Research Article

Cancer Epidemiology, Biomarkers & Prevention

Folate Pathway Gene Polymorphisms, Maternal Folic Acid Use, and Risk of Childhood Acute Lymphoblastic Leukemia

Elizabeth Milne[1], Kathryn R. Greenop[1], Rodney J. Scott[2,3,4], Michelle Haber[5], Murray D. Norris[5], John Attia[2,6], Sarra E. Jamieson[1], Margaret Miller[7], Carol Bower[1], Helen D. Bailey[1,8], Somer Dawson[1], Geoffrey B. McCowage[9], Nicholas H. de Klerk[1], Frank M. van Bockxmeer[10], and Bruce K. Armstrong[11,12]

Abstract

Background: Several studies suggest that maternal folic acid supplementation before or during pregnancy protects against childhood acute lymphoblastic leukemia (ALL). We investigated associations between ALL risk and folate pathway gene polymorphisms, and their modification by maternal folic acid supplements, in a population-based case–control study (2003–2007).

Methods: All Australian pediatric oncology centers provided cases; controls were recruited by national random digit dialing. Data from 392 cases and 535 controls were included. Seven folate pathway gene polymorphisms (*MTHFR* 677C>T, *MTHFR* 1298A>C, *MTRR* 66A>G, *MTR* 2756 A>G, *MTR* 5049 C>A, *CBS* 844 Ins68, and *CBS* 2199 T>C) were genotyped in children and their parents. Information on prepregnancy maternal folic acid supplement use was collected. ORs were estimated with unconditional logistic regression adjusted for frequency-matched variables and potential confounders. Case–parent trios were also analyzed.

Results: There was some evidence of a reduced risk of ALL among children who had, or whose father had, the *MTRR* 66GG genotype: ORs 0.60 [95% confidence interval (CI) 0.39–0.91] and 0.64 (95% CI, 0.40–1.03), respectively. The ORs for paternal *MTHFR* 677CT and TT genotypes were 1.41 (95% CI, 1.02–1.93) and 1.81 (95% CI, 1.06–3.07). ORs varied little by maternal folic acid supplementation.

Conclusions: Some folate pathway gene polymorphisms in the child or a parent may influence ALL risk. While biologically plausible, underlying mechanisms for these associations need further elucidation.

Impact: Folate pathway polymorphisms may be related to risk of childhood ALL, but larger studies are needed for conclusive results. *Cancer Epidemiol Biomarkers Prev; 24(1); 48–56. ©2014 AACR.*

[1]Telethon Kids Institute, University of Western Australia, Perth, Western Australia, Australia. [2]Hunter Medical Research Institute, John Hunter Hospital, New Lambton, New South Wales, Australia. [3]School of Biomedical Sciences and Pharmacy, Faculty of Health, University of Newcastle, Newcastle, New South Wales, Australia. [4]Hunter Area Pathology Service, HNEHealth, Newcastle, New South Wales, Australia. [5]Children's Cancer Institute Australia for Medical Research, Lowy Cancer Research Centre, University of New South Wales, Sydney, New South Wales, Australia. [6]School of Medicine and Public Health, Faculty of Health and Medicine, University of Newcastle, Newcastle, New South Wales, Australia. [7]School of Exercise and Health Sciences, Edith Cowan University, Mount Lawley, Western Australia, Australia. [8]Section of Environment and Radiation, International Agency for Research on Cancer, Lyon, France. [9]Oncology Unit, The Children's Hospital at Westmead, Sydney, Australia. [10]Department of Clinical Biochemistry, Royal Perth Hospital and the School of Surgery, University of Western Australia, Perth, Western Australia, Australia. [11]Sax Institute, Haymarket, New South Wales, Australia. [12]Sydney School of Public Health, University of Sydney, Sydney, New South Wales, Australia.

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

Corresponding Author: Elizabeth Milne, Telethon Kids Institute, PO Box 855, West Perth, Western Australia 6872, Australia. Phone: 618-9489-7756; Fax: 618-9489-7700; E-mail: Liz.Milne@telethonkids.org.au

doi: 10.1158/1055-9965.EPI-14-0680

Introduction

Acute lymphoblastic leukemia (ALL), the most common childhood malignancy, has a significant genetic basis for susceptibility (1–4). As well as contributing to the risk of childhood ALL, inherited genetic variation appears to predispose to specific ALL subtypes and response to chemotherapeutic intervention (2). As most cases of childhood ALL are thought to be initiated *in utero* (5), etiologic research has focused on dissecting the complex interaction between genetic susceptibility and fetal environmental exposures. Following the publication of findings from an Australian study suggesting that maternal folic acid supplementation during pregnancy protects against childhood common ALL (6), there has been growing interest in the possible role of folic acid and folate pathway genes. If folate is associated with risk of ALL, then it is biologically plausible that polymorphisms in these genes would also be associated with its risk.

Polymorphisms of the methylene tetrahydrofolate reductase (*MTHFR*) gene at 677C>T or 1298A>C are known to reduce enzyme activity, which is essential for the bioavailability and metabolism of folate (7, 8). Since 2001, more than 20 studies have reported on the association between the child's *MTHFR* genotype and risk of childhood ALL, and seven meta-analyses have been published (9–15). The results of these studies vary considerably,

but the most recent studies report either no association with either polymorphism (13) or a protective effect of *MTHFR* 677C>T alone (14).

Other folate gene polymorphisms that have been studied in relation to childhood ALL are 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*; refs. 16–23), 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*; refs. 17, 22, 24–28), methylenetetrahydrofolate dehydrogenase (*MTHFD1*; refs. 22, 25, 27, 29), thymidylate synthetase (*TS*/*TYMS*; refs. 17, 20, 22, 25, 29–32), and reduced folate carrier (*RFC1*; refs. 26, 27, 30, 32). A recent meta-analyses including *MTRR* and *MTR* polymorphisms indicated a possible protective effect of *MTRR* 66GG genotype (13).

The inconsistency in results of individual studies is attributed, at least partly, to limited sample sizes and ethnicity-related differences in allele frequencies in the populations. The impact of folate gene polymorphisms may also vary depending on folate status (33, 34). A French Canadian study found some combinations of *MTHFR* 677C>T and 1298A>C polymorphisms protected against childhood ALL only among children born before folic acid supplementation recommendations commenced (1998; ref. 35). Lupo and colleagues (21) also reported a similar difference before or after U.S. folate fortification in 1996 for maternal *MTR* genotype on ALL risk. Thus, differing folate status in populations may explain some of the discrepant results; it has been recommended that studies of folate polymorphisms and ALL risk should take account of folic acid intake (9, 14, 36).

Two studies have examined variation in folate pathway polymorphisms by maternal folic acid use. A Californian study reported interactions between maternal folic acid use and filial genotype at specific loci on cystathionine-beta-synthase (*CBS*), *MTRR*, and *TYMS* genes (25), while a French study found no evidence of such interactions with polymorphisms in the *MTHFR* or *MTRR* genes (24).

While most previously published studies of folate pathway gene polymorphisms have reported results for the child's genotype only, it is possible that the parents' genotype may also be important in determining fetal folate status. For example, several studies suggest that the risk of folate-related cardiac birth defects is affected by maternal genotype in folate pathway genes (37–40).

