | 30.0 |
| | Victoria/Tasmania | 68 | 28.7 | 173 | 27.5 |
| | SA/NT | 13 | 5.5 | 53 | 8.4 |
| | WA | 28 | 11.8 | 74 | 11.8 |
| | Queensland | 40 | 16.9 | 140 | 22.3 |
| Year of diagnosis/recruitment | 2005-2006 | 89 | 37.6 | 284 | 45.2 |
| | 2007-2008 | 77 | 32.5 | 180 | 28.6 |
| | 2009-2010 | 71 | 30.0 | 165 | 26.2 |
| Father's age at child's birth | <34 | 135 | 57.0 | 314 | 49.9 |
| | 34+ | 102 | 43.0 | 315 | 50.1 |
| Best education of either parent | <full high school | 19 | 8.0 | 33 | 5.2 |
| | Full high school/ trade qualification | 85 | 35.9 | 195 | 31.2 |
| | University/college | 133 | 56.1 | 400 | 63.6 |

| | | | | | |
|---|---|---|---|---|---|
| Area-based SES | 1 | 41 | 17.6 | 88 | 14.1 |
| (population quartiles) | 2 | 52 | 22.3 | 143 | 23.0 |
| | 3 | 64 | 27.5 | 189 | 30.4 |
| | 4 | 76 | 32.6 | 202 | 32.5 |
| Ethnic group[a] | European | 181 | 76.4 | 552 | 87.8 |
| | At least 50% European | 30 | 12.7 | 47 | 7.5 |
| | At least 50% non-European | 12 | 5.1 | 22 | 3.5 |
| | Indeterminate | 14 | 5.9 | 8 | 1.3 |
| Paternal alcohol consumption in | <21 drinks/week | 179 | 75.5 | 522 | 83.0 |
| year prior the pregnancy | 21+ drinks/week | 58 | 24.5 | 107 | 17.0 |
| Father had Folic acid supplements | No | 220 | 92.8 | 590 | 93.8 |
| 6 months prior the pregnancy | Yes | 17 | 7.2 | 39 | 6.2 |
| Father had B6/B12 supplements 6 | No | 220 | 92.8 | 581 | 92.4 |
| months prior the pregnancy | Yes | 17 | 7.2 | 48 | 7.6 |

NSW: New South Wales, ACT: Australian Capital Territory, SA: South Australia, NT: Northern Territory, WA: Western Australia.

[a]European: at least 3 European grandparents; 50% European: 2 European grandparents; at least 50% non-European: 2 non-European grandparents and ethnicity of 2 other grandparents unknown; indeterminate: no 2 grandparents of same ethnicity (i.e European or non-European) and 2+ grandparents of unknown ethnicity.

Table 2: Paternal preconceptional dietary folate, B6 and B12 and the risk of CBT.

| | Cases (n=237) | | Controls (n=629) | | | |
|---|---|---|---|---|---|---|
| | n | % | n | % | OR[a] | 95% CI |
| Energy-adjusted dietary folate (mcg) | | | | | | |
| < 394.9 | 99 | 41.8 | 210 | 33.4 | 1.00 | Referent |
| 394.9 – 509.5 | 71 | 30.0 | 210 | 33.4 | 0.82 | 0.55, 1.22 |
| >509.5 | 67 | 28.2 | 209 | 33.2 | 0.85 | 0.56, 1.28 |
| Energy-adjusted B6 (mg) | | | | | | |
| <1.53 | 83 | 35.0 | 211 | 33.6 | 1.00 | Referent |
| 1.53-1.71 | 80 | 33.8 | 208 | 33.1 | 0.92 | 0.63, 1.35 |
| >1.71 | 74 | 31.2 | 209 | 33.3 | 0.98 | 0.66, 1.47 |
| Energy-adjusted B12 (mcg) | | | | | | |
| ≤ 4.61 | 54 | 22.8 | 210 | 33.4 | 1.00 | Referent |
| 4.61-5.91 | 97 | 40.9 | 209 | 33.2 | 1.98 | 1.32, 2.99 |
| >5.91 | 86 | 36.3 | 210 | 33.4 | 1.74 | 1.14, 2.66 |

[a] Adjusted for matching variables (child's age, sex, State of residence), child's year of diagnosis/recruitment, paternal age, best parental education, child's ethnicity, paternal preconceptional high alcohol consumption.

