Breastfeeding and nutrition to 2 years of age and risk of childhood acute lymphoblastic leukemia and brain tumors

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Abstract (199 words)

Acute lymphoblastic leukemia (ALL) and childhood brain tumors (CBT) are two of the most common forms of childhood cancer, but little is known of their etiology. In two nationwide case-control studies we investigated whether breastfeeding, age of food introduction, or early diet are associated with the risk of these cancers. Cases aged 0-14 years were identified from Australian pediatric oncology units between 2003 and 2007 (ALL) and 2005 and 2010 (CBT) and population-based controls through nationwide random-digit dialling. Mothers completed questionnaires giving details of infant feeding up to the age of two years. Data from 322 ALL cases, 679 ALL controls, 299 CBT cases and 733 CBT controls were analysed using unconditional logistic regression. Breastfeeding was associated with a reduced risk of ALL (OR 0.52, 95% CI: 0.32, 0.84), regardless of duration. Introduction of artificial formula within 14 days of birth was positively associated with ALL (OR 1.57, 95% CI: 1.03, 2.37), as was exclusive formula feeding to 6 months (OR 1.81, 95% CI: 1.07, 3.05). No associations were seen between breastfeeding or formula use and risk of CBT. Our results suggest that breastfeeding and delayed introduction of artificial formula may reduce the risk of ALL but not CBT.

Keywords: child; leukemia; brain tumors, breastfeeding; infant diet; formula feeding; epidemiological

Introduction

Little is known about the causes of acute lymphoblastic leukaemia (ALL) or childhood brain tumors (CBT) which are, respectively, the first and second most common childhood cancers in the western world.

The majority of ALL cases are diagnosed before five years of age (1) which suggests that early life factors may play a role. Early exposure to infection is important for normal development of the immune system and delayed exposure may result in a rare response that triggers ALL (2). Breastfeeding may play an important part in normal immune system development as it involves the transmission of viruses and bacteria (2), thus priming the immune system. Breast milk also contains antibodies, providing passive immunity from acute infection during early life (2). Due to these advantages for the infant, the current recommendation is for infants to be exclusively breastfed for the first six months of life (3, 4). The association between breastfeeding and ALL has been the focus of three meta-analyses in the last decade (5-7) and all found an overall inverse association, but were cautious about attributing causation. Since these findings were published, two large studies - the Northern California Childhood Leukemia Study (NCCLS) and the Cross Canada Childhood Leukemia Study - reported no association between breastfeeding and ALL (8, 9).

For CBT, there is little evidence that breastfeeding plays a protective role, with six studies finding no association (10-15) and only one finding an inverse association (16). Another recent study found a protective association between breastfeeding and the risk of childhood cancer (OR for six months or more 0.5, 95% CI: 0.2-0.9), but did not separate cancer types (17). Only 34% of cases had ALL and 14% had CBT, making the interpretation of these findings with respect to specific cancer types difficult.

Less research has focussed on whether the child's nutritional intake during infancy is associated with risk of ALL or CBT. Investigations of early dietary patterns before the age of two years and risk of ALL have been undertaken by the NCCLS (8, 18) and a Texan group (19). While no association was seen with the age of introduction of solid foods (8), regular consumption of bananas, oranges or orange juice in the first two years appeared to reduce the risk of disease (18). These results suggest that some factor, such as vitamin C or potassium, in the first few years of life may reduce the risk of ALL. Schraw and colleagues found that longer duration of formula feeding and delayed introduction to solid foods was associated with an increased risk of ALL (19). For CBT, one study reported a protective association with fruit consumption in the

first year of life (20), and two other studies reported no associations with either food or vitamin intake in the first year of life (21) or the age at which solid foods were introduced (11).

The Australian Study of Acute Lymphoblastic Leukaemia (Aus-ALL) and the Australian Study of Childhood Brain Tumors (Aus-CBT) aimed to investigate genetic, dietary and environmental risk factors for ALL and CBT respectively. The aim of the current analysis was to conduct a detailed investigation of breastfeeding duration and timing of food introduction, together with other aspects of the child's nutritional intake up to the age of two years.

Material and Methods

Aus-ALL and Aus-CBT have been described previously (22, 23). Briefly, they were both national, population-based case-control studies, conducted between 2003 and 2006 and 2005 and 2010 respectively. Eligibility criteria were: diagnosis before 15 years of age and the availability of a biological mother with sufficient English to complete the questionnaires. Case families were identified and recruited through all ten pediatric oncology centers in Australia. ALL cases were eligible if they had been diagnosed between 1st July 2003 and 31st December 2006 and had reached remission. CBT cases were eligible if they were diagnosed between 2005 and 2010. Controls for both studies were recruited by random digit dialing and were frequency matched to cases by age, sex and State of residence in an approximate ratio of 3:1. Aus-ALL controls were recruited between 2003 and 2006 and CBT controls were recruited between 2007 and 2010. Aus-ALL controls recruited in 2005 and 2006 were also used as controls for Aus-CBT. Both studies had Human Research Ethics Committee approval from all participating hospitals.