In the Australian Study of the Causes of ALL (Aus-ALL), which was specifically powered to investigate the association between maternal folic acid supplementation and risk of ALL, we did not replicate the previous strong association between folic acid use during pregnancy and ALL risk (6). However, weak associations were seen with use prepregnancy; ORs were 0.84 [95% confidence interval (CI), 0.55–1.28] for 300.1 to 450 μg per day, and 0.77 (95% CI, 0.52–1.14) for >450 μg per day (41).

Our primary aim was to investigate associations between filial and parental folate pathway gene polymorphisms and risk of ALL, including by ALL subtype where possible. Our second aim was to investigate whether maternal use of folic acid prepregnancy modified any observed associations of folate pathway polymorphisms with ALL.

Materials and Methods

Aus-ALL was a national, population-based, multicenter case–control study conducted in Australia between 2003 and 2007. The study design and data collection methods have been described elsewhere (41, 42). Briefly, incident ALL cases were identified through all 10 Australian pediatric oncology centers, where the large majority of cases are treated. Inclusion criteria were: initial remission achieved, biological mother available and able to complete questionnaires in English, and Australian residence. Controls were prospectively recruited in the same time period by national random digit dialing (RDD), and frequency matched to cases by age (within 1 year), sex, and state of residence. For the genetic component of Aus-ALL, controls were frequency matched to cases on age, sex, and state of residence in a ratio of approximately 1.5:1. The study was approved by the Human Research Ethics Committees at all participating hospitals; parents of participating children provided written informed consent.

Parents were mailed questionnaires about key environmental and demographic variables. Mothers completed a questionnaire detailing folic acid supplement use with dose in the 3 months before and during pregnancy. Blood samples for DNA analysis were collected from case children and their parents at the treating hospital, while buccal cell samples were collected from control children and their parents at home using FTA cards (43). Case children also provided a buccal sample using this method. Punches taken from the cards were prepared for PCR as previously described (43), and samples were then subjected to whole genome amplification using the GenomiPhi V2 DNA Amplification Kit (GE Healthcare) according to the manufacturer's instructions.

Case and control children and their parents were genotyped for seven polymorphisms in folate pathway genes: *MTHFR* 677C>T (rs1801133), *MTHFR* 1298A>C (rs1801131), *MTRR* 66A>G (rs1801394), *CBS* 844 Ins68, *CBS* 2199T>C (rs706208), *MTR* 2756A>G (rs1805087), *MTR* 5049C>A (rs2853523).

The *MTHFR* 677C>T and *MTHFR* 1298A>C polymorphisms were analyzed as previously described (44). For *MTRR* 66A>G, *CBS* 2199T>C and *CBS* 844 Ins68 polymorphisms, genotyping was performed on an ABI Prism 7000 Sequence Detection System using TaqMan Universal PCR Master Mix (Applied Biosystems). The *MTR* 2756A>G and *MTR* 5049C>A polymorphisms were analyzed using a modification of the method of Morrison and colleagues (45).

For quality assurance purposes, 10% of samples were selected at random for repeat analysis within laboratories, and this was performed blinded in each laboratory. Between-laboratory concordance was also assessed at *MTHFR* 677C>T. Concordance between case children's genotypes derived using blood and WGA'd buccal samples was also examined.

Statistical analysis

Mendelian inconsistencies were identified in family data using the PedCheck software (46); 119 results from 28 case families and 76 control families were excluded. Hardy–Weinberg Equilibrium (HWE) analysis was performed in STATA v10 using unaffected, unrelated individuals. No polymorphisms were found to significantly deviate from HWE (data not shown).

The results of the quality assurance analyses were excellent with genotyping call rate 99%, within-laboratory concordance 99.7%, and between-laboratory concordance 98%. However, quality assurance analysis of genotypes derived from WGA'd buccal DNA samples showed discrepancies of 2.3% to 7.4% with those derived from lymphocyte DNA. Therefore, we applied a statistical adjustment method (using discordance estimates empirically derived from quality-control data in cases) in all our analyses. This method and the rationale for it have been described in detail elsewhere (47). Briefly, this method applies misclassification

probabilities to measured data, and then combines the results using 50 multiple imputations to minimize bias in the ORs.

We had previously seen weak evidence of an inverse association with ALL risk when mothers took folic acid ≥300 μg/day in the 3 months prepregnancy (41). We therefore categorized folic acid use for analysis as ≥300 μg/day versus none prepregnancy. As red cell folate levels are maintained for 3 to 4 months (48), this dose was also relevant to early pregnancy (90% of women taking folic acid prepregnancy also reported use in the first trimester). ORs and 95% CIs for main effects of each polymorphism were estimated using unconditional logistic regression analysis in R version 2.14.2 (2012, The R Foundation for Statistical Computing; www.r-project.org/). All case–control analyses were adjusted for the frequency matching factors—age, sex and state of residence. To ensure models were comparable, and because folic acid use may be confounded by some variables, all variables meeting the empirical criteria for confounding (independently associated with both the outcome and folic acid supplementation in the controls) were included in genotype models. They were birth order, ethnicity, maternal age, and parental education. Additional analyses were conducted for particular immunophenotypes and cytological subtypes of ALL (each of these models included all controls). To see whether there were different associations of genotypes with ALL in mothers who did and did not take folic acid, the main effects analyses of genotypes were repeated separately for these two groups.

Statistical interactions were assessed on the basis of the effect size and significance of the interaction term in the model (Wald test), and the resulting change in main effect estimates due to the presence of the interaction term. The interaction *P* values from these models are shown in the text, where appropriate, and results from corresponding stratified analyses are shown in the tables.

Case–parent trio analyses were undertaken using conditional logistic regression in StataIC version 11 (StataCorp) and genotype-based ORs were estimated. This method uses the genotypes of the parents to create pseudosiblings of case children using all possible genotypes the offspring could have had as controls that are matched on all environmental variables (i.e., no confounding variables need to be included as pseudosiblings have the same parents as cases; ref. 49). Data were prepared using "pseudocc" and "gtab" commands developed by David Clayton (50).

The results for all analyses were similar when children with birth defects (22 cases and 17 controls) were excluded, so these children were included in the final models.

Results

We were notified of 568 incident cases of ALL; 49 were ineligible to participate (three with no biological mother available 30 had mothers with insufficient English, 12 were nonresident, and four did not reach remission). Of the 519 eligible cases, parents of 416 (80.2%) consented to participate in the study, and genotyping results were obtained from 392 case children, 391 case mothers, and 344 case fathers. Child–parent genotyping trios were available for 276 case families.

Of the 2,947 eligible control families identified through RDD, 2,071 (70.3%) agreed to take part in the main study (41). In accordance with requirements for age and sex frequency matching, 1,067 control families were asked to provide DNA for the genetic component of the study; of these, 1,027 (96%) agreed. In all, genotyping results were available from 535 (50%) control children, 501 (47%) control mothers, and 447 (42%) control fathers. Supplementary Figure S1 shows the flow chart for DNA and data collection.