Table 3: Paternal preconceptional dietary folate, B6 and B12 and the risk of CBT by child's age at diagnosis.

| | Child age 0-4 | | | | | | Child age 5-14 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases (n=96) | | Controls (n=287) | | | | Cases (n=141) | | Controls (n=342) | | | |
| | n | % | n | % | OR[a] | 95% CI | n | % | n | % | OR[a] | 95% CI |
| Energy-adjusted dietary folate (mcg) | | | | | | | | | | | | |
| < 394.9 | 29 | 30.2 | 76 | 26.5 | 1.00 | Referent | 70 | 49.6 | 134 | 39.2 | 1.00 | Referent |
| 394.9 – 509.5 | 29 | 30.2 | 94 | 32.8 | 0.94 | 0.49, 1.81 | 42 | 29.8 | 116 | 33.9 | 0.76 | 0.45, 1.28 |
| >509.5 | 38 | 39.6 | 117 | 40.8 | 1.08 | 0.57, 2.03 | 29 | 20.6 | 92 | 26.9 | 0.71 | 0.40, 1.25 |
| P interaction term | | | | | | | | | | | | 0.78 |
| Energy-adjusted B6 (mg) | | | | | | | | | | | | |
| <1.53 | 34 | 35.4 | 87 | 30.4 | 1.00 | Referent | 49 | 34.8 | 124 | 36.3 | 1.00 | Referent |
| 1.53-1.71 | 28 | 29.2 | 101 | 35.3 | 0.58 | 0.31, 1.08 | 52 | 36.9 | 107 | 31.3 | 1.20 | 0.73, 1.97 |
| >1.71 | 34 | 35.4 | 98 | 34.3 | 0.85 | 0.46, 1.57 | 40 | 28.4 | 111 | 32.4 | 1.02 | 0.59, 1.76 |
| P interaction term | | | | | | | | | | | | 0.24 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Energy-adjusted B12 (mcg) | | | | | | | | | | | | |
| ≤ 4.61 | 19 | 19.8 | 104 | 36.2 | 1.00 | Referent | 35 | 24.8 | 106 | 31.0 | 1.00 | Referent |
| 4.61-5.91 | 35 | 36.5 | 94 | 32.8 | 2.57 | 1.29, 5.12 | 62 | 44.0 | 115 | 33.6 | 1.65 | 0.97, 2.79 |
| >5.91 | 42 | 43.8 | 89 | 31.0 | 3.29 | 1.67, 6.45 | 44 | 31.2 | 121 | 35.4 | 1.08 | 0.61, 1.91 |
| P interaction term | | | | | | | | | | | | 0.05 |

[a] Adjusted for matching variables (sex, State of residence), child's year of diagnosis/recruitment, paternal age, best parental education, child's ethnicity, paternal preconceptional alcohol consumption.

Supplementary Table 1: Paternal dietary nutrient percentiles in Aus-CBT[a]

| Nutrient | | 25th | 50th | 75th |
|---|---|---|---|---|
| Folate (mcg/day) | Cases | 318.5 | 447.8 | 546.5 |
| | Controls | 339.4 | 451.4 | 596.1 |
| B6 (mg/day) | Cases | 1.27 | 1.66 | 1.99 |
| | Controls | 1.30 | 1.64 | 2.07 |
| B12 (mcg/day) | Cases | 4.02 | 5.31 | 7.42 |
| | Controls | 3.79 | 5.20 | 6.99 |
| Energy (kj/day) | Cases | 7687 | 9445 | 11368 |
| | Controls | 7927 | 9456 | 11431 |