Dietary assessment

Mothers were asked to complete a questionnaire about their child's nutritional intake up to the age of two years; if the child was younger than 2 years mothers completed the relevant parts of the questionnaire up to diagnosis (or questionnaire completion for controls). Mothers were asked whether the child had ever been breastfed and for how long, and about the use of infant formulas, the main types of milk given, and the age of introduction of a range of foods. This information was used to calculate the length of exclusive breastfeeding. To assign the age at which formula feeding was commenced, we considered only formulas given for one month or longer. This was done to exclude short or atypical uses of formula likely to have had little influence on the infant's overall nutrition. Mothers were also asked to complete a food frequency table consisting of 28 food items about their children's diet between the ages of one and two years. Dietary folate intake was estimated by combining nutrient data from AUS-NUT

07 (24) and serving sizes based on those observed among 16-24 month old children in an Australian study (25). Voluntary fortification of individual foods (breakfast cereals; breads; fruit juices; yoghurts and milk) with folic acid was recorded and accounted for; wheat flour was mandatorily fortified from September 2009 (26).

Information was collected from manufacturers and product labels about the constituents of all supplements taken by the child up to two years of age. This information was used to assess whether the child had had a supplement containing multivitamins, folic acid, Vitamins A, B6, B12, or C or iron.

Subtype classification

We obtained information about immunophenotype and cytogenetic subtypes of ALL cases from the treating clinicians. The latter were determined using metaphase cytogenetics and fluorescence in situ hybridization screening. CBT subtypes were recorded by the treating clinician, and categorized independently by two pediatric oncologists as previously described (23).

Statistical analysis

For breastfeeding duration, we excluded children under 7 months of age at censoring date, as they could not contribute to the highest duration category. Two measures of ‘any breastfeeding’ (regardless of duration) were used: one which included all available children and the other with the above age restriction to allow comparison with the duration variables. The analysis of early food introduction included only children aged over 12 months as these were considered to be of a sufficient age to have been introduced to most common foods.

Correlations between the age of introduction of individual foods were evaluated with Pearson’s correlation coefficients. Age at initiation of formula feeding (for more than one month) was divided into groups based on tertiles among controls who received formula before six months. The information about the age at which specific solid foods were introduced was categorised into two groups, based on the distribution among the controls. Children who had never had that food before the age of two years were included in a separate group. The frequency of intake of key food groups was examined by collapsing clusters of nutritionally similar foods from the questionnaire, then dividing into frequency groups based on the tertile distribution among controls. As a large proportion of children were introduced to some foods at similar times, or had similar frequencies of food intake, some of the tertile-based groups were not equal.

Odds ratios (OR) and 95 percent confidence intervals (95% CI) were estimated using unconditional logistic regression in SPSS (IBM SPSS for Windows, version 20.0, Armonk, NY, IBM Corp, 2011). All models were adjusted for the study matching factors – age at diagnosis in months, sex and State of residence. Variables considered a priori to be potential confounders of the association between early life nutrition and the risk of ALL/CBT (ethnicity, maternal age at the child's birth, birth order, birth weight, family income and maternal education) were assessed for inclusion in the models. Variables that met the empirical definition of confounding (independently associated with both the exposure and the outcome) were included in the final models. As breastfeeding was found to be associated with ALL, it was also included in the final models for food exposures, as was proportion of optimum birth weight (POBW) (27). The final models for ALL analyses contained the matching factors, maternal age at the child's birth, birth order and maternal education, breastfeeding status and POBW. As having a birth defect was associated with both early life nutrition and risk of ALL, these children were excluded from ALL analyses. For CBT analyses, the potential confounders varied by exposure variable – those included in the final models are shown in the footnotes to tables.

We also analysed the breastfeeding data by immunophenotype and cytogenetic subtype (for ALL) and by tumor subtype (for CBT) for groups with more than 50 cases.

Results

We were notified of 519 eligible ALL cases between 2003 and 2007; parents of 416 (80.2%) consented to participate in the study, and the infant feeding questionnaire was completed and returned by 341 mothers (82.0%). Between 2005 and 2010, 730 eligible CBT cases were identified, of whom 568 (78%) were invited and 374 (66% of invited, 51% of eligible) consented to take part. Of these, 299 (80% recruited) case mothers completed an infant feeding questionnaire.

Between 2003 and 2010, 4,703 families eligible to be controls were identified, of whom 3,012 (64%) agreed to participate. In accordance with requirements for age and sex frequency-matching, we recruited 2,165 of these children to the study. Of these, 1,112 control mothers (51% of those recruited in both studies) completed an infant feeding questionnaire.