The distributions of demographic variables among cases and controls included in the analysis were generally similar, with some exceptions (Table 1). Case children were more likely to be first

Table 1. Demographic characteristics of cases and controls in the Australian Study of causes of acute lymphoblastic leukemia in children, 2003–2007

	Cases	Controls
***N* with child genotype**	**392**	**535**
Variable	***n* (%)**	***n* (%)**
Sex		
Male	176 (44.9)	252 (47.1)
Female	216 (55.1)	283 (52.9)
Age group		
0–1.9	35 (8.9)	43 (8.0)
2–4.9	178 (45.4)	239 (44.7)
5–9.9	111 (28.3)	182 (34.0)
10–14.9	68 (17.3)	71 (13.3)
State of residence		
NSW/ACT	123 (31.4)	164 (30.7)
Victoria/Tasmania	117 (29.8)	156 (29.2)
SA/NT	43 (11.0)	48 (9.0)
WA	40 (10.2)	61 (11.4)
Queensland	69 (17.6)	106 (19.8)
Birth order		
1	186 (47.4)	220 (41.1)
2	120 (30.6)	181 (33.8)
3+	86 (21.9)	134 (25.0)
Child's birth year		
1988–1993	54 (13.8)	62 (11.6)
1994–1999	127 (32.4)	207 (38.7)
2000–2006	211 (53.8)	266 (49.7)
Higher level of education of either parent		
Nontertiary	223 (56.9)	253 (47.3)
Tertiary	169 (43.1)	282 (52.7)
Ethnicity[a]		
European	282 (71.9)	427 (79.8)
At least 50% European	79 (20.2)	79 (14.8)
At least 50% non-European and not known if 50% European	14 (3.6)	11 (2.1)
Indeterminate	17 (4.3)	18 (3.4)
Household income		
<$20,000	28 (7.2)	21 (3.9)
$20,001–40,000	67 (17.2)	73 (13.9)
$40,001–70,000	115 (29.6)	185 (34.6)
$70,001–100,000	93 (23.9)	137 (25.7)
>$100,000 pa	86 (22.1)	117 (22.9)
Mother's age at child's birth		
<25 years	59 (15.1)	44 (8.2)
25–34 years	266 (67.9)	360 (67.3)
35+ years	67 (17.1)	131 (24.5)
Child had a birth defect		
Yes	22 (5.7)	17 (3.2)
Folic acid[b]		
None	261 (67.4)	326 (61.6)
>0–300 μg	44 (11.4)	50 (9.5)
300.1–450 μg	37 (9.6)	61 (11.5)
450+ μg	45 (11.6)	92 (17.4)

Abbreviations: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; SA, South Australia; 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 other 2 grandparents unknown; indeterminate = no 2 grandparents with the same ethnicity (i.e., European or non-European) and 2+ grandparents of unknown ethnicity.

[b]Maternal folic acid supplementation in the 3 months prior to the pregnancy.

born than controls and to have a birth defect. Control parents were more likely than case parents to be tertiary educated, have a higher income and to have been 35 years of age or older when the index child was born.

Overall, few associations were seen between folate pathway polymorphisms and risk of ALL. There was some evidence of reduced risk associated with the *MTRR* 66GG genotype in the child and the father, but not the mother (Table 2). ORs for *MTHFR* 677CT and TT genotypes were elevated for paternal genotype only. The case–parent trio analyses were mostly consistent with those of the case–control analysis of the child's genotype with two exceptions: *MTHFR* 1298CC and *MTR* 2756 GG (Table 2). The results of the case–control analysis were similar when restricted to the 276 children belonging to case–parent trios (data not shown).

Associations with the child's genotype were also examined for the main immunophenotypes and cytogenetic subtypes (Table 3). The inverse association with the *MTRR* 66GG genotype was observed for most ALL subtypes, whereas the ORs for *CBS* 844 Ins68 were elevated among cases with hyperdiploidy or Trisomy 21, and reduced among cases with ETV6-Runx-1 t(12, 21). *MTHFR* 677TT appeared to be positively associated with risk of T-cell ALL. The results for maternal and paternal genotype across subtypes seemed largely consistent with the overall findings (Supplementary Tables S1 and S2, respectively).

Results of gene polymorphisms stratified by folic acid supplementation are shown in Table 4 (for *MTHFR* and *MTRR*) and Supplementary Table S3 (for *MTR* and *CBS*). Evidence for gene–folic acid interactions was weak with all but one interaction *P* value being >0.05. ORs for *MTRR* 66GG genotype in the child were reduced irrespective of maternal folic acid use (Table 3; interaction *P* value = 0.35), whereas the OR for maternal *MTRR* 66GG genotype appeared reduced only among mothers who took ≥300 μg folic acid/day; however, the interaction *P* value was 0.17. There was some evidence that filial or maternal carriage of at least one T allele at *MTHFR* 677C>T was associated with an increased risk of ALL if the mother took ≥300 μg folic acid/day, but not otherwise (interaction *P* values 0.04 for the child's genotype and 0.09 for maternal genotype). Child's age, sex, and birth order were similar between those children who provided DNA and those who did not. However, in those who had provided DNA, mothers were older (92% vs. 82% were 25+), and parental education and income levels were higher (53% vs. 37% had tertiary education; 18% vs. 35% had annual income <$40,000), and more mothers used folic acid prepregnancy (36% vs. 23%; all $P < 0.001$). The above figures were similar where the control father had provided DNA. The median folic acid dose for the analyzed controls was 450 μg/day (interquartile range, 200) which was the same as the entire group previously reported (41).

Discussion

Our study of folate pathway gene polymorphisms and their association with ALL risk was the first to report results for polymorphisms in the child, mother, and father; this is important to have a comprehensive view of the potential role of genetic susceptibility in disease risk, and its source(s). Both case–control and case–parent trio analyses of associations with the child's genotype were undertaken, yielding largely consistent results. We also investigated possible interactions between child and maternal genotype and maternal use of folic acid in the preconception period.

Overall, we found little evidence of associations between folate pathway genotypes and risk of childhood ALL, or that maternal folic acid use in the period leading up to the pregnancy modified associations between genotype and ALL risk. However, there was some evidence of a reduced risk among children who had, or whose father had, the *MTRR* 66AG or GG genotype. In addition, paternal *MTHFR* 677TT genotype appeared to be associated with an increased risk, while there was also weak evidence of possible interactions between maternal prepregnancy use of folic acid and *MTHFR* 677CT/TT genotypes in the child and/or mother. Finally, the results of our subtype analyses suggested that some folate genotypes in the child may be associated with risk of specific disease subtypes.

Consistent with our findings for the child's *MTRR* 66A>G genotype, four of seven previous studies reported at least some evidence of a protective effect of the mutant allele (24, 26–28). In addition, a meta-analysis of three studies assessing this polymorphism (26–28) reported summary ORs for AG and GG of 0.76 (95% CI, 0.60–0.96) and 0.67 (95% CI, 0.52–0.88), respectively (13). For the *MTHFR* genotypes, however, a number of previous studies suggested that 677C>T may be protective, but that 1298A>C was not associated with risk (14, 15). We did not find evidence of a reduced risk with either polymorphism overall, similar to two recent studies (22, 24).

Seven out of eight previous studies investigating the child's *MTR* 2756A>G genotype and risk of childhood ALL reported null or inconclusive findings (16, 18, 20, 25–28), similar to our study. Only the UK Childhood Cancer Study reported elevated ORs: 1.24 (95% CI, 1.00–1.53) and 1.88 (95% CI, 1.16–3.07) for the AG and GG genotypes, respectively (19). Vijayakrishnan and Houlston (13) reported a null result in their meta-analysis of four studies (18, 26–28). The weight of the evidence would therefore suggest no association of the *MTR* 2756A>G genotype and risk of childhood ALL. No previous studies of childhood ALL and *MTR* 5049C>A, CBS 844 ins68, or *CBS* 2199T>C genotypes were found.