[a]Percentiles are based on values before energy adjustment

Supplemental Table 2: Paternal preconceptional dietary folate, B6 and B12 and the risk of CBT by level of paternal alcohol consumption in the 12 months prior to the pregnancy.

| | Alcohol <21 drinks/week[a] | | | | | | Alcohol ≥21 drinks/week | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases (n=179) | | Controls (n=522) | | | | Cases (n=58) | | Controls (n=107) | | | |
| | n | % | n | % | OR[b] | 95% CI | n | % | n | % | OR[b] | 95% CI |
| Energy-adjusted dietary folate (mcg) | | | | | | | | | | | | |
| < 394.9 | 61 | 34.1 | 156 | 29.9 | 1.00 | Referent | 38 | 65.5 | 54 | 50.5 | 1.00 | Referent |
| 394.9 – 509.5 | 57 | 31.8 | 175 | 33.5 | 0.91 | 0.58, 1.42 | 14 | 24.1 | 35 | 32.7 | 0.60 | 0.22, 1.61 |
| >509.5 | 61 | 34.1 | 191 | 36.6 | 0.92 | 0.58, 1.44 | 6 | 10.3 | 18 | 16.8 | 0.53 | 0.15, 1.84 |
| P interaction term | | | | | | | | | | | | 0.39 |
| Energy-adjusted B6 (mg) | | | | | | | | | | | | |
| <1.53 | 66 | 36.9 | 183 | 35.1 | 1.00 | Referent | 17 | 29.3 | 28 | 26.2 | 1.00 | Referent |
| 1.53-1.71 | 62 | 34.6 | 165 | 31.7 | 0.99 | 0.65, 1.52 | 18 | 31.0 | 43 | 40.2 | 0.72 | 0.27, 1.91 |
| >1.71 | 51 | 28.5 | 173 | 33.2 | 0.90 | 0.58, 1.41 | 23 | 39.7 | 36 | 33.6 | 1.46 | 0.52, 4.11 |
| P interaction term | | | | | | | | | | | | 0.70 |
| Energy-adjusted B12 (mcg) | | | | | | | | | | | | |
| ≤ 4.61 | 29 | 16.2 | 152 | 29.1 | 1.00 | Referent | 25 | 43.1 | 58 | 54.2 | 1.00 | Referent |
| 4.61-5.91 | 74 | 41.3 | 180 | 34.5 | 2.17 | 1.32, 3.57 | 23 | 39.7 | 29 | 27.1 | 1.66 | 0.69, 4.00 |
| >5.91 | 76 | 42.5 | 190 | 36.4 | 2.04 | 1.24, 3.36 | 10 | 17.2 | 20 | 18.7 | 1.03 | 0.35, 3.03 |
| P interaction term | | | | | | | | | | | | 0.53 |

[a] 1 standard drink contains 10g alcohol.

[b]Adjusted for matching variables (child's age, sex, State of residence), child's year of diagnosis/recruitment, paternal age, best parental education, child's ethnicity.

Supplemental Table 3: Paternal preconceptional dietary folate, B6 and B12 and the risk of CBT by paternal age group.