For ALL, 19 case and 23 control children with birth defects were excluded, leaving 322 cases and 679 controls for analysis. For CBT, 299 cases and 733 controls were available for analyses (Table 1). Due to small amounts of missing exposure or covariate data, the numbers available for analysis of different infant feeding factors varied.

Demographics

The demographic characteristics of cases and controls were generally similar, with some exceptions (Table 1). ALL case children were more likely to be first born than control children (50.5% versus 41.1%) while control mothers were more likely to have a college/university education and to have been aged 35 years or older when the child was born. CBT controls were more likely to be of European ethnicity.

Breastfeeding and formula feeding

‘Any breastfeeding’ results were similar whether we included all children or only those aged 7 months or more – the latter measure is reported henceforth. For ALL, the OR for having been breastfed was 0.52 (95% CI: 0.32, 0.84). However, this association was not stronger with increased duration of breastfeeding or exclusive breastfeeding. There was no association with formula feeding overall, and ALL was only associated with age at commencement of formula if it was introduced within 14 days of birth (OR: 1.57, 95% CI: 1.03, 2.37). In addition, the OR for exclusive use of formula to six months of age was 1.81 (95% CI: 1.07, 3.05) (Table 2).

For CBT, the ORs of any breastfeeding, breastfeeding duration, age starting on formula, and infant feeding method were all close to unity. The main type of milk given to the child between six months and one year and between one and two years of age did not appear to be associated with the risk of ALL or CBT (Table 2).

The ORs for breastfeeding by ALL subtype were all below the null, similar to the overall OR, apart from the *ETV6-Runx-1 t(12;21)* subtype (Supplementary Table 1). The highest OR for any formula feeding was in the hyperdiploidy subgroup. The ORs for breastfeeding and formula feeding among CBT subtypes (low-grade gliomas and embryonal tumors) were generally null, similar to the overall results. Most estimates were imprecise and potentially unstable due to small numbers of cases.

Introduction of Foods

The age of introduction of solid foods and fruit juice were all significantly inter-correlated (Supplemental Table 2), making the interpretation of findings for the introduction of individual foods difficult. Using ‘≤4 months’ as the reference group, the OR for starting food (any type) after four months of age was 1.40 (95% CI: 1.03, 1.90) for CBT. There was a suggestion that those who started vegetables later had an increased risk of ALL and CBT, and that those who started eggs later had a reduced risk of ALL (Table 3).

Frequency of foods eaten from one to two years of age

The frequency of intake of different food groups between the ages of one and two years is shown in Table 4. For ALL, the OR for more than 1.1 serves/day of meat/chicken/fish/eggs was 1.39 (95% CI: 0.98, 1.96, $P_{\text{trend}} = 0.07$). There were no associations evident between food group intake and risk of CBT.

Dietary folate and supplemental vitamins

There was no association between dietary folate intake between one and two years of age and risk of ALL or CBT (Supplemental Table 3). Few cases or controls had any vitamins or other supplements before two years of age, and no association was seen for ALL or CBT (Supplementary Table 4).

Sensitivity analyses

We repeated the analyses for breastfeeding excluding women who breastfed for 14 days or less, and the results for both ALL and CBT remained unchanged (data not shown).

Since controls recruited in 2005 and 2006 were used in the analyses for both ALL and CBT, we investigated whether the results differed when these controls were randomly allocated to either analysis (that is, independent controls used in each analysis); none of the results were materially different from those presented (data not shown).

Discussion

We collected data on dietary intake using detailed infant feeding questionnaires, which allowed us to determine breastfeeding duration and exclusivity, as well as other details of diet during infancy that have not been previously examined. Breastfeeding was associated with a reduced risk of ALL, but there was no evidence of a dose-dependent relationship with duration of breastfeeding or exclusive breastfeeding. Commencement of formula feeding (of ≥ 1 month's duration) was associated with an increased risk of ALL when started within 14 days of birth. There was no association between CBT risk and breastfeeding or use of infant formula.

Our findings for breastfeeding and ALL are consistent with three previous meta-analyses (5-7), although our OR (0.52, 95% CI: 0.32, 0.86) was lower than the pooled ORs in these reports. Our null finding for breastfeeding and CBT risk is similar to most previous studies (10-15, 28).

It has been proposed that B-cell ALL may be caused by an abnormal response to infection in children who have not been exposed to infectious agents early in life (2). It is possible that breastfeeding could guard against childhood ALL by providing passive immunity against infection whilst the postnatal immune system is developing, by the ingestion of maternal antibodies in breast milk (2). If the associations we observed were causal, our ORs suggest a

threshold effect rather than a dose-dependent effect; that is, even a brief period of breastfeeding may confer protection against ALL. A recent study reported an association between duration of formula feeding and risk of ALL, and the authors speculated that this may be because formula feeding increases insulin-like growth factor-1 levels more than breastfeeding, which in turn accelerates growth and causes proliferative stress, leading to higher ALL risk (19). These authors further hypothesized that earlier age of introduction to infant formula (which could not be investigated in their data) may be associated with increased risk of ALL; we found an increased risk among infants started on formula within 14 days of birth (about half of these were also breastfed to some extent) as well as infants exclusively fed formula to six months of age. Since initiation of breastfeeding and early use of infant formula are inversely correlated with each other, we cannot be sure whether the mechanism (if any) driving these associations is related to a benefit from breastfeeding or a detrimental effect of formula or both.