Three previous studies have investigated interactions between selected folate pathway polymorphisms and maternal folic acid supplementation. In our case-only analysis of 83 ALL cases from Western Australia (diagnosed between 1984 and 1992), we found no evidence of interactions with the child's *MTHFR* 677C>T or 1298A>C genotype (51). Metayer and colleagues (25) recently reported evidence of interactions between maternal folic acid use and specific loci on the *CBS*, *MTRR*, and *TYMS* genes; however, these loci were not investigated in our study. A recent French study (24) reported no interactions, although results were not presented in the article.

Only three previous studies have investigated maternal folate pathway genotype in relation to ALL risk; two found that the child's genotype was more closely associated with risk than the mother's (19, 35), while Lupo and colleagues found more associations with the mother's than the child's genotype (17). In our study, the findings for the child's genotype were generally consistent with those for maternal genotype. We were unable to identify any previous studies of paternal genotype with which to compare our findings.

Two previous studies have reported results for the child's folate pathway genotype by immunophenotype. The UKCCS reported null associations with *MTHFR* 677C>T and 1298A>C for both B- and T-cell ALL (19), while Tong and colleagues reported evidence

Table 2. Main effects of child's, mother's, and father's folate pathway genotypes on risk of childhood ALL

		Child genotype			Mother genotype		Father genotype	
		Case–control analysis ($n = 392/535$)		Case–parent trios ($n = 276$)	Case–control analysis ($n = 391/501$)		Case–control analysis ($n = 344/444$)	
***N* cases/controls** **Polymorphism**	**Genotype**	***N* cases/controls[a]**	**OR[b] (95% CI)**	**OR (95% CI)**	***N* cases/controls[a]**	**OR[b] (95% CI)**	***N* cases/controls[a]**	**OR[c] (95% CI)**
MTHFR 677 C>T	CC	166/244	1.00 (reference)	1.00 (reference)	176/212	1.00 (reference)	131/204	1.00 (reference)
(rs1801133)	CT	181/221	1.22 (0.92–1.63)	1.10 (0.80–1.51)	170/228	0.90 (0.67–1.20)	170/188	1.41 (1.02–1.93)
	TT	43/60	1.23 (0.77–1.97)	0.92 (0.54–1.56)	43/52	1.15 (0.71–1.86)	41/43	1.81 (1.06–3.07)
	CT/TT	224/281	1.23 (0.94–1.62)	1.08 (0.79–1.48)	213/280	0.94 (0.71–1.24)	211/231	1.48 (1.09–2.00)
MTHFR 1298 A>C	AA	195/264	1.00 (reference)	1.00 (reference)	177/228	1.00 (reference)	159/213	1.00 (reference)
(rs1801131)	AC	169/192	1.04 (0.78–1.38)	0.77 (0.57–1.05)	178/203	0.98 (0.73–1.31)	156/164	1.09 (0.80–1.49)
	CC	27/52	0.98 (0.54–1.79)	0.49 (0.28–0.87)	35/47	1.38 (0.79–2.42)	25/44	1.06 (0.56–2.01)
	AC/CC	196/244	1.02 (0.78–1.35)	0.74 (0.55–1.01)	213/250	1.03 (0.77–1.37)	181/208	1.08 (0.80–1.47)
MTRR 66A>G	AA	88/101	1.00 (reference)	1.00 (reference)	81/97	1.00 (reference)	82/75	1.00 (reference)
(rs1801394)	AG	208/233	0.84 (0.58–1.22)	0.95 (0.66–1.38)	195/229	0.82 (0.56–1.21)	160/204	0.62 (0.41–0.93)
	GG	88/174	0.60 (0.39–0.91)	0.71 (0.44–1.16)	105/143	0.94 (0.61–1.44)	88/129	0.64 (0.40–1.03)
	AG/GG	296/407	0.75 (0.53–1.06)	0.92 (0.63–1.32)	300/372	0.87 (0.60–1.24)	248/333	0.62 (0.42–0.93)
MTR 2756 A>G	AA	251/337	1.00 (reference)	1.00 (reference)	253/302	1.00 (reference)	219/274	1.00 (reference)
(rs1805087)	AG	130/158	1.00 (0.75–1.34)	1.12 (0.79–1.58)	118/142	0.86 (0.64–1.17)	106/119	0.93 (0.67–1.29)
	GG	10/19	1.70 (0.52–5.58)	0.47 (0.19–1.19)	19/29	1.82 (0.73–4.56)	12/19	1.93 (0.58–6.35)
	AG/GG	140/177	1.03 (0.78–1.37)	1.07 (0.76–1.52)	137/171	0.92 (0.69–1.23)	118/138	0.98 (0.71–1.34)
MTR 5049 C>A	CC	155/199	1.00 (reference)	1.00 (reference)	161/181	1.00 (reference)	131/160	1.00 (reference)
(rs285523)	CA	181/229	0.98 (0.72–1.32)	1.12 (0.80–1.57)	172/212	0.92 (0.68–1.25)	159/182	1.06 (0.76–1.49)
	AA	55/83	1.04 (0.67–1.61)	1.27 (0.76–2.13)	56/71	1.09 (0.69–1.70)	49/64	1.14 (0.70–1.86)
	CA/AA	236/312	1.01 (0.76–1.33)	1.13 (0.82–1.58)	228/283	0.96 (0.72–1.28)	208/246	1.08 (0.79–1.49)
CBS 2199 T>C	TT	118/147	1.00 (reference)	1.00 (reference)	110/143	1.00 (reference)	110/123	1.00 (reference)
(rs706208)	TC	179/253	0.82 (0.59–1.13)	0.92 (0.65–1.29)	188/238	0.95 (0.68–1.32)	166/200	0.89 (0.62–1.27)
	CC	88/105	1.23 (0.82–1.84)	1.34 (0.84–2.15)	85/85	1.47 (0.96–2.26)	53/81	0.81 (0.50–1.31)
	TC/CC	267/358	0.94 (0.69–1.28)	0.96 (0.69–1.35)	273/323	1.08 (0.79–1.47)	219/281	0.88 (0.63–1.24)
CBS 844 Ins 68[d]	WTWT	450/329	1.00 (reference)	1.00 (reference)	336/409	1.00 (reference)	288/360	1.00 (reference)
	WTIns/InsIns	55/69	0.79 (0.53–1.17)	1.03 (0.62–1.72)	49/71	0.67 (0.44–1.01)	48/69	0.67 (0.44–1.01)

[a]Numbers are from raw data before correction imputation method applied, individual polymorphism Ns may vary from total due to typing fails.
[b]ORs adjusted for matching variables (child's age, sex, and state of residence), mother's age group, parental education, birth order, and ethnicity.
[c]ORs adjusted for matching variables (child's age, sex, and state of residence), father's age group, parental education, birth order, and ethnicity.
[d]*N* for homozygous mutants insufficient to produce separate OR.