| | <34 years | | | | | | ≥34 years | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases (n=135) | | Controls (n=314) | | | | Cases (n=102) | | Controls (n=315) | | | |
| | n | % | n | % | OR[a] | 95% CI | n | % | n | % | OR[a] | 95% CI |
| Energy-adjusted dietary folate (mcg) | | | | | | | | | | | | |
| < 394.9 | 71 | 52.6 | 115 | 36.6 | 1.00 | Referent | 28 | 27.5 | 95 | 30.2 | 1.00 | Referent |
| 394.9 – 509.5 | 34 | 25.2 | 103 | 32.8 | 0.62 | 0.36, 1.07 | 37 | 36.3 | 107 | 34.0 | 1.26 | 0.68, 2.36 |
| >509.5 | 30 | 22.2 | 96 | 30.6 | 0.71 | 0.40, 1.27 | 37 | 36.3 | 113 | 35.9 | 1.19 | 0.63, 2.28 |
| P interaction term | | | | | | | | | | | | 0.09 |
| Energy-adjusted B6 (mg) | | | | | | | | | | | | |
| <1.53 | 50 | 37.0 | 114 | 36.3 | 1.00 | Referent | 33 | 32.4 | 97 | 30.9 | 1.00 | Referent |
| 1.53-1.71 | 49 | 36.3 | 111 | 35.4 | 0.96 | 0.58, 1.59 | 31 | 30.4 | 97 | 30.9 | 0.80 | 0.43, 1.48 |
| >1.71 | 36 | 26.7 | 89 | 28.3 | 0.89 | 0.50, 1.58 | 38 | 37.3 | 120 | 38.2 | 0.86 | 0.48, 1.57 |
| P interaction term | | | | | | | | | | | | 0.86 |
| Energy-adjusted B12 (mcg) | | | | | | | | | | | | |
| ≤ 4.61 | 33 | 24.4 | 98 | 31.2 | 1.00 | Referent | 21 | 20.6 | 112 | 35.6 | 1.00 | Referent |
| 4.61-5.91 | 56 | 41.5 | 113 | 36.0 | 1.51 | 0.86, 2.63 | 41 | 40.2 | 96 | 30.5 | 2.61 | 1.38, 4.93 |
| >5.91 | 46 | 34.1 | 103 | 32.8 | 1.46 | 0.82, 2.62 | 40 | 39.2 | 107 | 34.0 | 2.30 | 1.19, 4.42 |
| P interaction term | | | | | | | | | | | | 0.32 |

[a] Adjusted for matching variables (child's age, sex, State of residence), child's year of diagnosis/recruitment, best parental education, child's ethnicity.

Supplementary Table 4: Paternal preconceptional dietary folate, B6 and B12 and the risk of Low grade gliomas and Embryonal tumours.

| | Controls (n=629) | | Low grade gliomas (n=117) | | | | Embryonal tumors (n=56) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | OR[a] | 95% CI | n | % | OR[a] | 95% CI |
| Energy-adjusted dietary folate (mcg) | | | | | | | | | | |
| < 394.9 | 210 | 33.4 | 51 | 43.6 | 1.00 | Referent | 22 | 39.3 | 1.00 | Referent |
| 394.9 – 509.5 | 210 | 33.4 | 33 | 28.2 | 0.78 | 0.46, 1.31 | 16 | 28.6 | 0.83 | 0.39, 1.76 |
| >509.3 | 209 | 33.2 | 33 | 28.2 | 0.84 | 0.49, 1.46 | 18 | 32.1 | 1.04 | 0.48, 2.24 |
| Energy-adjusted B6 (mg) | | | | | | | | | | |
| <1.53 | 211 | 33.6 | 53 | 45.3 | 1.00 | Referent | 17 | 30.4 | 1.00 | Referent |
| 1.53-1.71 | 208 | 33.1 | 33 | 28.2 | 0.59 | 0.36, 0.97 | 21 | 37.5 | 1.27 | 0.62, 2.60 |
| >1.71 | 209 | 33.3 | 31 | 26.5 | 0.70 | 0.41, 1.17 | 18 | 32.1 | 1.25 | 0.59, 2.67 |
| Energy-adjusted B12 (mcg) | | | | | | | | | | |
| ≤ 4.61 | 210 | 33.4 | 25 | 21.4 | 1.00 | Referent | 16 | 28.6 | 1.00 | Referent |
| 4.61-5.91 | 209 | 33.2 | 48 | 41.0 | 2.07 | 1.20, 3.59 | 25 | 44.6 | 1.53 | 0.76, 3.10 |
| >5.91 | 210 | 33.4 | 44 | 37.6 | 2.00 | 1.14, 3.51 | 15 | 26.8 | 0.78 | 0.35, 1.73 |

[a] Adjusted for matching variables (child's age, sex, State of residence), child's year of diagnosis/recruitment, paternal age, best parental education, child's ethnicity, level of paternal preconceptional alcohol consumption.