There was some suggestion of an association between frequent consumption of meat/chicken/fish/eggs between the ages of one and two years and risk of ALL (but not CBT). Given the weakness of the association, it could be due to chance. The only previous study examining the association between food intake before two years of age and risk of ALL specifically examined cured meats and found no association (18).

Unlike our null finding, one previous study (19) reported a modest increased risk for ALL associated with the delayed introduction to solids. We did observe a positive association between introduction to food after four months and risk of CBT; the one other study to investigate this relationship in CBT found no association (11). We saw no associations between ALL or CBT and dietary folate intake or use of vitamin supplements.

It has been proposed that the inverse association between breastfeeding and ALL seen in many observational studies could be caused by selection bias and/or recall bias (5-7). However, despite the relatively low participation fraction among controls and the slightly higher socioeconomic status of controls relative to cases in our study, the proportion of ALL and CBT controls who were ever breastfed (94% and 93% respectively) were similar to the proportion in the 'Growing up in Australia' 2004 population cohort (92%) (29), suggesting that they were representative of the target population in this respect. In addition, when we stratified our results by education level, the ORs for breastfeeding were similar in both high and low education groups (interaction term *P*-value 0.72). Therefore, we consider it unlikely that the inverse association we observed for breastfeeding is entirely attributable to selection bias.

Exposure misclassification is also possible, especially when mothers are asked to recall details of infant feeding many years in the past. However, a study of recall of breastfeeding initiation and duration in Norway (where breastfeeding rates are high) showed very good recall ($\kappa=0.85$) up to 20 years after the event (30). Recall bias is also possible if case mothers report exposures differently from control mothers; however, it seems unlikely that this would occur for ALL but not CBT. Thus, we believe that recall bias is unlikely to account for the inverse relationship we observe for breastfeeding and ALL.

We were unable to accurately quantify portion sizes for food group intake – we were only able to assess the frequency that children received each food. Since portion sizes change rapidly with age during infancy, and assessment was based on intake frequency, considerable measurement error in the total quantity consumed may have occurred; however, such error is likely to be non-differential and would have biased the ORs to the null.

In Aus-ALL, only 4 cases (< 1% of those identified) did not reach remission. Therefore, it is unlikely that our findings for ALL were affected by survival bias. In Aus-CBT, however, approximately 9% of identified cases had died (and were therefore ineligible for study participation) and 10% of eligible cases were considered by their doctor to be too unwell to be invited to take part. If breastfeeding or infant nutrition were associated with survival or severity of CBT, our findings may be biased. However, we are unaware of any evidence that this may be the case, and it would be difficult to predict what direction such bias could take. Our findings may only be generalizable to less severe CBT cases.

In conclusion, our results suggest that breastfeeding may reduce the risk of ALL, and that early introduction of regular use of artificial formula may increase the risk of ALL. If causal, these results could be due to the nutritional/immune effects of breast milk or the impact of formula on growth, or a combination of these factors. We saw no association between breastfeeding or formula use and risk of CBT, while there was weak evidence that the introduction of foods later than four months of age may be associated with an increased risk.

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Table 1: Demographic characteristics of cases and controls