Table 3. Child's folate pathway genotypes and risk of ALL by immunophenotype and cytogenetic subtype

Polymorphism	Genotype	PreB cell ($n = 346$) OR[a] (95% CI)	T cell ($n = 37$) OR[a] (95% CI)	ETV6-Runx-1 [$t(12, 21)$; ($n = 61$)] OR[a] (95% CI)	Hyperdiploidy ($n = 115$) OR[a] (95% CI)	Trisomy or higher 21 ($n = 67$) OR[a] (95% CI)
MTHFR 677 C>T	CC	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
	CT	1.21 (0.90–1.63)	1.39 (0.63–3.06)	0.96 (0.53–1.72)	1.48 (0.95–2.30)	2.19 (1.22–3.93)
	TT	1.11 (0.68–1.83)	2.57 (0.92–7.19)	0.42 (0.12–1.52)	0.86 (0.37–1.98)	0.73 (0.20–2.65)
	CT/TT	1.19 (0.90–1.58)	1.63 (0.78–3.39)	0.85 (0.48–1.50)	1.37 (0.89–2.11)	1.95 (1.10–3.47)
MTHFR 1298 A>C	AA	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
	AC	1.00 (0.74–1.35)	1.58 (0.74–3.36)	1.41 (0.78–2.56)	0.81 (0.52–1.28)	1.05 (0.58–1.87)
	CC	0.87 (0.46–1.63)	1.20 (0.29–4.91)	1.00 (0.29–3.39)	1.18 (0.55–2.56)	1.27 (0.47–3.45)
	AC/CC	0.98 (0.74–1.31)	1.51 (0.73–3.12)	1.35 (0.76–2.40)	0.86 (0.56–1.32)	1.07 (0.62–1.85)
MTRR 66A>G	AA	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
	AG	0.83 (0.57–1.21)	0.87 (0.33–2.30)	1.25 (0.57–2.74)	0.77 (0.45–1.34)	0.84 (0.41–1.73)
	GG	0.58 (0.37–0.89)	0.99 (0.33–2.95)	0.61 (0.24–1.55)	0.46 (0.24–0.90)	0.63 (0.27–1.44)
	AG/GG	0.73 (0.51–1.05)	0.88 (0.35–2.26)	0.99 (0.46–2.11)	0.65 (0.38–1.10)	0.79 (0.40–1.58)
MTR 2756 A>G[b]	AA	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
	AG/GG	1.08 (0.80–1.44)	0.63 (0.28–1.39)	1.20 (0.66–2.17)	0.95 (0.61–1.49)	1.25 (0.71–2.19)
MTR 5049 C>A	CC	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
	CA	0.99 (0.72–1.35)	1.21 (0.57–2.62)	1.24 (0.68–2.27)	1.04 (0.65–1.67)	1.09 (0.59–1.99)
	AA	1.05 (0.67–1.65)	0.71 (0.19–2.66)	0.51 (0.16–1.61)	1.34 (0.70–2.55)	1.25 (0.53–2.94)
	CA/AA	1.01 (0.75–1.35)	1.12 (0.54–2.35)	1.07 (0.60–1.92)	1.11 (0.71–1.73)	1.14 (0.64–2.03)
CBS 2199 T>C	TT	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
	TC	0.80 (0.57–1.12)	1.23 (0.50–3.03)	0.93 (0.47–1.84)	0.81 (0.50–1.32)	0.58 (0.32–1.05)
	CC	1.25 (0.82–1.89)	1.37 (0.46–4.10)	1.07 (0.45–2.54)	0.92 (0.50–1.72)	0.32 (0.12–0.85)
	TC/CC	0.92 (0.67–1.26)	1.30 (0.55–3.07)	0.98 (0.52–1.87)	0.87 (0.55–1.37)	0.53 (0.30–0.92)
CBS 844 Ins 68[b]	WTWT	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
	WTIns/InsIns	0.80 (0.53–1.19)	0.99 (0.37–2.67)	0.26 (0.08–0.89)	1.63 (0.97–2.73)	1.61 (0.83–3.13)

[a]All ORs adjusted for matching variables (child's age, sex, state of residence), maternal age, child ethnicity, birth order, parental education, and corrected for genotype method discordance.
[b]N for homozygous mutants insufficient to produce separate OR.

of a protective association between *MTHFR* 677TT genotype and both ALL subtypes (52). We found no evidence of associations between either SNP and B-cell ALL, and only weak evidence that the *MTHFR* 677TT genotype was associated with an elevated risk of T-cell ALL. The UKCCS reported ORs of 1.82 (95% CI, 1.09–3.03) and 1.63 (95% CI, 0.55–4.88) for B- and T-cell ALL (respectively) associated with *MTR* 2756GG genotype (19), while we found no association with either type of ALL. The UK study also found that *MTHFR* 677TT was associated with an increased risk, and the 1298CC genotype a decreased risk, of the ETV-Runx-1 cytogenetic subtype (19), while we observed no evidence of either association with this ALL subtype.

As previously suggested, the inconsistency among published results for folate pathway polymorphisms is probably due to the small sample sizes in most studies and the fact that both genotype frequencies and folate status vary among study populations.

A biologic role for polymorphisms in folate metabolizing genes in the etiology of ALL is certainly plausible, despite some of the inconsistent findings reported in this area. The folate pathway is critical to cancer development, being involved in DNA synthesis, repair, and methylation (53), and low folate status and/or aberrant folate metabolism are known to potentiate disease development (54). Polymorphisms in folate pathway genes cause altered enzyme kinetics and thereby alter folate availability; for example, polymorphisms in genes coding for folate metabolic enzymes have been shown to affect the synthesis of S-Adenosyl methionine and result in hyper- and hypomethylation states of DNA (55). Thus, folate pathway gene polymorphisms may contribute to disease susceptibility through epigenetic regulation of gene expression involving DNA hypomethylation (56).

Our finding of a potential increased risk of ALL associated with paternal *MTHFR* 677TT genotype is consistent with what is known about the impact of this polymorphism on MTHFR activity. Thus, relative to individuals with the wild-type *MTHFR* 677CC genotype, those with the TT genotype have lower levels of genomic DNA methylation (57). In mice, sperm hypomethylation has been shown to increase disease susceptibility in the offspring (58), and there is growing evidence from human studies that paternal folate status can affect metabolic gene expression in the offspring through epigenetic mechanisms involving chromatin packaging in sperm (59, 60). Such mechanisms may explain our findings for paternal *MTHFR* 677C>T, but it is not clear why similar overall associations were not seen with the child's or mother's genotype. We did observe elevated ORs for ALL with the *MTHFR* 677C>T genotype in the child and mother when the mother took $\geq$300 μg folic acid in the prepregnancy period. As folic acid supplementation would at least partly counter the tendency to hypomethylation associated with carriage of the mutant allele at *MTHFR* 677C>T, and therefore mitigate the potentially increased risk of ALL, the possible mechanisms underlying these findings are not clear and require further investigation.

The *MTRR* gene is involved in the folate metabolic cycle, and reactivates oxidized cobalamin–MTR complex (which itself catalyses the remethylation of homocysteine to methionine) by reductive methylation (54). It is not clear why the *MTRR* 66GG genotype should have a protective association with ALL; however, alterations in the activity of MTRR are likely to have a profound effect on epigenetic programming which may explain a protective effect due to a reduction in the ability to provide methyl groups required for hypermethylation (61).

Table 4. ORs for folate pathway genotypes stratified by maternal use of folic acid (≥300 μg) in the 3 months before pregnancy

N cases/controls			Child (354/495[a])		Mother (353/461[a])	
Polymorphism	**Folic acid**	**Genotype**	***n* case/control[a]**	**OR[b] (95% CI)**	***n* case/control[a]**	**OR[b] (95% CI)**
MTHFR 677 C>T	None	CC	117/144	1.0 (reference)	127/129	1.0 (reference)
		CT	115/134	1.03 (0.72–1.50)	104/137	0.74 (0.51–1.08)
		TT	27/41	0.94 (0.52–1.71)	28/33	1.02 (0.55–1.90)
		CT/TT	142/175	1.03 (0.72–1.45)	132/170	0.80 (0.56–1.13)
	≥300 μg	CC	32/80	1.0 (reference)	32/63	1.0 (reference)
		CT	50/67	1.92 (1.07–3.43)	48/75	1.26 (0.69–2.29)
		TT	11/19	1.63 (0.65–4.11)	12/15	2.01 (0.77–5.24)
		CT/TT	61/86	1.85 (1.06–3.23)	60/90	1.36 (0.77–2.41)
MTHFR 1298A>C	None	AA	130/164	1.0 (reference)	119/139	1.0 (reference)
		AC	111/116	1.03 (0.71–1.49)	114/131	0.86 (0.59–1.26)
		CC	19/36	0.90 (0.43–1.89)	27/26	1.69 (0.78–3.68)
		AC/CC	130/152	1.01 (0.71–1.43)	141/157	0.94 (0.65–1.36)
	≥300 μg	AA	47/83	1.0 (reference)	42/69	1.0 (reference)
		AC	43/61	1.09 (0.62–1.90)	44/58	1.10 (0.61–2.00)
		CC	3/13	0.64 (0.14–2.98)	6/17	0.89 (0.27–2.95)
		AC/CC	46/74	1.05 (0.61–1.82)	50/75	1.08 (0.61–1.91)
MTRR 66A>G	None	AA	57/63	1.0 (reference)	56/61	1.0 (reference)
		AG	135/146	0.89 (0.55–1.45)	126/148	0.73 (0.45–1.19)
		GG	63/104	0.74 (0.43–1.27)	71/81	1.05 (0.60–1.82)
		AG/GG	198/250	0.82 (0.51–1.31)	197/229	0.84 (0.53–1.33)
	≥300 μg	AA	24/32	1.0 (reference)	22/29	1.0 (reference)
		AG	51/69	0.65 (0.32–1.34)	50/65	0.74 (0.36–1.54)
		GG	17/60	0.34 (0.15–0.79)	19/51	0.47 (0.20–1.10)
		AG/GG	68/129	0.53 (0.27–1.06)	69/116	0.65 (0.33–1.30)

[a]All numbers are before genotype correction imputation (cases/controls where mother took >0 and <300 μg folic acid are excluded, Ns for individual polymorphisms may vary due to sample fails).
[b]ORs adjusted for matching variables, mother's age group, parental education, birth order, and ethnicity, and each main effect corrected for genotype error.

Aus-ALL has both strengths and limitations. Cases were ascertained from participating oncology centers where virtually all children with ALL in Australia are treated, and 75% of eligible cases participated in Aus-ALL. Only four cases did not reach remission and were therefore ineligible for inclusion. We have previously shown that the control families in our study had higher socioeconomic status than the general population, and that those who returned the questionnaires and provided DNA samples were higher still (42). In addition, DNA was provided from only 51% of families from whom it was requested, and these families tended to be of higher SES, have older mothers, and be more likely to use folic acid. Therefore, although we adjusted for factors that may be related to selection and/or to completion of questionnaires and provision of DNA, such as parental education and ethnicity, there may be residual confounding by these factors. While these differences should not influence the main analysis of folate pathway genes, or the gene by folic acid use interactions, it may increase the frequency of folic acid users in the control group. Because of the dependence on self-reported data, there is likely to have been misclassification of folic acid use. Misclassification, however, is likely to have been nondifferential because we used standardized questionnaires and little is known in the community about a possible relationship between folic acid and risk of ALL.

Some of the observed associations may be due to chance. We investigated seven folate gene polymorphisms in three individuals, examined interactions of genotypes with maternal use of folic acid and did subgroup analyses by ALL immunophenotype and cytogenetic subtypes; hence, a large number of ORs were estimated. Buccal samples were used for controls, and statistical correction of known blood–buccal discordance was required for these genotypes, which adds to the potential imprecision of results. Estimates also lacked precision because of the relatively small numbers in the subgroup analyses; these factors should be considered when interpreting our findings. Some associations, however, were consistently observed across family members and subgroup analyses, and are concordant with most previous studies; for example, those with *MTRR* 66A>G.

In conclusion, our findings suggest that some folate pathway polymorphisms in the child and parents are related to risk of childhood ALL, and that some of these associations vary by maternal folate status. The apparent lack of an overall protective effect of the *MTHFR* 667C>T polymorphism against ALL in our study, in contrast with most previous studies, may reflect adequacy of dietary folate and supplemental folic acid intake in Australia (62, 63). In light of growing evidence of a paternal contribution to disease susceptibility in the offspring, further studies of paternal genotype are needed. Pooling data from similar studies internationally would substantially strengthen our ability to investigate folate gene–environment interactions in the etiology of childhood ALL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: E. Milne, R.J. Scott, J. Attia, C. Bower, N.H. de Klerk, B.K. Armstrong

Development of methodology: E. Milne, K.R. Greenop, R.J. Scott, M. Haber, M.D. Norris, J. Attia, M. Miller, C. Bower, F.M. van Bockxmeer, B.K. Armstrong

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Milne, R.J. Scott, H.D. Bailey, S. Dawson, G.B. McCowage, F.M. van Bockxmeer

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E. Milne, K.R. Greenop, R.J. Scott, S.E. Jamieson, M. Miller, C. Bower, S. Dawson, G.B. McCowage, N.H. de Klerk

Writing, review, and/or revision of the manuscript: E. Milne, K.R. Greenop, R.J. Scott, M. Haber, M.D. Norris, J. Attia, S.E. Jamieson, M. Miller, C. Bower, H.D. Bailey, S. Dawson, G.B. McCowage, N.H. de Klerk, F.M. van Bockxmeer, B.K. Armstrong
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Milne, K.R. Greenop, S. Dawson
Study supervision: E. Milne, R.J. Scott, J. Attia
Other (development of dietary assessment methods, supervision of dietary data collection, analysis, and interpretation): M. Miller

Acknowledgments

The authors thank their laboratory technical staff (Lan Nguyen, Stewart Smith, Jayne Murray, and Raewyn Billings) for valuable contribution.

Consortium Statement

The Aus-ALL consortium conducted the study and the Telethon Kids Institute, University of Western Australia, was the coordinating centre. Bruce Armstrong (Sydney School of Public Health), Elizabeth Milne (Telethon Kids Institute), Frank van Bockxmeer (Royal Perth Hospital), Michelle Haber (Children's Cancer Institute Australia), Rodney Scott (University of Newcastle), John Attia (University of Newcastle), Murray Norris (Children's Cancer Institute Australia), Carol Bower (Telethon Kids Institute), Nicholas de Klerk (Telethon Kids Institute), Lin Fritschi (WA Institute for Medical Research), Ursula Kees (Telethon Kids Institute), Margaret Miller (Edith Cowan University), and Judith Thompson (WA Cancer Registry) were the research investigators and Helen Bailey (Telethon Kids Institute) was the project coordinator. The clinical Investigators were: Frank Alvaro (John Hunter Hospital, Newcastle); Catherine Cole (Princess Margaret Hospital for Children, Perth); Luciano Dalla Pozza (Children's Hospital at Westmead, Sydney); John Daubenton (Royal Hobart Hospital, Hobart); Peter Downie (Monash Medical Centre, Melbourne); Liane Lockwood (Royal Children's Hospital, Brisbane); Maria Kirby (Women's and Children's Hospital, Adelaide); Glenn Marshall (Sydney Children's Hospital, Sydney); Elizabeth Smibert (Royal Children's Hospital, Melbourne); and Ram Suppiah (previously Mater Children's Hospital, Brisbane).