	ALL				CBT			
	Cases		Controls		Cases		Controls	
	n	%	n	%	n	%	n	%
Total available	322		679		299		733	
Sex								
Boys	176	54.7	356	52.4	177	59.2	381	52.0
Girls	146	45.0	323	47.6	122	40.8	352	48.0
Age ¹								
0-1	28	8.7	45	6.6	48	16.1	86	11.7
2-4	146	45.3	287	42.3	69	23.1	224	30.6
5-9	93	28.9	239	35.2	74	24.7	190	25.9
10-14	55	17.1	108	15.9	108	36.1	233	31.8
State of residence ²								
NSW/ACT	100	31.1	205	30.2	102	34.1	220	30.0
Queensland	56	17.4	142	20.9	56	18.7	169	23.1
SA/NT	35	10.9	63	9.3	18	6.0	57	7.8
Victoria/Tasmania	95	29.5	197	29.0	83	27.8	203	27.7
Western Australia	36	11.2	72	10.6	40	13.4	84	11.5
Birth order								
1	161	50.0	279	41.1	136	45.5	302	41.2
2	93	28.9	231	34.0	97	32.4	267	36.4
3+	68	21.1	169	24.9	66	22.1	164	22.4
POBW ³								
≤113% (mean+1SD)	269	85.1	578	86.9	257	89.5	628	86.6
>113%	47	14.9	87	13.1	30	10.5	97	13.4
Multiple birth								
Singleton	315	97.8	661	97.3	289	96.7	705	96.2
Multiple	7	2.2	18	2.7	10	3.3	28	3.8
In NICU >2 weeks ⁴								
No	292	93.9	529	94.6	250	89.9	672	92.6
Yes	19	6.1	30	5.4	28	10.1	54	7.4
Child's birth year								
1988 – 1993/90-98	42	13.0	83	12.2	85	28.4	180	24.6
1994 – 1999/98-03	107	33.7	248	36.5	122	40.8	357	48.7
2000 – 2006/04-10	173	53.7	348	51.3	92	30.8	196	26.7
Mother's age at child's birth								
<25 years	45	14.0	59	8.7	46	15.4	50	6.8
25 to 34 years	219	68.0	459	67.6	185	62.1	465	63.4
35+ years	58	18.0	161	23.7	67	22.5	218	29.7
Mother's education ⁵								
Didn't complete secondary	87	27.0	149	21.9	70	23.5	126	17.2
Complete secondary or trade qualification	114	35.4	243	35.8	100	33.6	248	33.8
University/college	121	37.6	287	42.3	128	43.0	359	49.0
Ethnicity ⁶								
European ethnicity	243	75.7	528	77.8	187	62.5	565	77.1
At least 50% European	59	18.3	116	17.1	71	23.7	121	16.5
At least 50% non-European and unknown if 50%	12	3.7	15	2.2	12	4.0	22	3.0

European								
Indeterminate	8	2.5	20	2.9	29	9.7	25	3.4

¹Age groups are based on the child's age at the censoring date. For cases, this was the date of diagnosis and for controls it was the date the questionnaire was returned.

²ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; SA, South Australia.

³POBW data missing for 6 ALL cases, 14 ALL controls, 12 CBT cases, 8 CBT controls.

⁴Neonatal Intensive Care Unit stay data missing for 11 ALL cases, 120 ALL controls, 21 CBT cases and 7 CBT controls.

⁵Maternal education missing for 1 CBT case.

⁶European = at least 3 European grandparents; 50% European = 2 European grandparents; At least 50% non-European and not known if 50% European = 2 non-European grandparents and ethnicity of other 2 grandparents unknown; Indeterminate = no 2 grandparents with the same ethnicity (ie European or non-European) and 2+ grandparents of unknown ethnicity.

Table 2: Breastfeeding, infant feeding method, and main milks and risk of childhood ALL and CBT.

[illegible]

Only breastfed	114	36.8	255	38.7	Reference	65	28.9	194	32.4	Reference
Breastfed and infant formula	161	51.9	364	55.2	0.91 (0.67, 1.22)	136	60.4	359	59.5	1.07 (0.75, 1.54)
Only infant formula	35	11.3	40	6.1	1.81 (1.07, 3.05)	24	10.7	49	8.1	1.33 (0.73, 2.44)
Main Milks										
Main milk between 6 months-1 year of age ⁶										
Cow's milk or cow's milk-based infant formula	180	58.3	338	51.4	Reference	145	55.1	350	50.6	Reference
Breast milk	115	37.2	289	44.0	0.90 (0.67, 1.22)	106	40.3	315	45.5	0.88 (0.64, 1.20)
Other milk or other infant formula	14	4.5	30	4.6	0.99 (0.50, 1.94)	12	4.6	27	3.9	0.99 (0.47, 2.06)
Main milk between 1-2 years of age ⁶										
Cow's milk or cow's milk-based infant formula	260	85.2	546	84.9	Reference	223	84.8	589	85.1	Reference
Breast milk	24	7.9	59	9.2	1.01 (0.61, 1.69)	20	7.6	64	9.2	0.85 (0.49, 1.46)
Other milk or other infant formula	21	6.9	38	5.9	1.33 (0.76, 2.35)	20	7.6	39	5.6	1.18 (0.66, 2.12)

¹Adjusted for matching variables (child's age in months at diagnosis for cases and date of questionnaire return for controls, sex and State), maternal education, maternal age at birth, birth order, proportion of optimum birth weight.

² The reference group are those who were never breast fed.

³Adjusted for matching variables (child's age in months at diagnosis for cases and date of questionnaire return for controls, sex and State), parental education, year of birth, maternal age, ethnicity, NICU stay of >2 days.

⁴ Two ALL cases and two ALL controls, and 1 CBT control are missing data about the duration of breastfeeding; analysis of duration excludes infants under 7 months of age.

⁵ Exclusive breastfeeding is defined as only breast milk given. This was calculated using the information about the age that the infant first had formulas, other milks or juice or solid foods.

⁶ALL models were additionally adjusted for any breastfeeding.