Grant Support

This study was supported by the Australian National Health and Medical Research Council (Grant ID 254539), National Health and Medical Research Council Career Development Award and Cancer Council Western Australia Research Fellowship (to E. Milne), National Health and Medical Research Council PhD Scholarship (to H. Bailey), and National Health and Medical Research Council Fellowship and Program Grant (to C. Bower).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 16, 2014; revised September 22, 2014; accepted November 9, 2014; published OnlineFirst November 13, 2014.

References

1. Greaves M. Infection, immune responses and the aetiology of childhood leukaemia. Nat Rev Cancer 2006;6:193–203.
2. Trevino LR, Yang W, French D, Hunger SP, Carroll WL, Devidas M, et al. Germline genomic variants associated with childhood acute lymphoblastic leukemia. Nat Genet 2009;41:1001–5.
3. Enciso-Mora V, Hosking FJ, Sheridan E, Kinsey SE, Lightfoot T, Roman E, et al. Common genetic variation contributes significantly to the risk of childhood B-cell precursor acute lymphoblastic leukemia. Leukemia 2012;26:2212–5.
4. Xu H, Yang W, Perez-Andreu V, Devidas M, Fan Y, Cheng C, et al. Novel susceptibility variants at 10p12.31-12.2 for childhood acute lymphoblastic leukemia in ethnically diverse populations. J Natl Cancer Inst 2013;105:733–42.
5. Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G, et al. Prenatal origin of acute lymphoblastic leukaemia in children. Lancet 1999;354:1499–503.
6. Thompson JR, FitzGerald P, Willoughby ML, Armstrong BK. Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. Lancet 2001;358:1935–40.
7. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
8. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998;64:169–72.
9. Pereira TV, Rudnicki M, Pereira AC, Pombo-de-Oliveira MS, Franco RF. 5,10-Methylenetetrahydrofolate reductase polymorphisms and acute lymphoblastic leukemia risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15:1956–63.
10. Zintzaras E, Koufakis T, Ziakas PD, Rodopoulou P, Giannouli S, Voulgarelis M. A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in acute lymphoblastic leukemia. Eur J Epidemiol 2006;21:501–10.
11. Koppen IJ, Hermans FJ, Kaspers GJ. Folate related gene polymorphisms and susceptibility to develop childhood acute lymphoblastic leukaemia. Br J Haematol 2010;148:3–14.
12. Wang J, Zhan P, Chen B, Zhou R, Yang Y, Ouyang J. MTHFR C677T polymorphisms and childhood acute lymphoblastic leukemia: a meta-analysis. Leuk Res 2010;34:1596–600.
13. Vijayakrishnan J, Houlston RS. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. Haematologica 2010;95:1405–14.
14. Wang H, Wang J, Zhao L, Liu X, Mi W. Methylenetetrahydrofolate reductase polymorphisms and risk of acute lymphoblastic leukemia-evidence from an updated meta-analysis including 35 studies. BMC Med Genet 2012;13:77.
15. Yan J, Yin M, Dreyer ZE, Scheurer ME, Kamdar K, Wei Q, et al. A meta-analysis of MTHFR C677T and A1298C polymorphisms and risk of acute lymphoblastic leukemia in children. Pediatr Blood Cancer 2012;58:513–8.
16. Nikbakht M, MalekZadeh K, Kumar Jha A, Askari M, Marwaha RK, Kaul D, et al. Polymorphisms of MTHFR and MTR genes are not related to susceptibility to childhood ALL in North India. Exp Oncol 2012;34:43–8.
17. Lupo PJ, Nousome D, Kamdar KY, Okcu MF, Scheurer ME. A case-parent triad assessment of folate metabolic genes and the risk of childhood acute lymphoblastic leukemia. Cancer Causes Control 2012;23:1797–803.
18. Kamel AM, Moussa HS, Ebid GT, Bu RR, Bhatia KG. Synergistic effect of methyltetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphism as risk modifiers of pediatric acute lymphoblastic leukemia. J Egypt Nat Cancer Inst 2007;19:96–105.
19. Lightfoot TJ, Johnston WT, Painter D, Simpson J, Roman E, Skibola CF, et al. Genetic variation in the folate metabolic pathway and risk of childhood leukemia. Blood 2010;115:3923–9.
20. Rahimi Z, Ahmadian Z, Akramipour R, Vaisi-Raygani A, Rahimi Z, Parsian A. Thymidylate synthase and methionine synthase polymorphisms are not associated with susceptibility to childhood acute lymphoblastic leukemia in Kurdish population from Western Iran. Mol Biol Rep 2012;39:2195–200.
21. Lupo PJ, Dietz DJ, Kamdar KY, Scheurer ME. Gene–environment interactions and the risk of childhood acute lymphoblastic leukemia: exploring the role of maternal folate genes and folic acid fortification. Pediatr Hematol Oncol 2014;31:160–8.
22. Lautner-Csorba O, Gezsi A, Erdelyi DJ, Hullam G, Antal P, Semsei AF, et al. Roles of genetic polymorphisms in the folate pathway in childhood acute lymphoblastic leukemia evaluated by bayesian relevance and effect size analysis. PLoS ONE 2013;8:e69843.
23. Yu K, Zhang J, Zhang J, Dou C, Gu S, Xie Y, et al. Methionine synthase A2756G polymorphism and cancer risk: a meta-analysis. Eur J Hum Genet 2010;18:370–8.
24. Amigou A, Rudant J, Orsi L, Goujon-Bellec S, Leverger G, Baruchel A, et al. Folic acid supplementation, MTHFR and MTRR polymorphisms, and the

risk of childhood leukemia: the ESCALE study (SFCE). Cancer Causes Control 2012;23:1265–77.
25. Metayer C, Scelo G, Chokkalingam AP, Barcellos LF, Aldrich MC, Chang JS, et al. Genetic variants in the folate pathway and risk of childhood acute lymphoblastic leukemia. Cancer Causes Control 2011;22: 1243–58.
26. de Jonge R, Tissing WJ, Hooijberg JH, Jansen G, Kaspers GJ, Lindemans J, et al. Polymorphisms in folate-related genes and risk of pediatric acute lymphoblastic leukemia. Blood 2009;113:2284–9.
27. Gast A, Bermejo JL, Flohr T, Stanulla M, Burwinkel B, Schrappe M, et al. Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukemia: a case-control study. Leukemia 2007;21:320–5.
28. Petra BG, Janez J, Vita D. Gene-gene interactions in the folate metabolic pathway influence the risk for acute lymphoblastic leukemia in children. Leuk Lymphoma 2007;48:786–92.
29. Chan JY, Ugrasena DG, Lum DW, Lu Y, Yeoh AE. Xenobiotic and folate pathway gene polymorphisms and risk of childhood acute lymphoblastic leukaemia in Javanese children. Hematol Oncol 2011;29: 116–23.
30. Silva RM, Fontes AC, Silva KA, Sant'ana TA, Ramos FJ, Marques-Salles Tde J, et al. Polymorphisms involved in folate metabolism pathways and the risk of the development of childhood acute leukemia. Genet Test Mol Biomark 2013;17:147–52.
31. Nazki FH, Masood A, Banday MA, Bhat A, Ganai BA. Thymidylate synthase enhancer region polymorphism not related to susceptibility to acute lymphoblastic leukemia in the Kashmir population. Genet Mol Res 2012;11:906–17.
32. Yeoh AEJ, Lu Y, Chan JYS, Chan YH, Ariffin H, Kham SKY, et al. Genetic susceptibility to childhood acute lymphoblastic leukemia shows protection in Malay boys: results from the Malaysia-Singapore ALL Study Group. Leuk Res 2009;34:276–83.
33. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996;93:7–9.
34. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–102.
35. Krajinovic M, Lamothe S, Labuda D, Lemieux-Blanchard E, Theoret Y, Moghrabi A, et al. Role of MTHFR genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Blood 2004;103: 252–7.
36. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol 2003;157:571–82.
37. Zhu H, Yang W, Lu W, Etheredge AJ, Lammer EJ, Finnell RH, et al. Gene variants in the folate-mediated one-carbon metabolism (FOCM) pathway as risk factors for conotruncal heart defects. Am J Med Genet A 2012; 158A:1124–34.
38. van Beynum IM, Kapusta L, den Heijer M, Vermeulen SH, Kouwenberg M, Daniels O, et al. Maternal MTHFR 677C>T is a risk factor for congenital heart defects: effect modification by periconceptional folate supplementation. Eur Heart J 2006;27:981–7.
39. Hobbs CA, Cleves MA, Macleod SL, Erickson SW, Tang X, Li J, et al. Conotruncal heart defects and common variants in maternal and fetal genes in folate, homocysteine, and transsulfuration pathways. Birth Defects Res A Clin Mol Teratol 2014;100:116–26.
40. Lupo PJ, Goldmuntz E, Mitchell LE. Gene-gene interactions in the folate metabolic pathway and the risk of conotruncal heart defects. J Biomed Biotechnol 2010;2010:630940.
41. Milne E, Royle JA, Miller M, Bower C, de Klerk N, Bailey H, et al. Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukemia in the offspring. Int J Cancer 2010; 126:2690–9.
42. Bailey H, Milne E, de Klerk N, Fritschi L, Bower C, Attia J, et al. Representativeness of child controls recruited by random digit dialing. Paediatr Perinat Epidemiol 2010;24:293–302.
43. Milne E, van Bockxmeer FM, Robertson L, Brisbane JM, Ashton LJ, Scott RJ, et al. Buccal DNA collection: comparison of buccal swabs with FTA cards. Cancer Epidemiol Biomarkers Prev 2006;15:816–9.
44. Reeves SG, Meldrum C, Groombridge C, Spigelman AD, Suchy J, Kurzawski G, et al. MTHFR 677 C>T and 1298 A>C polymorphisms and the age of onset of colorectal cancer in hereditary nonpolyposis colorectal cancer. Eur J Hum Genet 2009;17:629–35.
45. Morrison K, Papapetrou C, Hol FA, Mariman EC, Lynch SA, Burn J, et al. Susceptibility to spina bifida; an association study of five candidate genes. Ann Hum Genet 1998;62:379–96.
46. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998; 63:259–66.
47. Cooper MN, de Klerk NH, Greenop KR, Jamieson SE, Anderson D, van Bockxmeer FM, et al. Statistical adjustment of genotyping error in a case-control study of childhood leukaemia. BMC Med Res Methodol 2012; 12:141.
48. Bakker DJ, de Jong-van den Berg LT, Fokkema MR. Controlled study on folate status following folic acid supplementation and discontinuation in women of child-bearing age. Ann Clin Biochem 2009;46:231–4.
49. Cordell HJ, Barratt BJ, Clayton DG. Case/pseudocontrol analysis in genetic association studies: a unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. Genet Epidemiol 2004;26:167–85.
50. Clayton DG. Programs for analysis of genetic association studies. Cambridge, United Kingdom: Cambridge University, 2011.
51. Milne E, de Klerk NH, van Bockxmeer F, Kees UR, Thompson JR, Baker D, et al. Is there a folate-related gene-environment interaction in the etiology of childhood acute lymphoblastic leukemia? Int J Cancer 2006;119: 229–32.
52. Tong N, Fang YJ, Li J, Wang ML, Lu Q, Wang SZ, et al. Methylenetetrahydrofolate reductase polymorphisms, serum methylenetetrahydrofolate reductase levels, and risk of childhood acute lymphoblastic leukemia in a Chinese population. Cancer Sci 2010;101:782–6.
53. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull 1999;55:578–92.
54. Nazki FH, Sameer AS, Ganaie BA. Folate: metabolism, genes, polymorphisms and the associated diseases. Gene 2014;533:11–20.
55. Weiner AS, Boyarskikh UA, Voronina EN, Mishukova OV, Filipenko ML. Methylenetetrahydrofolate reductase C677T and methionine synthase A2756G polymorphisms influence on leukocyte genomic DNA methylation level. Gene 2013;533:168–72.
56. Castro R, Rivera I, Martins C, Struys EA, Jansen EE, Clode N, et al. Intracellular S-adenosylhomocysteine increased levels are associated with DNA hypomethylation in HUVEC. J Mol Med 2005;83:831–6.
57. Castro R, Rivera I, Ravasco P, Camilo ME, Jakobs C, Blom HJ, et al. 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C–>T and 1298A–>C mutations are associated with DNA hypomethylation. J Med Genet 2004;41:454–8.
58. Lambrot R, Xu C, Saint-Phar S, Chountalos G, Cohen T, Paquet M, et al. Low paternal dietary folate alters the mouse sperm epigenome and is associated with negative pregnancy outcomes. Nat Commun 2013;4:2889.
59. Guilmatre A, Sharp AJ. Parent of origin effects. Clin Genet 2012;81:201–9.
60. Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. Bioessays 2014;36:359–71.
61. Padmanabhan N, Jia D, Geary-Joo C, Wu X, Ferguson-Smith AC, Fung E, et al. Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development. Cell 2013;155:81–93.
62. Brown RD, Langshaw MR, Uhr EJ, Gibson JN, Joshua DE. The impact of mandatory fortification of flour with folic acid on the blood folate levels of an Australian population. Med J Aust 2011;194:65–7.
63. Australian Institute of Health and Welfare. Mandatory folic acid and iodine fortification in Australia and New Zealand: supplement to the baseline report for monitoring. Cat. no. PHE 153; [cited May 20 2014] Canberra, Australia: AIHW, 2011 Available from: http://aihw.gov.au/publication-detail/?id=10737420085.

Folate Pathway Gene Polymorphisms, Maternal Folic Acid Use, and Risk of Childhood Acute Lymphoblastic Leukemia

Elizabeth Milne, Kathryn R. Greenop, Rodney J. Scott, et al.

Cancer Epidemiol Biomarkers Prev 2015;24:48-56. Published OnlineFirst November 13, 2014.