Table 3: Age of introduction of main foods and risk of childhood ALL and CBT

Food	ALL				OR ¹	95% CI	CBT				OR ²	95% CI
	case n	%	control n	%			case n	%	control n	%		
Any Food ³												
≤ 4 months	165	56.5	362	57.2	1.00	Referent	111	41.3	322	47.4	1.00	Referent
> 4 months	126	43.2	270	42.7	1.09	0.82, 1.46	158	58.7	357	52.5	1.40	1.03, 1.90
Not yet/not before 2 years	1	0.3	1	0.2			0	0	1	0.1		
Any Solid ⁴												
≤ 4 months	160	54.2	355	56.0	1.00	Referent	108	40.1	314	46.2	1.00	Referent
> 4 months	134	45.4	278	43.8	1.14	0.86, 1.52	161	59.9	365	53.7	1.38	1.01, 1.88
Not yet/not before 2 years	1	0.3	1	0.2			0	0	1	0.1		
Cereal												
≤ 5 months	182	60.1	411	63.8	1.00	Referent	146	53.3	377	55.0	1.00	Referent
> 5 months	114	37.6	222	34.5	1.23	0.92, 1.64	123	44.9	297	43.3	1.04	0.77, 1.41
Not yet/not before 2 years	7	2.3	11	1.7	1.42	0.53, 3.83	5	1.8	12	1.7	1.20	0.40, 3.59
Vegetables												
≤ 6 months	256	84.5	565	87.6	1.00	Referent	215	78.2	573	83.5	1.00	Referent
> 6 months	46	15.2	78	12.1	1.36	0.91, 2.04	60	21.8	110	16.0	1.31	0.91, 1.90
Not yet/not before 2 years	1	0.3	2	0.3	1.13	0.10, 13.23	0	0	3	0.4		
Fruit												
≤ 6 months	238	78.5	521	80.7	1.00	Referent	199	72.4	537	78.4	1.00	Referent
> 6 months	63	20.8	123	19.0	1.13	0.79, 1.60	73	26.5	145	21.2	1.26	0.90, 1.77
Not yet/not before 2 years	2	0.7	2	0.3	1.80	0.24, 13.33	3	1.1	3	0.4	2.05	0.35, 12.06
Fruit juice												
≤ 7 months	163	54.5	319	49.5	1.00	Referent	103	37.6	266	38.9	1.00	Referent
> 7 months	115	38.5	260	40.4	0.88	0.65, 1.19	137	50.0	312	45.7	1.26	0.90, 1.75
Not yet/not before 2 years	21	7.0	65	10.1	0.64	0.37, 1.10	34	12.4	105	15.4	0.98	0.61, 1.58
Meat/chicken												
≤ 7 months	175	57.9	384	59.7	1.00	Referent	144	52.6	383	55.9	1.00	Referent
> 7 months	122	40.4	248	38.6	1.14	0.85, 1.52	122	44.5	290	42.3	1.03	0.77, 1.39
Not yet/not before 2 years	5	1.7	11	1.7	1.08	0.36, 2.32	8	2.9	12	1.8	1.59	0.60, 4.24

Fish												
≤ 8 months	140	46.8	307	48.1	1.00	Referent	106	39.0	289	42.3	1.00	Referent
> 8 months	103	34.4	245	38.4	0.93	0.68, 1.27	113	41.5	281	41.1	1.03	0.75, 1.43
Not yet/not before 2 years	56	18.7	86	13.5	1.27	0.85, 1.92	53	19.5	113	16.5	1.07	0.70, 1.62
Egg												
≤ 10 months	148	49.7	290	45.3	1.00	Referent	112	41.3	297	43.5	1.00	Referent
> 10 months	108	36.2	284	44.4	0.75	0.55, 1.01	127	46.9	309	45.2	1.12	0.82, 1.53
Not yet/not before 2 years	42	14.1	66	10.3	1.15	0.73, 1.79	32	11.8	77	11.3	1.00	0.61, 1.62

¹OR adjusted for matching variables, mothers age, maternal education, birth order, percentage of optimal birth weight, ever breastfed, excludes children with birth defects. Analysis includes children over 12 months of age at diagnosis/recruitment.

²OR adjusted for matching variables, year of birth, mothers age, ethnicity, maternal education, maternal dietary folate intake during pregnancy. Analysis includes children over 12 months of age at diagnosis/recruitment.

³ Includes cereal, fruit, fruit juice, vegetables, meat/chicken, fish, egg.

⁴ Includes cereal, fruit, vegetables, meat/chicken, fish, egg (not fruit juice).

Table 4: Frequency of food types eaten between ages 1-2 and risk of childhood ALL and CBT

Food Group	Times/day	ALL			CBT		
		N case/ control	OR ¹	95% CI	N case/ control	OR ²	95% CI
Cereals ³	0-2.7	91/212	1.00	Referent	98/245	1.00	Referent
	>2.6-3.6	115/197	1.40	0.99, 1.97	98/245	1.12	0.79, 1.58
	>3.6	96/231	1.00	0.71, 1.42	87/202	1.10	0.77, 1.58
Fruits ⁴	0-1.7	105/217	1.00	Referent	104/234	1.00	Referent
	>1.7-2.6	78/200	0.86	0.60, 1.23	93/261	0.87	0.62, 1.23
	>2.6	121/222	1.18	0.85, 1.65	86/197	1.10	0.76, 1.59
Vegetables ⁵	0-1.1	125/232	1.00	Referent	100/247	1.00	Referent
	>1.1-2.0	125/286	0.86	0.63, 1.17	123/295	1.07	0.77, 1.49
	>2.0	54/124	0.86	0.58, 1.29	60/150	0.99	0.66, 1.47
Dairy products ⁶	0-2.1	89/218	1.00	Referent	105/236	1.00	Referent
	>2.1-3.7	111/212	1.21	0.86, 1.71	100/225	1.00	0.71, 1.41
	>3.7	102/212	1.13	0.80, 1.61	77/230	0.79	0.55, 1.14
Meat/fish/chicken/egg	0-0.7	100/222	1.00	Referent	108/243	1.00	Referent
	>0.7-1.1	93/230	0.96	0.68, 1.35	91/251	0.91	0.64, 1.28
	>1.1	110/191	1.39	0.98, 1.96	83/198	0.93	0.65, 1.34

¹ Adjusted for matching variables (child's age at IFQ return, sex, state of residence), maternal age, maternal education, birth order, POBW, child ever breast fed.

² Adjusted for matching variables (child's age, sex, state of residence), child's year of birth, maternal age, maternal education, maternal dietary folate intake, child's ethnicity.

³ Group includes breakfast cereal, bread, rice, crackers, rusks, pasta.

⁴ Group includes stewed/pureed fruit, citrus fruit, other fruits, fruit juice.

⁵ Group includes raw vegetables, cooked green and other vegetables (except potato).

⁶ Group includes cow or goats milk, cheese, yoghurt, custard.

Supplemental Table 1: Breastfeeding and formula feeding and risk of ALL and CBT by subtypes.

		Any breastfeeding			Any formula feeding	
		case n	exposed cases/controls	OR ^{1,2} (95% CI)	exposed cases/controls	OR ^{1,3} (95% CI)
ALL						
	Pre-B cell	274	243/619	0.51 (0.31, 0.85)	214/479	1.22 (0.87, 1.73)
	T cell	31	27/619	0.38 (0.12, 1.25)	16/479	0.36 (0.17, 0.79)
	Normal karyotype	105	93/619	0.52 (0.25, 1.06)	83/479	1.24 (0.74, 2.08)
	Any genetic feature	198	176/619	0.52 (0.30, 0.92)	147/479	1.01 (0.69, 1.47)
	Chromosomal deletions	49	43/619	0.41 (0.15, 1.08)	35/479	0.91 (0.46, 1.78)
	ETV6-Runx-1 t(12;21)	49	46/619	1.33 (0.37, 4.81)	33/479	0.58 (0.29, 1.15)
	Hyperdiploidy	89	77/619	0.37 (0.18, 0.77)	71/479	1.45 (0.82, 2.55)
	Trisomy (or greater) of chromosome 21 ⁴	51	44/619	0.34 (0.14, 0.87)	38/479	1.01 (0.51, 2.00)
CBT						
	Low-grade gliomas	134	123/657	0.96 (0.46, 2.01)	102/512	1.25 (0.79, 1.98)
	Embryonal tumors	62	58/657	1.17 (0.39, 3.53)	46/512	1.02 (0.55, 1.90)

¹Adjusted for matching variables (child's age in months at diagnosis for cases and date of questionnaire return for controls, sex and State), maternal education, maternal age at birth, birth order, proportion of optimum birth weight.

² The reference group are those who were never breast fed. Excludes children under 7 months age.

³ The reference group are those who were never formula fed. Excludes children under 7 months age.

⁴ Refers to somatic (not constitutive) changes in tumor cells; children with Down syndrome were excluded from the analysis.

Supplemental Table 2: Correlations (Pearson's r) between the ages of introduction of key solid foods

	Cereal	Fruit	Vegetables	Meat or chicken	Fish	Fruit juice	Egg
ALL cases and controls							
Cereal	1.00	0.48**	0.49**	0.41**	0.19**	0.24**	0.24**
Fruit		1.00	0.78**	0.54**	0.30**	0.27**	0.29**
Vegetables			1.00	0.62**	0.31**	0.28**	0.31**
Meat or chicken				1.00	0.45**	0.23**	0.35**
Fish					1.00	0.15**	0.40**
Fruit juice						1.00	0.30**
Egg							1.00
CBT cases and controls							
Cereal	1.00	0.36**	0.44**	0.38**	0.14**	0.24**	0.19**
Fruit		1.00	0.70**	0.50**	0.29**	0.23**	0.32**
Vegetables			1.00**	0.57**	0.29**	0.26**	0.32**
Meat or chicken				1.00	0.48**	0.21**	0.38**
Fish					1.00	0.09*	0.47**
Fruit juice						1.00	0.19**
Egg							1.00

*P values <0.01 **P values <0.001

Supplemental Table 3: Odds ratios and 95% confidence intervals for the risk of ALL and CBT associated with the level of daily dietary folate equivalents between 1-2 years of age

ALL				CBT			
	Cases ¹ n= 305	Controls ¹ n= 645			Cases ¹ n= 271	Controls ¹ n= 671	
Daily dietary folate equivalents(μg)	n (%)	n (%)	OR ^{2,3} (95% CI)	Daily dietary folate equivalents(μg)	n (%)	n (%)	OR ^{3,4} (95% CI)
0 to 97	56 (18.4)	116 (18.0)	Reference	0 to 106.5	57 (21.0)	135 (20.1)	Reference
>97-138	66 (21.6)	135 (20.9)	1.00 (0.64, 1.56)	>106.5 to 153.0	56 (20.7)	136 (20.3)	1.03 (0.65, 1.64)
>138-181	66 (21.6)	130 (20.2)	1.04 (0.66, 1.62)	>153.0 to 193.9	52 (19.2)	130 (19.4)	1.05 (0.65, 1.68)
>181-228	55 (18.0)	133 (20.6)	0.82 (0.51, 1.30)	>193.9 to 237.5	61 (22.5)	135 (20.1)	1.31 (0.82, 2.07)
>228	62 (20.3)	131 (20.3)	0.95 (0.60, 1.50)	>237.5	45 (16.6)	135 (20.1)	1.02 (0.62, 1.67)

¹Only subjects with full dietary information aged over 1 year of age are included in these analyses

²Adjusted for matching variables (child's age in months at diagnosis for cases and date of questionnaire return for controls, sex and State), maternal education, maternal age at birth, birth order, proportion of optimum birth weight and ever breast fed.

³ The reference group are those in the lowest intake quintile.

⁴Adjusted for matching variables (child's age in months at diagnosis for cases and date of questionnaire return for controls, sex and State), parental education, child's year of birth, ethnicity, maternal folic acid supplementation 1 month prior to pregnancy, maternal alcohol consumption during pregnancy, maternal dietary folate intake during pregnancy.

Supplemental Table 4: Odds ratios and 95% confidence intervals for the risk of ALL associated with having vitamins and other supplements until the age of two years

	ALL			CBT		
	Cases n= 316 n (%)	Controls n= 663 n (%)	OR ^{1,2} (95% CI)	Cases n= 292 n (%)	Controls n= 728 n (%)	OR ^{2,3} (95% CI)
Any supplements						
No	288 (91.1)	589 (88.8)	Reference	254 (87.0)	627 (86.1)	Reference
Yes	28 (8.9)	74 (11.2)	0.81 (0.51, 1.29)	38 (13.0)	101 (13.9)	0.99 (0.65, 1.50)
Multivitamin ⁴	24 (7.7)	46 (7.2)	1.10 (0.65, 1.85)	24 (8.6)	59 (8.6)	1.06 (0.63, 1.79)
Folate	7 (2.4)	13 (2.2)	1.04 (0.40, 2.70)	16 (5.9)	26 (4.0)	1.43 (0.73, 2.80)
B12	6 (2.0)	17 (2.8)	0.69 (0.26, 1.80)	17 (6.3)	27 (4.1)	1.52 (0.79, 2.93)
Vitamin C	18 (5.9)	30 (4.8)	1.29 (0.70, 2.38)	11 (4.2)	43 (6.4)	0.68 (0.34, 1.36)
Vitamin A	16 (5.3)	21 (3.4)	1.64 (0.84, 3.24)	8 (3.1)	30 (4.6)	0.73 (0.32, 1.66)
Iron	18 (5.9)	32 (5.2)	1.14 (0.62, 2.09)	27 (9.6)	56 (8.2)	1.25 (0.75, 2.08)

¹Adjusted for matching variables (child's age in months at diagnosis for cases and date of questionnaire return for controls, sex and State), maternal education, maternal age at birth, birth order, proportion of optimum birth weight and ever breast fed.

²The reference group are those who did not have any vitamins, minerals or other dietary supplements before the age of two years.

³Adjusted for matching variables, year of birth, parental education, child's ethnicity, maternal alcohol consumption during pregnancy, maternal use of folic acid supplements 1 month before pregnancy.

⁴For ALL, all these products and only these contained B6. For CBT, only one child had B6 that was not part of a multivitamin.